Handbook of PREBIOTICS AND PROBIOTICS INGREDIENTS

Health Benefits and Food Applications

Edited by
SUSAN SUNGSOO CHO
E. TERRY FINOCCHIARO

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Preface

Prebiotics and probiotics have been proven to promote gastrointestinal health and immune function. The concept behind probiotics is to enhance good bacteria and discourage bad bacteria in the human gastrointestinal tract. Prebiotics, which enhance the growth of beneficial bacteria in the lower intestine, are primarily fibers naturally found in food. The food industry is in a position to recognize that prebiotics and probiotics may contribute to helping improve public health by promoting gastrointestinal health as well as immune function. However, it is important to find prebiotics and probiotics that are fully compatible with formulation, processing, packaging, and distribution. This *Handbook of Prebiotics and Probiotics Ingredients* is comprehensive in the field of prebiotics and probiotics; it includes the most current biological research findings and food applications. The handbook also includes global aspects of both prebiotics and probiotics with chapters contributed by experts from around the world. It will serve as a thorough reference for product developers, nutritionists, health professionals, and government agencies worldwide.

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CHAPTER 1

Analysis of Dietary Fiber and Nondigestible Carbohydrates

Betty W. Li

CONTENTS

1.1 INTRODUCTION

In this chapter, we present several analytical methods, mostly the official methods that have been approved by AOAC International (Association of Official Analytical Chemists) and American Association of Cereal Chemists (AACC), for the determination of dietary fiber and specific nondigestible carbohydrates that have purported health-promoting properties and that could be classified as "prebiotics." During the past three decades, there have been a number of published analytical methods for measuring dietary fiber (DF). Most were developed based on a physiological definition proposed by Trowell et al.[1] in 1976: "Dietary fibre consists of the plant polysaccharides and lignin, which are resistant to hydrolysis, by digestive enzymes of man." Between 1975 and 1983, several analysts in Europe and the United States were developing gravimetric procedures using a combination of pepsin, pancreatin, α-amylase, and amyloglucosidase to remove protein and starch from test samples. Through the joint efforts of scientists at U.S. Food and Drug Administration (FDA), members of AOAC International, and other analysts in North America and Europe, a collaborative study was completed and published as an enzymatic–gravimetric method. This method was adopted as official AOAC method 985.29. Subsequently, it has been modified and simplified by other groups in the United States and Canada. By 1994, four other methods were also collaboratively studied and adopted as official methods by AOAC and AACC. Need for implementation of the Nutrition Labeling and Education Act of 1990 has led to a de facto definition of DF as the material isolated by AOAC method 985.29 as modified in 1988 (FDA-DHHS, 1990).[2] Table 1.1 lists all the approved methods with corresponding number, name, and reference. All five currently approved methods for total dietary fiber (TDF) require a step in which the fiber fraction that is soluble in enzyme digestate is presumed to precipitate in 78 to 80 percent ethanol, and thus is

Table 1.1 Approved Methods for Total Dietary Fiber

Method Number		
AOAC	**AACC**	**Method Name**
985.29	32-05	Total Dietary Fiber in Foods. Enzymatic-Gravimetric Method (Prosky et al., 1985)[5]
991.43	32-07	Total, Soluble, and Insoluble Dietary Fiber in Foods—Enzymatic-Gravimetric Methods, MES-TRIS Buffer (Lee, et al., 1992)[6]
992.16	32-06	Total Dietary Fiber, Enzymatic-Gravimetric Method (Mongeau and Brassard, 1993)[7]
993.21		Total Dietary Fiber in Foods and Foods Products with ≤ 2% Starch, Nonenzymatic-Gravimetric Method (Li and Cardozo, 1994)[9]
994.13	32-25	Total Dietary Fiber (Determined as Neutral Sugar Residues, Uronic Acid Residues, and Klason Lignin) Gas Chromatographic–Calorimetric–Gravimetric Method (Theander et al., 1995)[10]

Table 1.2 Approved Methods for Nondigestible Carbohydrates

Method Number		
AOAC	**AACC**	**Method Name**
997.08	32-31	Fructans in Food Products, Ion Exchange Chromatographic Method (Hoebregs, 1997)[11]
999.03	32-32	Measurement of Total Fructan in Foods by Enzymatic/ Spectrophotometric Method (McCleary et al., 2000)[16]
2000.11	32-28	Polydextrose in Foods, Ion Chromatographic Method (Craig et al., 2001)[12]
2001.02	32-33	Determination of *trans*-galactooligosaccharides in Selected Food Products by IC (Slegte, 2002)[13]
2001.03	32-41	Determination of Resistant Maltodextrins and Total Dietary Fiber in Selected Foods by LC–Enzymatic–Gravimetric Method (Gordon and Ohkuma, 2002)[14]

recovered along with the insoluble fraction via filtration. There are, however, certain naturally occurring or manufactured oligosaccharides and polysaccharides, that is, nondigestible carbohydrates, that remain soluble in the dilute alcohol medium and, hence, are not recovered as part of the TDF residue. Since 1997, methods have been developed and approved by AOAC and AACC for separate determinations of fructans and fructo-oligosaccharides, polydextrose, galacto-oligosaccharides, and resistant maltodextrins (Table 1.2). In 2002, the Institute of Medicine of the National Academy of Sciences[3] proposed a definition stating:

> *Dietary Fiber* consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants. *Functional Fiber* consists of isolated, nondigestible carbohydrates that have beneficial physiological effect in humans. *Total Fiber* is the sum of Dietary Fiber and Functional Fiber.

If and when the above definition is accepted by the FDA, the scientific community, and consumers, then the existing analytical methods need to be modified to measure all the components as defined above.

1.2 ANALYTICAL PROCEDURES FOR TOTAL DIETARY FIBER

The approved methods can be classified as either gravimetric or chemical procedures. Regardless of this distinction, all ground, dried food samples containing >10 percent fat and/or sugar, should be extracted sequentially with hexane or petroleum ether to remove fat, and with 80 percent ethanol or methanol to remove sugar. Detailed descriptions of each method under discussion can be found in an AOAC publication.[4]

Table 1.3 Enzymatic–Gravimetric Methods: Their Buffers and Enzymes

Method	Buffer	Enzymes
AOAC 985.29	Phosphate	α-Amylase (heat-stable termamyl), protease, amyloglucosidase
AOAC 991.43	MES-TRIS	α-Amylase (heat-stable termamyl), protease, amyloglucosidase
AOAC 992.16	Phosphate, acetate	α-Amylase (heat-stable termamyl), protease, amyloglucosidase, "-amylase
AOAC 994.13	Acetate	α-Amylase (heat-stable termamyl), amyloglucosidase

1.2.1 Enzymatic–Gravimetric Methods

AOAC methods 985.29,[5] 991.43,[6] and 992.16[7] fall under this classification and are based on the principle that a combination of enzymes in specific buffers will hydrolyze starch and protein when present in a particular food sample. By adding to the digestate four times its volume of 95 percent ethanol, soluble and insoluble DF along with other minor food components is precipitated and collected by filtration. The isolated residues are corrected for crude protein and ash, and the final weights are taken to be TDF content of the test samples. In 2007, Kanaya et al.[8] published studies using newly developed enzymes to further shorten the analysis time for AOAC method 991.43. For foods containing < 2 percent starch, AOAC method 993.21[9] does not require any enzyme treatment. Table 1.3 lists approved TDF methods with their respective buffers and enzymes.

1.2.2 Enzymatic–Chemical Method

AOAC method 994.13[10] is the only approved method that quantifies, as monosaccharides, the carbohydrate constituents of DF residues are isolated similarly to those from the enzymatic–gravimetric procedures. Test samples are treated with enzymes to remove starch, then insoluble materials, recovered from dilute alcohol, are hydrolyzed stepwise in concentrated and then dilute sulfuric acid. Neutral sugars in the hydrolyzate are derivatized, first by reduction, followed with acetylation; the resulting alditol acetates are separated and quantified by gas chromatography (GC) or analyzed as free sugars by high-performance liquid chromatography (HPLC) after a sample cleanup step. Uronic acids are determined by a colorimetric procedure. Klason lignin content is calculated as acid insoluble organic matter lost upon ashing.

1.3 ANALYTICAL PROCEDURES FOR NONDIGESTIBLE CARBOHYDRATES

As mentioned before, there are naturally occurring or manufactured oligo- and polysaccharides that are not recovered by any of the approved AOAC/AACC methods

for measuring TDF. Some of these dilute, alcohol-soluble nondigestible carbohydrates do possess physiological characteristics similar to DF, such as fermentation to short-chain fatty acids, effect on fecal bulking, and transit time. In some cases, they may be considered "prebiotics."

At present, there are five approved methods for the determination of nondigestible carbohydrates. These methods can be classified as chromatographic or spectrophotometric procedures; in general, they all require initial extraction with hot (80°C) or boiling water and centrifugation in an ultrafiltration device when appropriate. In 2008, a new method was published for the determination of fructo-oligosaccharides using ion cyclotron resonance mass spectrometry.

1.3.1 Ion Chromatographic Method

1.3.1.1 For Fructans and Fructo Oligosaccharides

Fructans are polysaccharides consists of fructose linked by β-(2-1) bonds with degree of polymerization (DP) range from 2 to 60 as in inulin, and 2 to 10 as in fructo-oligosaccharides.

AOAC method 997.08/AACC 32-31[11] was the first method approved by AOAC International and AACC specifically for the determination of fructans and their oligomers. Test samples are extracted with boiling water; the extract is hydrolyzed sequentially with amyloglucosidase and inulinase. Free fructose, glucose, and sucrose are separated and quantified by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) in the extract before hydrolysis, then glucose and fructose after each of the two enzyme hydrolysis steps. Fructan content in the test sample is calculated by difference from the amount of each sugar in different solutions.

1.3.1.2 For Polydextrose

Polydextrose is a manufactured polysaccharide prepared by acid catalyzed vacuum thermal polymerization of glucose and sorbitol. The average DP is 12 with range of molecular weight between 162 and 20,000. AOAC method 2000.11[12] incorporates hot water extraction and ultrafiltration. The filtrate is treated with a mixture of isoamylase, amylogluco-sidase, and fructanase. Polydextrose standards are treated in similar manner, and used to quantify a high-molecular-weight fraction of polydextrose using HPAEC-PAD.

1.3.1.3 For trans-Galacto-Oligosaccharides

trans-Galacto-oligosaccharides (TGOS) are manufactured oligosaccharides produced from lactose by enzymatic transgalactosylation and with DP range from 2 to 7. AOAC method 2001.02[13] employs hot (80°C) phosphate buffer for the extraction of TGOS and lactose from test samples. The extract is treated with β-galactosidase to hydrolyze the di- and oligosaccharides to yield glucose and galactose. Free galactose

and lactose are determined before and after enzyme hydrolysis, and their concentrations are used to calculate the total TGOS content of the test samples.

1.3.2 High-Performance Liquid Chromatographic Method

1.3.2.1 For Resistant Maltodextrins

Resistant maltodextrins (RM) are mixtures of oligo- and polysaccharides produced by a combination of heat and enzyme treatment of cornstarch with a wide range of molecular weight averaging about 2,000. The lower-molecular-weight fraction is soluble in dilute alcohol. The AOAC method 2001.03[14] measures first a nondigestible carbohydrate fraction recovered from 78 percent alcohol solution using AOAC method 985.29. Then the dilute alcohol filtrate is concentrated on a rotary evaporator, and passed through ion exchange resins for the removal of salts and proteins. Low-molecular-weight RM is quantified by HPLC with reflective index detector. This method measures both the dilute alcohol soluble and insoluble nondigestible carbohydrates.

1.3.2.2 For Lactulose

Using a Waters carbohydrate analysis column, separation and quantification of a solution containing galactose, tagatose, lactose, and lactulose was achieved by elution with a mixture of water and acetonitrile as described by Parrish et al.[15] This is not an official method; however, it is applicable for the analysis of samples containing mono- and disaccharides.

1.3.3 Spectrophotometric Method

1.3.3.1 For Total Fructan

AOAC method 999.03[16] incorporates enzyme treatments with spectrophotometric determination for the measurement of fructan and fructo-oligosaccharides. Test samples are extracted into hot water (80°C) with pH maintained above 5.5. Extracts are incubated with a solution of sucrase/amylase, followed by reduction with sodium borohydride. The mixtures containing sugar alcohol are then incubated with fructanase, followed by the addition of PAHBAH (p-hydroxybenzoic acid hydrazide) reagent and the absorbance is measured at 410 nm against a reagent blank. Total fructan content is calculated from the concentration of fructose in the hydrolyzate.

1.3.4 Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

1.3.4.1 For Fructo-Oligosaccharides (FOS)

A relatively new method[17] for precise quantification of fructo-oligosaccharides has been published utilizing matrix-assisted laser desorption/ionization Fourier

transform ion cyclotron resonance mass spectrometry. The method was used to monitor the consumption of fructo-oligosaccharides in bacterial fermentation samples to better understand the role of inulin and FOS as prebiotics.

1.4 NEEDS

1.4.1 Reliable Methods for Determining Lignin as a Component of Dietary Fiber

In any enzymatic–gravimetric method, DF as oligo- and polysaccharides that are nonhydrolyzable by the specific enzymes are usually recovered along with lignin and other associated substances, such as waxes, cutin, and suberin from 78 percent alcohol. In the enzymatic–chemical method, only the constituent sugars and lignin represent DF. However, there is no accurate method for routine measurement of lignin, whose structure as a phenyl-propanoid polymer has not been well defined. Klason lignin determined by AOAC method 994.13, as the acid insoluble organic matter in the DF residue, may include some tannins and Maillard reaction products. A modified permanganate method has been shown to be more reproducible and the values are lower when compared with those obtained after acid detergent fiber extraction followed by permanganate treatment or after Klason lignin treatment.[18]

1.4.2 Methods to Determine Resistant Starch, Naturally Occurring and Added

The fraction of starch that escapes digestion in the small intestine and is fermented in the large intestine is known as resistant starch (RS).[19] Analytically, the amount of RS isolated as part of DF varies depending on the food and the method. At present, all AOAC methods for TDF include a certain amount of RS in their DF values for starchy foods. AOAC method 2002.02/AACC method 32-40[20] specifically measures RS. Test samples are incubated with a mixture of pancreatic α-amylase and amyloglucosidase at 37°C for 16 hours. A pellet is obtained by centrifugation, then dissolved in 2 *M* KOH; the alkaline solution is neutralized with acetate buffer, and treated with amyloglucosidase. The absorbance of glucose in the enzyme hydrolyzate is measured at 510 nm after the addition of glucose oxidase-peroxidase reagent. RS content is calculated from the amount of glucose in the hydrolyzate.

1.4.3 Integrated Methods to Determine Alcohol-Soluble and Alcohol-Insoluble Nondigestible Carbohydrates

With the exception of AOAC method 2001.03 for the determination of resistant maltodextrins and TDF, all the existing methods mentioned above are applicable for the determination of either alcohol-soluble or alcohol-insoluble nondigestible carbohydrates, but not both simultaneously in the same test portion. Integrated methods ought to be developed to do just that. Such methods should also be able to quantify

a variety of alcohol-soluble nondigestible carbohydrates when present in the same food, for example, fructo-oligosaccharides, polydextrose, and other naturally occurring or manufactured oligosaccharides.

1.4.4 Methods to Distinguish Naturally Occurring from Added Nondigestible Carbohydrates

Fructo-oligosaccharides and higher-molecular-weight fructans occur naturally in many plant foods; however, in a number of processed foods, they have been isolated from natural sources and added as food ingredients. This is analogous to processed sucrose from sugar beets or canes. At present, there is no method by which one can quantify the amount of sucrose that comes from a plant food and that which was added, for example, in sweetened canned fruits. Similarly, there is no method for determining any given nondigestible carbohydrate as naturally occurring DF or as added fiber.

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PART I

Sources of Prebiotics

CHAPTER 2

Short-Chain Fructo-Oligosaccharide
A Low Molecular Weight Fructan

Anne M. Birkett and Coni C. Francis

CONTENTS

2.1 INTRODUCTION

2.1.1 What Is scFOS?

Fructans have been defined as "any compound where one or more fructosyl–fructose linkages constitutes a majority of linkages … fructan is used to name molecules that have a majority of fructose residues, whatever the number is" (Roberfroid, 2005a). Fructans therefore represent a heterogeneous group, and as such, many different possible chemical entities exist. Fructans can vary with respect to the following (Roberfroid, 2005a):

- Source—Plant, bacteria, and fungi
- Chain composition—All fructose or mostly fructose
- Linkages—2,1 and 2,6
- Degree of polymerization (DP)—Plant fructans do not exceed DP of 200; however, bacterial fructans can have a DP as high as 100,000
- Architecture—Linear, branched, or cyclic
- Functionality—Physiology and food science

Because of the heterogeneity of the fructan family, subclass classifications have evolved with their own set of chemical and physiological properties. Figure 2.1 represents different classes of linear fructans, categorized according to chain length. The subclass called inulin represents a higher-molecular-weight group, with DP < 200. In contrast, the subclass called oligofructose has a lower molecular weight, with DP < 10 (Roberfroid, 2005a). The oligofructose subgroup can be further subdivided into the group called short-chain fructo-oligosaccharides (scFOS).

Commercially, scFOS consists of low-molecular-weight linear chains synthesized by enzymatic fermentation from sucrose; however, the short chains also exist in nature. scFOS is clearly a unique subset of the broader oligofructose group because the fermentation process results in linear chains of three to five sugar units only, with every chain terminated by glucose. In the broader oligofructose group, DP can extend to 10, and chains can be terminated by either glucose or fructose, which

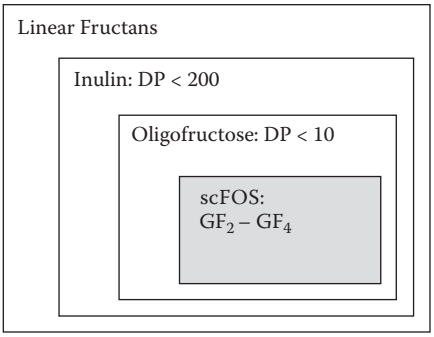

Figure 2.1 Classes of linear plant fructans, categorized by chain length.

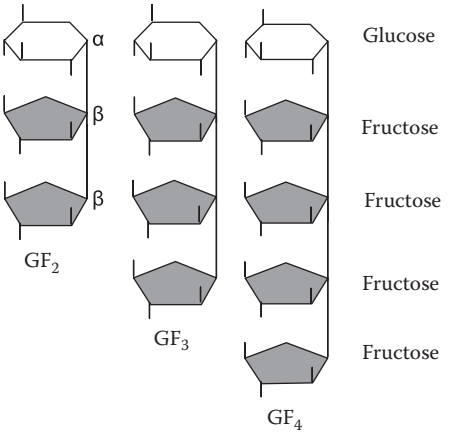

Figure 2.2 Structure of scFOS. (Adapted from Spiegel et al., 1994.)

influences food application properties, such as participation in Maillard browning reactions. The nomenclature for the scFOS chains can be abbreviated to: GF_2 (= 1-kestose); GF_3 (= nystose); GF_4 (= fructosylnystose or 1F-β-fructofuranosylnystose), as shown in Figure 2.2 (Hidaka et al., 1986; Kono, 1993; Spiegel et al., 1994). Bonds between the scFOS monomers are not hydrolyzed between the mouth and small intestine: the fructosyl–glucose linkage is always β–(2<–>1) as in sucrose, and the fructosyl-fructose linkages are β–(1→2) (Roberfroid, 2005a).

Owing to differences in structure, it is important to characterize and understand the collective nutritional, chemical, and food science properties of scFOS as a separate fructan subgroup. In this chapter, nutritional studies cited used scFOS not oligofructose, except where otherwise indicated. Thus, the breadth of evidence on scFOS is presented. Also in this review, the properties of scFOS have been compared with other fructan ingredients. Various commercial sources of fructan ingredients are available, with chicory being the primary raw material used for inulin and oligofructose (Roberfroid, 2005a). Examples of commercial ingredients include:

Inulin
- Orafti: ST, ST-gel, GR, HP, HP-gel, HPX, HIS, HIS Ultra (BENEO-Orafti, www.orafti.com)
- Oliggo-Fiber: XL, DS2, Instant, Instant Premium, S20 (Cargill, www.cargillhft.com)
- Fibruline: XL, DS2, Instant, S20 (Cosucra, www.cosucra.com)
- Frutafit: HD, IQ, CLR, TEX (Sensus, www.sensus.nl)

Oligofructose
- Orafti: L60, L85, L95, P95, Synergy 1 (BENEO-Orafti, www.orafti.com)
- Oliggo-Fiber: F97, F97 Premium (Cargill, www.cargillhft.com)
- Fibrulose: F97 (Cosucra, www.cosucra.com)
- Frutalose: L60, L85, L92 (Sensus, www.sensus.nl)

scFOS
NutraFlora® (GTC Nutrition, www.nutraflora.com)
Actilight (Beghin Meiji and Syral, www.beghin-meiji.com)
Meioligo (Meiji Seika Kaisha Ltd., www.meiji.co.jp)

2.1.2 Sources of scFOS

Fructans serve storage and protective functions in many commonly consumed plants. Thus, fructans are a typical part of the diet. Some food sources of fructans are higher in scFOS, while others are richer in high-molecular-weight fructans, such as inulin. scFOS is present in selected foods that include onion, artichoke, garlic, wheat, and banana, and is typically present at low levels (Table 2.1). In contrast, some prepared meals are particularly high in total fructan content. For example, a bowl of French onion soup could contain 6 to 18 g of fructans (Van Loo et al., 1995).

Estimated daily intakes of fructans in the United States have been calculated by applying analytical values for various foods to food consumption databases. According to the three references below, mean total fructan intake likely ranges between 1 to 5 g/day with scFOS intake < 1 g/day.

- Van Loo et al. (1995) estimated that consumption of fructans ranged between 1 to 4 g/day, mostly coming from wheat (76 to 78 percent), onion (10 to 18 percent), and banana (3 to 5 percent); 10 percent of the population was estimated to eat double this amount, between 2 and 8 g/day.
- Moshfegh et al. (1999) estimated the separate consumption of oligofructose and inulin in the United States using the U.S. Department of Agriculture database, 1994–1996 Continuing Survey of Food Intakes by Individuals. Estimated mean intakes were 2.5 g/day (range 1 to 4 g) for oligofructose and 2.6 g/day (range 1 to 4 g) for inulin. Thus, the combined total intake of fructans was estimated to be similar to that of Van Loo et al. (1995), and approximately 50 percent of fructans consumed would be DP < 10. Food sources contributing oligofructose were mostly wheat (71 percent), onion (24 percent), banana (2 percent), and garlic (2 percent).

Table 2.1 Food Sources of scFOS

Plant	Fructan, g/100 g, as is	scFOS Content (DP ≤ 5)
Onion	1–8, raw	DP 2–12 = 100%; most frequently occurring DP is 5
Jerusalem artichoke	17–21, raw	DP <10 = 52%
Garlic	16	DP <5 = 25%
Wheat	1–4	DP <5 = 50%
Globe artichoke	2	DP <4 = 5%
Banana	1	DP <5 = 100%

Source: Adapted from Van Loo et al., 1995.

- Spiegel et al. (1994) specifically estimated scFOS intake, using consumption data from the Environmental Protection Agency's Dietary Risk Evaluation System. Their estimation for scFOS intake was 0.8 g/day, and the two primary food sources of scFOS were tomato (0.6 g) and banana (0.2 g). Interestingly, tomato was not listed as a primary source of fructans by Van Loo et al. (1995) or Moshfegh et al. (1999).

Estimations of fructan or specifically scFOS intake are only available for a few countries. Van Loo et al. (1995) estimated fructan intake in Europe and reported a higher intake than for the United States, at 3 to 11 g/day. Most of the fructans would likely come from wheat (63 to 69 percent), onion (14 to 16 percent), and garlic (5 to 9 percent), similar to the United States. Intakes of fructans would vary regionally, due to different food preferences. For example, the estimated fructan intake in Belgium ranged from 3 to 10 g/day, and the estimated fructan intake in Spain ranged from 6 to 17 g/day.

2.1.3 Recognition of scFOS as a Fiber

Dietary fiber is unique among nutrients in that it is generally accepted as a physiological concept rather than a chemical entity. That is, the dietary fiber in a food could represent a collection of different components varying in chemical and physical attributes, and varying in relative proportions. At this time, there is no globally utilized definition for dietary fiber, but most definitions in use include or assume the following criteria (Roberfroid, 2005c):

- Is present in edible plant cells
- Is a carbohydrate
- Resists hydrolysis by human/mammalian intestinal enzymes
- Resists absorption in the small intestine
- Is fermented (partially or totally) by large intestinal bacteria

scFOS meets all of these criteria and, therefore, can be considered a dietary fiber.

In the United States, there has been a reliance on methodology to identify and measure fiber components. This is rather arbitrary for many nondigestible carbohydrates meeting the above criteria, particularly for fructans, such as scFOS, which do not measure as a fiber using standard Association of Official Analytical Chemists (AOAC) enzymatic–gravimetric methods (e.g., AOAC 985.29, AOAC 991.43). scFOS is not measured by these methods because it is soluble in aqueous ethanol; however, it can be measured by alternative methods, such as the enzymatic–chemical method AOAC 999.03 and the enzymatic–HPAEC (high-performance anion-exchange chromatography) method AOAC 997.08 (McCleary, 2003). The latter method is based on a DP of 10, so it can be corrected for the lower DP of scFOS for a more accurate measurement if required.

As a result of the methodological issues described above, the following two definitions of fiber are often used as a guideline in the United States to assess whether

a food component is a dietary fiber. According to both definitions, scFOS would be considered a component of fiber.

1. **American Association of Cereal Chemists (AACC, 2001):** "Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation." In the discussion of this definition, the authors referred to oligosaccharides with a DP between 3 and 10 and stated that they are "clearly included in this definition." scFOS would be classified as an analogous carbohydrate.
2. **Food and Nutrition Board (FNB) of the U.S. Institute of Medicine of the National Academy of Sciences (2005):** "Dietary fiber consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants. Functional fiber consists of isolated, nondigestible carbohydrates that have beneficial physiological effects in humans." In the discussion of this definition, the authors clearly state that "fructans could be classified as functional fibers." Indeed, the report specifically describes fructo-oligosaccharides as DP 2 to 4.

The Food Chemical Codex (2006) has recognized scFOS with a separate published monograph. The monograph differentiates and defines "fructo-oligosaccharides, scFOS" produced from sucrose compared with that which is made from inulin.

2.1.4 Manufacturing Process for scFOS

scFOS is manufactured by a bioenzymatic (or fermentation) process, using sucrose from sugar beet or cane sugar as the starting raw material. There are several key advantages of this process relative to extracting scFOS directly from plant sources:

- The composition and architecture of the scFOS chains are more consistent.
- All chains are glucose terminated, which influences functionality (see later).
- The process is more efficient than extracting directly from known plant sources. For example, the fructans in banana are 100 percent DP < 5, but banana only contains 1 percent fructans.

The process is considered natural, non-genetically modified, kosher, halal, and allergen free.

The bioenzymatic process uses a β-fructofuranosidase enzyme from the fungus *Aspergillus niger*. This is a transfructosylating enzyme that links fructose from one sucrose molecule to another, thereby sequentially building up the fructose backbone of the scFOS chain. To increase yield, residual-free sucrose and glucose, as well as the enzyme, are removed after the fermentation process by chromatographic separation (Kono, 1993).

Three linear chains are produced by this bioenzymatic process (Figure 2.2). Their approximate relative proportions are as follows (Bornet, 1994; Bouhnik et al., 2006; Hidaka et al., 1990):

- GF_2 (1-kestose): ~35 to 40 percent of scFOS
- GF_3 (nystose): ~45 to 50 percent of scFOS
- GF_4 (fructosylnystose): ~10 percent of scFOS

2.2 PHYSIOLOGICAL EFFECTS OF scFOS

2.2.1 Digestibility of scFOS

The chemical and physical nature of scFOS is quite similar to sucrose, but the physiological action is very different. Both *in vivo* and *in vitro* models have been used to demonstrate that scFOS is not digested between the mouth and small intestine, prior to the large intestine. This is because neither the pancreas nor the small intestine mucosa secrete enzymes capable of hydrolyzing the β–(1→2) fructosyl-fructose linkages.

Digestibility of scFOS has been assessed in various ways, including simulated salivary and intestinal enzyme digestion, measurement of glucose and insulin response, fate of labeled scFOS, and breath hydrogen determination.

- ***In vitro:*** Digestion in the mouth was simulated by incubating scFOS *in vitro* with human salivary enzymes at 37°C for 24 hours. Compared with sucrose and maltose, the scFOS was not digested (Hidaka et al., 1986).
- ***In vitro:*** Digestion in the human small intestine was simulated by incubating scFOS with rat pancreatic homogenate and small intestinal mucosa at 37°C for 2 hours. The scFOS was not digested (Hidaka et al., 1986).
- **Rats:** Digestibility was tested by feeding ^{14}C labeled scFOS to germ-free, antibiotic-treated, and conventional rats. In the germ-free rats, ^{14}C was not detected in exhaled carbon dioxide within the first 4 hours, and hardly detected within the first 8 hours, indicating that scFOS is not digested in the small intestine (Tokunaga, 2004).
- **Humans:** Digestibility was tested indirectly *in vivo* in healthy male subjects using a glucose response test. In the test, 25 g scFOS was consumed after overnight fasting, and blood glucose, fructose, and insulin were measured over a 2-hour period. Response was compared with a 25 g sucrose challenge. The glucose, fructose, and insulin response curves were all flat following scFOS consumption, indicating that scFOS is not digested or absorbed within the small intestine (Hidaka et al., 1991a).
- **Humans:** Digestibility of scFOS was tested by comparing changes in breath hydrogen following ingestion of 10 g scFOS relative to 10 g lactulose, a nondigestible carbohydrate. In the test, 6-hour breath hydrogen area under the curve measurements were similar for scFOS and lactulose, indicating that scFOS is not digested. Peak response occurred between 3 and 5 hours after ingestion of scFOS (Stone-Dorshow and Levitt, 1987).

Although scFOS is not digested, it is fermented in the large intestine, so it contributes some energy to the body via short-chain fatty acids (SCFAs). Acetate is metabolized in muscle, kidney, heart, and brain; propionate is cleared by the liver, and is reported to be a glucogenic precursor and suppressor of cholesterol synthesis; and butyrate is metabolized by the colonic epithelium where it regulates cell growth and differentiation (Tuohy et al., 2006). Hosoya et al. (1988) measured the caloric value of scFOS by combining data from two radiochemical balance studies. In the first study, they adapted subjects to 6.1 g/day of [^{14}C] labeled scFOS for 7 days, then collected breath, flatus, urine, and fecal samples for 48 hours to determine partitioning of the ^{14}C. In the study, 58 and 67 percent of the ^{14}C was recovered within 24 and 48 hours, respectively. Most of this was recovered in respiratory gas, with 40 percent recovered within the first 12 hours. Over the 48-hour period, 10 percent was recovered in feces, 2 percent in urine, and less than 0.05 percent in flatus. The second study used in the caloric value calculation was an *in vitro* human fecal incubation study, which measured bacterial SCFA production. Following the 8-hour incubation, 89 percent of the ^{14}C was recovered; 10 percent was found in $^{14}CO_2$, mostly produced within the first 4 hours, and 58 percent of the ^{14}C was converted to SCFA. The primary SCFAs were acetate, propionate, and butyrate, with ^{14}C present in the ratios 42:35:20. Combining these two studies, Hosoya et al. (1988) calculated the caloric value of scFOS to be 1.5 kcal/g, less than half that of sucrose. The presence of labeled SCFAs and CO_2 indicates that scFOS is utilized by the intestinal bacteria to generate SCFAs, and that these SCFAs are further metabolized.

2.2.2 Bacterial Utilization of scFOS

As described above, there is direct evidence that bacteria utilize scFOS, demonstrated by the production of labeled SCFAs from labeled scFOS (Hosoya et al., 1988). However, SCFAs are not accepted as validated biomarkers of prebiotic activity, that is, selected bacterial growth or activity; hence, well-designed clinical studies with bacterial enumeration are preferred (Roberfroid, 2005d). Selective utilization of scFOS by intestinal bacteria has been demonstrated *in vitro* using pure cultures of selected bacterial species or using mixed fecal flora inoculations, and also in animal and human studies by measuring the bacterial composition of the feces. This section describes *in vitro* prebiotic studies and the next section describes clinical prebiotic evidence.

scFOS is one of only three recognized prebiotics—inulin-type fructans, *trans*-galacto-oligosaccharides, and lactulose (Gibson et al., 2004). It has been accepted as a prebiotic because it meets the following three criteria:

1. It resists gastric acidity, hydrolysis by mammalian enzymes, and intestinal absorption.
2. It is fermented by the intestinal microflora.
3. It selectively stimulates the growth of large intestinal bacteria associated with health and well-being.

In vitro culture studies have been used to demonstrate that scFOS is selectively utilized by bacteria, particularly by bifidobacteria and lactobacilli (Table 2.2, Table 2.3, and Table 2.4). McKellar et al. (1993) tested the growth of 43 species/strains of bifidobacteria at 37°C for 48 hours and reported that all grew on scFOS, as measured by optical density (Table 2.4). Separately, Kaplan and Hutkins (2000) tested the ability of 28 species/strains of lactic acid bacteria to ferment the isolated pure scFOS, with fermentation measured as a colored zone around the colonies growing on the agar

Table 2.2 Bacterial Utilization of scFOS

Species	No. of Strains	Growth[a]	Species	No. of Strains	Growth[a]
Bifidobacterium adolescentis	4	++	*Bacteroides melaninogenicus*	1	++
Bifidobacterium longum	3	++	*Fusobacterium varium*	2	–
Bifidobacterium breve	3	+	*Megamonas hypermegas*	2	++
Bifidobacterium infantis	2	++	*Mitsuokella multiacidus*	2	Variable
Bifidobacterium bifidum	2	–	*Escherichia coli*	2	–
Lactobacillus acidophilus	3	–	*Klebsiella pneumoniae*	1	++
Lactobacillus fermentum	4	–	*Enterococcus faecalis*	1	+
Lactobacillus salivarius	2	+	*Enterococcus faecium*	1	+
Lactobacillus casei	1	–	*Streptococcus intermedius*	2	++
Lactobacillus plantarum	1	+	*Peptostreptococcus prevotii*	1	–
Eubacterium aerofaciens	1	+	*Peptostreptococcus parvulus*	1	++
Eubacterium limosum	1	–	*Clostridium perfringens*	4	–
Eubacterium lentum	1	–	*Clostridium difficile*	2	–
Propionibacterium acnes	1	–	*Clostridium paraputrificum*	2	–
Bacteroides fragilis	4	++	*Clostridium clostridiforme*	2	+
Bacteroides thetaiotaomicron	3	++	*Clostridium ramosum*	2	+
Bacteroides vulgatus	2	++	*Clostridium butyricum*	1	++
Bacteroides distasonis	1	++	*Veillonella dispar*	2	–
Bacteroides ovatus	1	++	*Megasphaera elsdenii*	1	–

[a] Bacterial growth after 48-hour incubation; growth score judged by measurement of optical density and pH. ++, same level of growth compared to glucose; +, weaker growth compared to glucose; –, no growth.
Source: Adapted from Hidaka et al., 1986.

Table 2.3 Lactic Acid Bacteria Utilization of scFOS

Species and Strain	Growth on Agar Containing scFOS[a]
Lactobacillus bulgaricus B734	–
Lactobacillus bulgaricus CR5	–
Lactobacillus bulgaricus CR14	+
Lactobacillus acidophilus 33200	+
Lactobacillus acidophilus 837	+
Lactobacillus acidophilus DDS-1	+
Lactobacillus acidophilus NCFM	+
Lactobacillus plantarum 4008	+
Lactobacillus plantarum 1195	+
Lactobacillus plantarum 12006	+
Lactobacillus plantarum MR240	+
Lactobacillus lactis 448	–
Lactobacillus casei 685	+
Lactobacillus casei MR191	+
Lactobacillus strain GG	–
Streptococcus thermophilus 19987	–
Streptococcus thermophilus 14485	–
Streptococcus thermophilus 19258	–
Streptococcus thermophilus MTC321	–
Bifidobacterium adolescentis 15705	+
Bifidobacterium adolescentis 15706	+
Bifidobacterium breve 15698	+
Bifidobacterium breve 15700	+
Bifidobacterium bifidum 15696	–
Bifidobacterium infantis 17930	+
Bifidobacterium infantis 25962	+
Bifidobacterium longum 15708	+

[a] + indicated when colonies were surrounded by a yellow zone; – indicated when no zone was apparent.
Source: Adapted from Kaplan and Hutkins, 2000.

after 24-hour incubation. Of the species/strains tested, 19 could ferment the scFOS (Table 2.3), indicating interspecies and interstrain differences. This highlights the need to identify and test specific species/strains, particularly when pairing probiotics with prebiotics in symbiotic combinations.

Recent studies have explored the mechanism by which lactic acid bacteria utilize scFOS, to provide greater understanding of the selectivity shown by specific lactic acid bacteria. Both *Lactobacillus plantarum* 1995 and *Lactobacillus* strain GG were able to utilize GF_2 and GF_3 but not GF_4 as measured by optical density

Table 2.4 Growth of Bifidobacteria on scFOS and Inulin

Species	Strain (*n*)	scFOS Growth (A600)	Inulin Growth (A600)	Difference
B. boum	1	0.256	0.024	0.232
B. pseudolongum	1	0.542	–0.051	0.593
B. globosum	1	0.659	–0.092	0.751
B. pullorum	1	0.774	–0.068	0.842
B. ruminantium	1	0.889	–0.106	1.049
B. choerinum	1	0.900	–0.214	1.114
B. animalis	2	0.957	–0.016	0.973
B. gallinarum	1	1.09	0.071	1.019
B. bifidum	8	1.10	–0.032	1.132
B. breve	2	1.13	–0.070	1.200
B. longum	6	1.18	0.002	1.178
B. species	4	1.21	0.045	1.165
B. suis	1	1.22	–0.051	1.271
B. breve/longum	1	1.28	0.029	1.251
B. merycicum	1	1.43	0.042	1.388
B. magnum	1	1.47	–0.051	1.521
B. adolescentis	2	1.54	0.041	1.499
B. infantis	2	1.57	0.300	1.270
B. minimum	1	1.85	0.670	1.180
B. cuniculi	1	2.05	0.578	1.472
B. thermophilum	4	2.13	0.390	1.740
	43	Average growth	Average growth	
		1.258[a]	0.0937	1.164

[a] Significantly different, $p \leq 0.05$.
Source: Adapted from McKellar et al., 1993.

(Kaplan and Hutkins 2000). Similarly McKellar and Modler (1989) explored the relationship between chain length and β-fructosidase activity in various bifidobacteria species (*Bifidobacterium adolescentis* ATCC 15703, *B. longum* ATCC 15070, *B. thermophilum* ATCC 25525) and observed maximum cell-associated enzyme activity for scFOS versus inulin. This suggests that at least some bifidobacteria and lactobacilli selectively use different fructans according to chain length, and that scFOS, particularly the smaller scFOS chains, are selectively utilized by certain bacteria. FOS transporters have been identified on *L. paracasei* 1995 (Kaplan and Hutkins, 2003) and *L. acidophilus* (Barrangou et al., 2003). Transporter assays suggest that FOS transport is selective for chain length, as studies with *L. paracasei* 1195 revealed that the uptake of GF_2 and GF_3 was rapid, whereas little GF_4 uptake occurred (Kaplan and Hutkins, 2003). Selective transport could explain selective utilization of the shorter chains, specifically GF_2 and GF_3.

Not only is scFOS selectively used by health-promoting bacteria, such as bifidobacteria and lactobacilli, but it is also important to note that it is not utilized by selected harmful bacteria, thereby providing a second mechanism by which scFOS can contribute to a healthy colonic microbial balance. For example, in Table 2.2, scFOS was not utilized by *Escherichia coli* or *Clostridium difficile* (Hidaka et al., 1986). Rousseau et al. (2005) demonstrated in a 48-hour *in vitro* incubation study that *Candida albicans* did not utilize scFOS. Using *in vitro* incubation techniques with mixed fecal flora, scFOS was shown to produce less total gas than other fructans (Probert and Gibson, 2002). *In vitro* incubation studies with isolated bacteria show that most bacteria tested did not produce gas from scFOS compared with glucose, particularly 10 species/strains of bifidobacteria and 8 species/strains of lactobacilli (Kawaguchi et al., 1993). As bifidobacteria and lactobacilli are primary users of scFOS, this could explain the observations from Probert and Gibson (2002) when mixed fecal flora were used. Further, in a human study, rectal gas samples were collected and measured after scFOS consumption. N_2 was the primary gas produced, followed by CO_2 and H_2. H_2S, which is the major sulfur-containing compound in feces and is correlated with odor, was reduced following scFOS consumption compared with lactulose or no added fiber (Kawaguchi et al., 1993). This could explain why no difference in stool odor was observed when human subjects were fed up to 5 g/day scFOS (Tokunaga et al., 1993).

scFOS also acts to inhibit the growth of harmful bacteria and the production of potentially harmful metabolites. Mechanistic understanding of how scFOS inhibits pathogenic growth and activity is evolving; however, it is known that scFOS fermentation generates SCFAs that lower pH, and thereby inhibit the growth of selected pathogenic bacteria. Further, by providing a source of carbohydrate energy to intestinal bacteria, scFOS shifts the intestinal metabolic balance toward carbohydrate versus protein fermentation, reducing the production of potentially harmful by-products like phenols. Studies demonstrating protective effects of scFOS against the growth and activity of pathogens, such as *E. coli*, *Salmonella typhimurium,* and *C. difficile* (a causative agent of pseudomembranous colitis), are listed below. In general, animals fed scFOS while exposed to antibiotics and pathogens have reduced pathogenic effects including disease symptoms, toxin levels, and pathogen levels.

2.2.2.1 Escherichia coli

- **Pigs:** Piglets were given a milk replacer with or without scFOS for 6 days, after which they received an *E. coli* challenge. Of 8 piglets not fed scFOS, 6 developed diarrhea within 36 hours of the *E. coli* challenge, but only 1 of 8 piglets fed scFOS developed diarrhea. Survival rates were 62.5 percent without scFOS and 100 percent with scFOS. Bifidobacteria counts were nonsignificantly higher and *E. coli* counts were nonsignificantly lower in piglets fed scFOS (Bunce et al., 1995).

2.2.2.2 *Salmonella typhimurium*

- **Pigs:** Piglets were given formula with or without scFOS for 14 days during which they received an *S. typhimurium* challenge. scFOS reduced severity of the infection-associated symptoms, shown by greater activity, $p < 0.05$ (Correa-Matos et al., 2003).

2.2.2.3 *Clostridium difficile*

- **Hamsters:** Antibiotic-compromised hamsters were given a *C. difficile* challenge while fed diets with and without scFOS. Hamsters consuming the scFOS diet had increased survival time, at 15 days versus 13.5 days for the control group, $p < 0.001$ (Wolf et al., 1997).
- **Mice:** Antibiotic-compromised mice were given diets with and without scFOS over 10 days during which they received a *C. difficile* challenge. In the scFOS-fed group, toxin A titers were lower, $p < 0.05$; animals had more culturable bacteria, $p < 0.05$; and experienced less incidence of detectable toxin A and diarrhea (Gaskins et al., 1996).
- **Pigs and *in vitro*:** In an *in vitro* fermentation study using pig fecal inoculum, growth of acidogenic bacteria increased when scFOS was present, yielding SCFAs, particularly acetate, and decreasing pH. *C. difficile* growth and activity is pH sensitive, hence, no culturable counts of *C. difficile* were obtained, nor was toxin A detected (May et al., 1994).

2.2.2.4 Other

- **Human:** Elderly subjects were given 8 g/day of scFOS for a 2-week period. Bifidobacteria counts increased, $p < 0.05$, with increases first noted after 4 days. There was a significant negative correlation between the average count of bifidobacteria and the occurrence of *C. perfringens* ($r = -0.837$, $p < 0.05$), indicating that bifidobacteria may suppress the growth of this organism in the human large intestine (Hidaka et al., 1986).
- **Rats:** In a rat study where diets contained high levels of tyrosine and tryptophan, production of phenols was reduced when scFOS was fed, indicating a shift in metabolic balance with reduced protein fermentation (Hidaka et al., 1986).

2.2.3 Clinical Prebiotic Evidence for scFOS

As mentioned previously, fructan fibers are one of only three recognized prebiotic fibers (Gibson et al., 2004). This recognition for scFOS primarily comes from a number of clinical observations, as clinically observed changes in microflora are the best-accepted biological marker for prebiotics. At least 13 published references are available in the public domain. Some reported on multiple studies (total of 16 studies) and multiple doses. Therefore, collectively from the 10 references and 16 studies, there are 32 observations on possible prebiotic effects of scFOS (Table 2.5 and Table 2.6). These studies were conducted in various groups that included healthy adults, elderly individuals, and people with metabolic syndrome and renal failure.

Table 2.5 Prebiotic Effect of scFOS

References	Treatment Duration	scFOS Dose, g/d	Bifidobacteria Effect	Lactobacilli Effect
Bouhnik et al., 1999	7 days	2.5	No	
		5.0	Yes	
		10	Yes	
		20	Yes	
Bouhnik et al., 2004	7 days	10	Yes ($p = 0.056$)	
	7 days	2.5	No	
		5.0	No	
		7.5	No	
		10	No	
Bouhnik et al., 2006	7 days	2.5	Yes	No
		5.0	Yes	No
		7.5	Yes	No
		10.0	Yes	No
Bouhnik et al., 1996	4, 8, 12 days	12.5	Yes	
Bouhnik et al., 2007	4 weeks	8	Yes	
Buddington et al., 1996	25 days	4	Yes	
Garleb et al., 1996	14 days	15	Yes	
		31	Yes	
Guigoz et al., 2002	3 weeks	8	Yes	No
Hidaka et al., 1986	4, 8, 11, 14 days	8	Yes	Yes
	Not defined	1	Yes, no stats	
		2	Yes, no stats	
		4	Yes, no stats	
Mitsuoka et al., 1986	8 weeks	1	Yes	
		2	No	
		4	Yes	
	6–12 months	6.1	Yes, no stats	
Mitsuoka et al., 1987	4, 14 days	8	Yes	No
Tokunaga et al., 1993	2 weeks	1	Yes	
		3	Yes	
		5	Yes	
Williams et al., 1994	14 days	4	Yes	No

Table 2.6 Summary Table of Prebiotic Observations (References in Table 2.5)

scFOS Dose, g/d	No. of Positive Observations	Effective Duration	Ineffective Duration
1 g	3 of 3	14–56 days	
2 g	1 of 2		56 days
2.5 g	1 of 3	7 days	7 days
3 g	1 of 1	14 days	
4 g	4 of 4	14–56 days	
5 g	3 of 4	7–14 days	7 days
6.1 g	1 of 1	365 days	
7.5 g	1 of 2	7 days	7 days
8 g	4 of 4	4–28 days	
10 g	3 of 4	7 days	7 days
12.5 g	1 of 1	4–12 days	
15 g	1 of 1	14 days	
20g	1 of 1	7 days	
31 g	1 of 1	14 days	

Table 2.5 and Table 2.6 show that:

- 26 of 32 (or 81 percent) of observations were positive for an effect of scFOS on bifidobacteria.
- scFOS was an effective prebiotic at doses ranging from 1 to 31 g/day.
- scFOS was a stronger substrate for bifidobacteria than lactobacilli according to fecal bacteria measurements. Only 1 of 8 observations was positive for lactobacilli.
- scFOS was effective as a prebiotic for bifidobacteria at 14 days with 1 g/day, at 7 days with 2.5 g/day, and at 4 days with 8 g/day, indicating a dose effect.
- All 9 of 9 (100 percent) observations were positive following 12 to 14 days of scFOS consumption.

As a prebiotic, scFOS selectively feeds the bifidobacteria. Bouhnik et al. (1999) demonstrated that not only did counts of bifidobacteria increase with 10 g/day scFOS, but also the percent bifidobacteria among total anaerobes. In a second study, Bouhnik et al. (1996) found an increase in bifidobacteria counts with no effect on total fecal anaerobes.

The prebiotic effect of scFOS appears to be dependent on dose and treatment duration. A positive correlation between scFOS dose and fecal bifidobacteria counts was noted in three studies (Bouhnik et al., 1999; 2004; 2006) and Bouhnik et al. (1999) found that 5 g/day scFOS increased bifidobacteria counts in 75 percent of subjects, but with 10 g/day, scFOS bifidobacteria counts increased in 100 percent of subjects. While some studies did not observe a prebiotic effect at 7 days (refer to Table 2.5), there were three observations of a prebiotic effect after only 4 days (Bouhnik et al., 1996; Hidaka et al., 1986; Mitsuoka et al., 1987). The rapid response

could reflect the higher dose used in these studies: 12.5, 8, and 8 g/day, respectively. scFOS is a more effective prebiotic in people with lower starting bifidobacteria counts (Guigoz et al., 2002; Hidaka et al., 1986, Tokunaga et al., 1993). This could explain why all 5 of 5 (100 percent) observations in elderly subjects were positive for a bifidogenic effect of scFOS (Bouhnik et al., 2007; Guigoz et al., 2002; Hidaka et al., 1986; Mitsuoka et al., 1987).

Bifidobacteria do not seem to adapt to the presence of scFOS over time, such that the bifidogenic effect does not diminish with continued scFOS consumption. In the study by Mitsuoka et al. (1986), bifidobacteria counts and percent bifidobacteria increased after 1 month, and continued over 12 months throughout the study. However, when scFOS consumption ceased, bifidobacteria counts returned to baseline levels, indicating the need for continued prebiotic intake (Bouhnik et al., 2007; Buddington et al., 1996).

2.2.4 Consequences for Health

The benefit of scFOS for digestive health extends beyond balancing the microflora composition, to having a positive impact on various aspects of digestive health that span from inflammation and immune response to diarrhea. Health effects are likely due to the promotion of selected bacteria that are known to be immunostimulatory (e.g., bifidobacteria and lactobacilli) and/or increased concentrations of selected scFOS metabolites, such as SCFAs (e.g., butyrate), which are known to promote healthy colonic tissue and function. More mechanistic studies are required to understand the role of scFOS in digestive health, but in the meantime there is a consistent relationship between dietary scFOS and improved immune and inflammatory function. Table 2.7 summarizes the effects of scFOS for compromised groups, and details are provided below.

2.2.4.1 Diarrhea

- **Children:** In Indonesia, children 1 to 14 years of age with diarrhea from various causes were given a control formula or one with 2.5 to 5 g scFOS depending on age. The children who consumed scFOS had a shorter duration of diarrhea, reduced from 4.2 days to 2.7 days, $p = 0.001$ (Juffrie, 2002).
- **Pigs:** Pigs with acute diarrhea induced by cholera enterotoxin were given an oral electrolyte solution (OES) with and without scFOS. Standard OES is formulated to replenish lost water and electrolytes, but does not reduce stool volume or the duration of diarrhea. scFOS did not reduce the duration of diarrhea and associated loss of water (possibly because the toxin rather than the live pathogen was used), but scFOS promoted intestinal bacterial recovery (lactobacilli) within 24 hours, $p = 0.0001$ (Oli et al., 1998).
- **Pigs:** As described earlier, scFOS reduced incidence of diarrhea and increased survival in piglets exposed to *E. coli* relative to piglets given diets without scFOS (Bunce et al., 1995).

Table 2.7 Benefits of scFOS for Compromised Groups

Group	Test supplement (scFOS or Multiingredient Formula Containing scFOS)	Health Benefit	Ref.
Infants/children with diarrhea	scFOS	Reduced diarrhea duration	Juffrie, 2002
Seniors	Multiingredient formula	Heightened immune response	Langkamp-Henken et al., 2004
Seniors	Multiingredient formula	Heightened immune response	Langkamp-Henken et al., 2006
Seniors	scFOS	Modified immune markers; increased bifidobacteria	Guigoz et al., 2002
Seniors	scFOS	Increased bifidobacteria	Bouhnik et al., 2007
Seniors	scFOS	Increased bifidobacteria	Hidaka et al., 1986
Seniors	scFOS	Increased bifidobacteria	Mitsuoka et al., 1987
Ulcerative colitis	Multiingredient formula	Reduced use of inflammatory medication	Seidner et al., 2005
Pancreatitis	Multiingredient formula	Suppressed acute inflammatory response	Karakan et al., 2007
Minor functional bowel disorder	scFOS	Reduced intensity and frequency (trend) of symptoms; improved quality of life	Paineau et al., 2008
Constipation	scFOS	Increased fecal frequency (no stats)	Hidaka et al., 1991b
Renal failure	Multiingredient formula	Reduced constipation	Cockram et al., 1998
Renal failure	scFOS	Increased bifidobacteria	Mitsuoka et al., 1986
Hyperlipidemia, diabetes, high blood pressure, peripheral arterial occlusion	scFOS	Increased bifidobacteria	Mitsuoka et al., 1986

2.2.4.2 Constipation

- **Humans:** Patients with end-stage renal disease were given a renal formula with or without 16 to 19 g/day scFOS for 2 weeks. Patients receiving the formula with scFOS had less constipation (Cockram et al., 1998).

2.2.4.3 Inflammation

- **Humans—Pancreatitis:** Hospitalized patients with severe pancreatits were given a control enteral nutrition formula or a multifiber-enriched formula containing scFOS for 48 hours. Acute phase inflammatory response was suppressed with the test formula, as shown by a reduced time to normalize C-reactive protein levels (from 10 to 7 days, $p < 0.05$), and two scores of inflammation: APACHE II score (from 6.5 to 4 days, $p < 0.05$) and contrast abdominal computed tomography (CT) score (16 to 12 days, $p < 0.05$). Overall complications, which included multiorgan failure, cholangitis, sepsis, pseudocyst, and death, were reduced with the test formula, $p < 0.05$, and hospital stay was also shortened from 15 to 10 days, $p < 0.05$ (Karakan et al., 2007).
- **Humans—Minor Functional Bowel Disorder:** Hospitalized patients with minor functional bowel disorder assessed by questionnaire (abdominal discomfort, constipation) were given a control or test formula with 5 g scFOS over a 6-week period. Patients consuming scFOS had reduced intensity of digestive disorders (by 44 percent, $p = 0.026$), a trend for reduced frequency of symptoms (by 75 percent of subjects, $p = 0.064$), and improved quality of life as shown by activity scores, $p = 0.011$ (Paineau et al., 2008).
- **Humans—Ulcerative Colitis:** Subjects with ulcerative colitis were provided a control formula or a test formula with 6.7 g scFOS in combination with other nutrients. Subjects consuming the test formula had no worsening of their disease activity index or histology index, yet were able to reduce their use of antiinflammatory medication, $p < 0.001$ (Seidner et al., 2005).
- **Rats—Colitis:** Rats with trinitrobenzene sulfonic acid (TNBS)-induced colitis were given diets with or without scFOS for 7 to 14 days. scFOS inhibited weight loss; reduced mucosal damage and promoted healing, shown by reduced macroscopic damage at 14 days, $p < 0.05$; and reduced colonic mucosal myeloperoxidase (MPO) activity, $p < 0.05$, an enzyme marker of polymorphonuclear neutrophil primary granules. scFOS increased cecal butyrate and reduced pH, $p < 0.05$, which could contribute to the effects because intracolonic butyrate infusion decreased inflammation and MPO activity (Cherbut et al., 2003).
- **Rats—Colitis:** Rats with colitis induced by a peptidoglycan-polysaccharide derived from streptococci were given diets with or without scFOS for 1 week prior to and 3 weeks after induction. scFOS exhibited antiinflammatory action similar in efficacy to sulfasalazine, shown by reduced liver weight, $p < 0.05$, and less inflamed liver, spleen, and colonic mucosal tissue (Grisham et al., 1996).
- **Pigs:** Pigs were given a control diet or a multinutrient ulcerative colitis formula (UCNF) containing scFOS for 21 days. The UCNF diet suppressed synthesis of proinflammatory prostaglandins, prostaglandin E ($p < 0.0001$), 6-keto-prostaglandin $F_{1\alpha}$ ($p < 0.05$), and thromboxane B_2 ($p < 0.0001$) (Campbell et al., 1997).

2.2.4.4 Immune Response

- **Humans—Vaccine Response:** Two studies reported improved vaccine and immune response when seniors were given a test formula with 4.4 g/day scFOS in combination with other nutrients versus a control formula. In the first study, the test formula was consumed for 183 days during which time the influenza vaccine was given. Subjects consuming the test formula had fewer days of upper respiratory tract infection (median 3 days, range 0 to 69 days per completed subject versus median 0 days, range 0 to 49 days per completed subject, $p = 0.049$), had greater lymphocyte proliferation to the influenza vaccine ($p = 0.013$), and had greater increase in serum antibody titer ($p = 0.012$) (Langkamp-Henken et al., 2004). In the second study by the same group, the seniors consumed the test formula for 4 weeks before and 6 weeks after the influenza vaccination. Subjects consuming the test formula had greater lymphocyte and antibody response to the vaccine ($p = 0.008$, $p = 0.047$), reduced cytokine production (interleukin-6, $p = 0.045$), and fewer subjects were treated for fever ($p = 0.02$) (Langkamp-Henken et al., 2006).
- **Humans—Immune Response:** In a pretest/posttest study, seniors were given 8 g scFOS for 3 weeks. Changes in nonspecific immunity were observed that included decreased phagocytic activity of granulocytes and monocytes ($p < 0.001$) and decreased expression of interleukin-6 mRNA in blood monocytes ($p = 0.018$) (Guigoz et al., 2002).
- **Dogs:** Pregnant dogs were given diets with and without scFOS from the 35th day of gestation until weaning. Those given scFOS exhibited higher colostrum and milk immunoglobulin M (IgM), $p < 0.01$, without concomitant effect on IgG_1, IgG_2, and IgA. Puppies of these dogs tended to have higher *Bordetella bronchiseptica*-specific IgM immune response, $p = 0.018$ (Adogony et al., 2007).
- **Mice:** Antibiotic-compromised mice were given diets with and without scFOS for 10 days during which they received a *Clostridium difficile* challenge. The cecal macrophage number was higher in the scFOS-fed group, $p < 0.01$, with no change in dendritic cells (Gaskins et al., 1996).
- **Mice:** Mice were fed diets with and without scFOS for 4 to 6 weeks. scFOS increased intestinal IgA secretion, $p < 0.001$, and there was a dose-dependent increase in IgA secretion from Peyer's patches, $p < 0.05$, and interferon-γ and interleukin 10 from Peyer's patches $CD4^+$ helper/inducer T cells, $p < 0.05$ (Hosono et al., 2003).
- **Mice:** Newborn mice and their dams were fed diets with and without scFOS preweaning, then for up to 23 days postweaning. Mice fed scFOS had increased intestinal IgA, $p < 0.05$, increased percentage of $B220^+IgA^+$ cells in Peyer's patches, $p < 0.05$, and increased pIgR expression, $p < 0.05$, which is important for transepithelial transport of intestinal IgA onto the mucosal surface (Nakamura et al., 2004).

2.2.4.5 Mineral Absorption

- **Humans—Calcium Absorption:** scFOS fermentation is known to increase large intestinal SCFA production, resulting in a lowering of intestinal pH. Lower pH increases mineral solubility rendering the minerals more absorbable, which has been proposed as a mechanism whereby fermentable fibers increase large intestinal mineral absorption. Increased calcium absorption was observed in three acute studies when 3 g scFOS was given in a breakfast meal to men and young women,

$p < 0.05$ (Fukushima et al., 2002; Ohta et al., 1999; Uenishi et al., 2002). Increased absorption was first measured at 4 hours and extended out to 8 and 12 hours. The rapid fermentation of scFOS could influence this short-term effect. In these three studies, increased calcium absorption was measured in urine calcium, which is positively correlated with calcium absorption from the digestive tract (Ohta et al, 1999). Although not yet repeated in humans, increased calcium absorption promoted by scFOS could enhance bone mineral content, as rat studies have demonstrated increased bone calcium stores when fed scFOS (summarized in Ohta et al., 1998b).

- **Rats:** A second mechanism has been proposed whereby scFOS could enhance mineral absorption. Active calcium absorption requires the participation of a calcium transporter protein called calbindin-D9k, and there is a high correlation between this protein and calcium absorption (Ohta et al., 1998a). Intact and gastrectomized rats fed diets containing scFOS exhibited increased levels of calbindin-D9k in the large intestine (Ohta et al., 1998a, 1998b). Studies on this biomarker have not yet been repeated in humans.

2.3 COMMERCIAL FOOD APPLICATION OF scFOS

scFOS ingredients have a long history of global food use. scFOS was first made available as a commercial ingredient by Meiji Seika Kaisha Ltd. in Japan (Beghin-Meiji, 2008). It was initially launched with a lower fiber content, but now has at least 95 percent fiber on a dry weight basis, with the residual ≤5 percent (dry weight) consisting of the sugars sucrose, glucose, and fructose. scFOS is currently marketed under three different trade names globally: NutraFlora® in North America, South America, Australia; Actilight in Europe; and Meioligo (formerly Neosugar) in Asia.

scFOS has been an approved food ingredient in Japan since 1980, and has approved FOSHU (Foods for Specified Health Uses) status. In the European Union (EU), scFOS has been recognized as a food ingredient since 1991, and has been approved as a bifidogenic ingredient since 1997. scFOS was first made commercially available in the United States in 1988, with the first food product containing scFOS launched in 1994. In the United States, scFOS is considered GRAS approved and natural, and is on the National Organic Standards Board list of approved substances. Currently, scFOS can be found in more than 500 food products worldwide (Macfarlane et al., 2008; Spiegel et al., 1994).

scFOS is a useful food ingredient for three distinct reasons:

1. **Nutritional enrichment.** With a high fiber content of ≥95 percent (dry basis), it is an efficient and economical way to enrich with fiber. The fiber in scFOS is 100 percent soluble, so scFOS is an ideal "invisible fiber." For example, 10 g of scFOS can easily be mixed into an 8-ounce glass of water. Also, because scFOS is approximately 30 percent as sweet as sucrose, yet contains only 1.5 kcal/g, it is an effective ingredient for calorie reduction, particularly sugar reduction.
2. **Structure–function claims.** Due to the extensive body of scientific evidence underpinning scFOS, it has become a popular ingredient for structure–function claims relating to digestive health and bone health. Example claims for digestive health

include prebiotic, promotes digestive function, increases levels of good bacteria, and reinforces immune system function. Example claims for bone health include enhances calcium absorption; and supports bone health.

3. **Application benefits.** scFOS is a unique ingredient because, although it is non-digested, it has properties and functional benefits similar to sucrose and glucose syrup (summarized in Table 2.8). The combination of nutritional and technical benefits of scFOS make it an ideal ingredient for inclusion in most food systems and food processes. However, two limitations exist: (1) yeast-leavened bakery products (as the yeast ferments the scFOS) and (2) low-pH (below 4), shelf-stable beverages, where scFOS can be hydrolyzed. Loss of scFOS can be prevented with refrigeration or freezing.

Table 2.8 Properties of scFOS

Nutritional properties	≥95% fiber (dry basis), ≤5% moisture
	1.5 kcal/g
Physical properties	White powder; odorless
	Has a small particle size (100% passes through U.S. 40 mesh)
	Is completely soluble
	Has similar density and refractive index to sucrose
	Heat stable
	30% as sweet as sucrose; has a clean taste without lingering effects
Functional benefits	Does not contribute to viscosity
	Does not contribute to Maillard browning
	Rounds the sweetness profile and enhances the potency of high-intensity sweeteners
	Masks off notes
	Enhances flavors, e.g., fruit flavors
	Balances the cooling effect of sugar alcohols
	Improves texture, e.g., crispiness of extruded cereals
	Enhances the mouthfeel and creaminess of low-fat and fat-free dairy products
	Contributes to product shine, e.g., breakfast cereals
	Has humectant properties, e.g., maintains bar softness thereby extending shelf life
	Affects freezing point, e.g., creates a creamy frozen dessert
	Does not require process modification, e.g., when extruding breakfast cereals
	Aids in the dispersion of gums, proteins, and other hard-to-disperse systems/products
	Reduces water activity

scFOS has been included in a wide variety of foods and supplements globally that have been marketed for children, adults, and hospital/institutional use. Food products available on the market include the following:

- Beverages: Soy milk, smoothies, juice
- Dairy products: Yogurt, ice cream, frozen yogurt
- Desserts: Pudding, jelly/jello
- Fruit products: Fruit preparations
- Bakery products: Snack bars, biscuits/cookies, waffles, pancakes
- Breakfast cereal: Extruded cereals, instant oatmeal
- Confectionery: Chocolate, gummy candy
- Infant and toddler foods
- Specialty nutrition products: Liquid supplements

2.4 COMPARATIVE EFFECTS OF scFOS AND OTHER FRUCTANS

2.4.1 Biological Outcomes

Various studies have directly compared scFOS with other fructans and found differences in biological outcomes that include:

- Selective bacterial utilization
- Mechanism for bacterial utilization
- Rate of fermentation
- Gas production
- Tolerance

McKellar et al. (1993) conducted *in vitro* incubation studies across a broad selection of bifidobacteria species and strains by comparing growth on various carbohydrate sources at 37°C for 48 hours, as measured by absorbance at 600 nm. Averaged across all 43 species/strains tested, glucose and sucrose were the best growth factors: average growth on each of the mono- and disaccharides was sucrose (1.767 ± 0.0643 SE) > glucose (1.704 ± 0.0489) > fructose (1.208 ± 0.0767). By comparing the fructan substrates tested, scFOS (1.258 ± 0.0324) was a better growth factor than inulin (0.0937 ± 0.232), $p \leq 0.05$ (see Table 2.4). Across all 19 species of bifidobacteria, scFOS was a better growth factor. In fact, every species utilized scFOS, but 10 species could not utilize inulin. Hence, scFOS is a more generic growth substrate for bifidobacteria than inulin.

Mechanistic studies suggest that scFOS may be a better substrate for intestinal bacteria than oligofructose or inulin due to its shorter and more specific DP.

- Two species of lactobacilli were better able to utilize GF_2 and GF_3 than GF_4 (Kaplan and Hutkin s, 2000).
- β-Fructosidase activity in various bifidobacteria species showed higher cell-associated enzyme activity for scFOS versus inulin (McKellar et al., 1989).
- scFOS uptake by a lactobacilli transporter is more rapid for GF_2 and GF_3 than GF_4 (Kaplan and Hutkins, 2003).

Inulin is reported to be more slowly fermented than oligofructose (Roberfroid, 2005b). It was recently confirmed that scFOS is also more rapidly fermented than inulin. Using *in vitro* batch fermentation and human fecal inoculum, Stewart et al. (2008) compared the fermentation profile of scFOS, two types of oligofructose and three types of inulin. After a 4-hour fermentation period, total SCFA concentration was higher for scFOS than for the three inulins tested, nonsignificant for two, significant for one. This trend continued across the first 12 hours of the incubation. Further, the rate of SCFA production was higher for scFOS than for inulin, particularly in the first 4-hour period, $p < 0.05$. Differences in concentration and rate were largely due to acetate production which is not surprising as scFOS is generally more bifidogenic than inulin and bifidobacteria produce acetate.

In addition to SCFA production, fructans also differ in gas production, with scFOS producing less gas than other fructans. Probert and Gibson (2002) used an *in vitro* fermentation system with human fecal flora inoculum to compare gas production by four fructans: scFOS, oligofructose, branched FOS, and levan. scFOS produced significantly less gas than oligofructose within the first 4 hours ($p = 0.01$), less gas than oligofructose and branched FOS within 8 hours ($p = 0.01$, $p = 0.05$, respectively), and by 24 hours scFOS produced less gas than all other fructans tested ($p = 0.01$). As gas/flatus is typically the highest reported symptom of gastrointestinal (GI) distress following fructan consumption (Bouhnik et al., 1999, 2004, 2006) and is typically experienced by more people than for other symptoms of GI distress (Bouhnik et al. 1999, 2006), differences in gas production between fructans should be considered when selecting ingredients to formulate consumer-accepted food products.

Several authors have tested GI tolerance to scFOS and other fructans. Where tolerance to different fructans was compared within the same study, differences between different types of fructans emerged. Bouhnik et al. (2004) tested the GI tolerance of 10 g of seven different nondigestible carbohydrates for 7 days, two of which included scFOS and inulin. When scFOS was consumed, changes in GI distress symptoms were similar to or lower than the control with the exception of bloating (Table 2.9). scFOS was better tolerated than inulin, with a 12-fold lower effect on flatus and 6-fold lower effect on bloating. On average, subjects reported no change in abdominal pain with scFOS, but this was increased with inulin. Therefore, 10 g/day of scFOS was well tolerated with little effect compared with the control, and less GI distress compared with inulin. Bouhnik et al. (1999, 2006) also showed that that scFOS is well tolerated up to 10 g/day in two dose-response studies. In the 1999 study, they assessed doses of 0, 2.5, 5, 10, 20 g/day scFOS over 7 days. With respect to flatus, the 2.5, 5.0, and 10 g/day scFOS doses were well tolerated, with no significant difference between the doses. However, the flatus observed with the 20 g/day dose was significantly higher, $p < 0.05$. No significant differences were reported amongst the 0 to 20 g/day doses for bloating, borborygmi, or abdominal pain. In the 2006 study, doses of 0, 2.5, 5, 7.5, and 10 g/day scFOS were consumed for 7 days. Flatus, borborygmi, and abdominal pain did not differ between the doses. However, some bloating was observed at the lower doses.

Table 2.9 Tolerance to Fructans

	Day 8[a]	Day 15	Change after 7 Days
Placebo			
Excess flatus	1.25	2.63	1.38
Bloating	0.75	0.25	–0.50
Borborygmi	0.25	2.25	2.00
Abdominal pain	0.50	1.25	0.75
scFOS			
Excess flatus	3.50	3.88	0.38
Bloating	2.00	2.38	0.38
Borborygmi	1.38	2.25	0.87
Abdominal pain	1.50	1.50	0.00
Inulin			
Excess flatus	0.63	5.25	4.62
Bloating	0.38	2.63	2.25
Borborygmi	0.25	1.13	0.88
Abdominal pain	0.00	1.25	1.25

[a] Days 1–7 were the baseline period, but subjects excluded fructans from their diet; and days 8–14 were the treatment period. Symptom intensity was graded as 0 = no symptoms, 1–7 = mild symptoms, 8–14 = moderate symptoms, 15–21 = severe symptoms.

Source: Adapted from Bouhnik et al., 2004.

2.4.2 Food Application Outcomes

In terms of food applications, scFOS is more similar to oligofructose than to inulin. Relative to inulin, scFOS has higher solubility and dispersibility and lower viscosity. As a result of their respective physicochemical properties, scFOS is better suited for beverage applications, whereas inulin is a good gel former, contributing creamy mouthfeel and functioning as a fat replacer.

scFOS differs from the broader oligofructose subclass of fructans because of its chemical structure:

- scFOS chains are specifically GF_2, GF_3, and GF_4, whereas oligofructose is DP < 10.
- scFOS chains are all terminated by glucose (i.e., GF_n), whereas oligofructose can be terminated by either glucose or fructose (i.e., GF_n or FF_n).

Glucose or fructose termination determines the extent to which the chains participate in nonenzymatic Maillard browning. This is a complex series of reactions that involves food proteins and reducing sugars during thermal processing. Tuohy et al.

(2006) reviewed the potential biological importance of Maillard reaction products (MRP) for health. They resist digestion in the small intestine so can alter bacterial growth and activity in the large intestine; they could contribute to increased levels of bacterially produced toxic metabolites from amino acids; a number of MRPs have been reported to possess mutagenic or carcinogenic properties; and upon absorption MRPs can induce inflammatory responses. Further, Maillard reaction results in losses of protein via cross-linking (which renders them indigestible), essential amino acids (e.g., lysine), certain vitamins (e.g., vitamin C and thiamin), and some metals via complexation (e.g., copper, zinc, and iron).

Unpublished research in our lab has shown that browning is more likely to occur with fructose termination, such that browning is more apparent in the order oligofructose > inulin > scFOS when fructans are heated in the presence of lysine, one of the amino acids known to participate in these reactions. Conditions used to obtain this effect were 1.25 percent lysine + 5 percent fructan in distilled water, heated with stirring for 90 minutes. Separately, Huebner et al. (2008) compared browning of different fructans (e.g., scFOS, oligofructose, and inulin) under different Maillard reaction conditions: 10 percent short-chain and 2 percent long-chain fructan in citrate-phosphate buffer pH7 with 1 percent glycine, heated at 85°C with shaking for 0 to 6 hours, and absorption measured at 420 nm compared with glucose. More browning was observed with oligofructose than scFOS at 1, 2, and 3 hours, with browning fourfold greater at each time point (Huebner et al., 2008).

2.5 ADDITIONAL SOURCES OF INFORMATION

Additional information about scFOS can be obtained from the following references and Web sites:

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CHAPTER 3

Inulin and Oligosaccharides
A Special Focus on Human Studies

Damien Paineau, Frédérique Respondek, and Yoram Bouhnik

CONTENTS

3.1 INTRODUCTION

The intestinal habitat, mainly the large intestine of an individual, contains 300 to 500 different species of bacteria, and the number of microbial cells within the gut lumen is about 10 times larger than the number of eukaryotic cells in the human body (Salminen et al., 1998; Segain et al., 2000; Guarner and Malagelada, 2003). In this complex and dynamic microbial ecosystem, living bacteria achieve concentrations of up to 10^{11} to 10^{12} per gram of luminal content (Guarner and Malagelada, 2003).

This ecosystem interacts with the host health, in various domains including the protection against pathogens (barrier effect), inflammatory bowel diseases, colonic cancers, and others (Guarner and Malagelada, 2003). Some gut bacteria, including subspecies of *Clostridium perfringens,* sulfate-reducing and amino acid fermenting species are considered harmful. On the other hand, others are considered as beneficial. The main potentially health-enhancing bacteria are the bifidobacteria and lactobacilli, both of which belong to the lactic acid bacteria group (Salminen et al., 1998). These two genera do not include any significant pathogenic species and their potentially prophylactic and therapeutic beneficial effects are now well demonstrated in human and animal studies (Picard et al., 2005).

Modulation of the microflora composition by "functional foods" with the objective to improve the colonic environment is a challenge. A prebiotic is defined as a "nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon" (Gibson et al., 2004; Macfarlane et al., 2006). They have been widely tested, in animal and human studies, for their beneficial actions in the prevention or treatment of a broad spectrum of gastrointestinal disorders, from impairment of colonic transit to colonic carcinogenesis (Macfarlane et al., 2006). Probiotics are defined as "live microorganisms, which confer a health benefit on the host when administered in adequate amounts" (Guarner and Schaafsma, 1998). Synbiotics are products in which both a probiotic and a prebiotic are combined.

The aim of this chapter is to focus on the physiological effects of oligosaccharides and inulin (fructans) in the gastrointestinal tract, with a special focus on human studies.

3.2 CHARACTERISTICS AND PHYSIOLOGICAL EFFECTS OF FRUCTANS

The only known components for which convincing evidence in favor of a prebiotic effect has been reported are carbohydrates that resist digestion in the upper

Table 3.1 Fructans Used as Prebiotics

Name	Composition	Method of Manufacture	Degree of Polymerization
Short-chain fructo-oligosaccharides	β(2–1) linear fructans	Tranfructosylation from sucrose, or hydrolysis of chicory inulin	3–5
Fructo-oligosaccharides		Tranfructosylation from sucrose, or hydrolysis of chicory inulin	2–10
Inulin		Hydrolysis of chicory inulin	2–60
Long-chain inulin		Hydrolysis of chicory inulin	10–60

gastrointestinal tract (nondigestible carbohydrates or NDCHs), but that are hydrolyzed and fermented in the large bowel. Three types of carbohydrates, essentially nondigestible oligosaccharides, fulfill the criteria for prebiotic classification: fructans (inulin and fructo-oligosaccharides (FOS)), (*trans*-)galacto-oligosaccharides (TOS or GOS), and lactulose (Macfarlane et al., 2006). The aim of this chapter is to focus on fructans (Table 3.1); TOS, GOS, and lactulose are presented in other chapters.

3.2.1 Effects of Fructans on Intestinal Microflora Composition

3.2.1.1 Bifidogenic Effect

Over the past decade, it has emerged that some NDCHs have the potential to increase the concentration of bifidobacteria in the colon (Bornet and Brouns, 2002). The intensity of this bifidogenic effect depends on the chemical structure of the prebiotic, leading to differences in efficient doses. The results of the main human studies carried out to assess bifidogenic properties of fructans are summarized in Table 3.2.

In a recent randomized controlled study, Bouhnik et al. (2004) found that lactulose, long-chain inulin, and isomalto-oligosaccharides (IMO) were not bifidogenic at 10 g/day for 7 days on the contrary to short-chain fructo-oligosaccharides (sc-FOS), soybean oligosaccharides (SOS), and galacto-oligosaccharides (GOS). The three nonbifidogenic substrates were further studied in a dose–response relationship using higher doses (Bouhnik et al., 2004). In the study, 80 volunteers were randomized in three groups of 24 subjects who received one of the three nonbifidogenic NDCHs at a dose of 10, 15, and 20 g/day for 7 days (8 volunteers per dose) and a fourth group of 8 subjects who received the placebo. Bifidobacteria counts increased when using lactulose at 20 g/day ($P < 0.05$) and inulin at 15 g/day ($P < 0.01$) and 20 g/day ($P < 0.05$) (Table 3.2). A dose relationship was demonstrated for sc-FOS (Bouhnik et al., 1999, 2004, 2006), but not for other bifidogenic substrates.

When focused on the fructans, sc-FOS were found bifidogenic at doses ranging from 2.5 to 10 g/day (Bouhnik et al., 1999, 2004, 2006), and inulin at doses ranging from 5 to 15 g/day (Bouhnik et al., submitted (a); Gibson and Roberfroid, 1995;

Table 3.2 Selection From Our Randomized Controlled Trials Using Fructan Prebiotic for Evaluation of Bifidogenic Effect in Healthy Adults

Subjects	Study Design	Prebiotic Type, Consumption	Method/end Points	Main Results	Ref.
$n = 200$	Double-blind parallel-group study ($n = 64$)	Prebiotic at 10 g/d for 7 days • sc-FOS • SOS • GOS • Type III resistant starch • Lactulose, long-chain inulin, IMOS • Placebo	Fecal bacterial count at d0 and d8	Increase of bifidobacteria • $P = 0.008$ • $P = 0.006$ • $P < 0.0001$ • $P = 0.02$ • NS, NS, NS —	Bouhnik et al., 2004
	Double-blind parallel-group, dose–response relation (DRR) study ($n = 136$)	Prebiotic at dose of 2.5, 5.0, 7.5, and 10 g/d vs. placebo for 7 days • sc-FOS • SOS • GOS • Type III resistant starch	DRR between scFOS and fecal bifidobacteria counts	• DRR found for scFOS • No DRR for SOS, GOS, type III resistant starch • A low baseline bifidobacteria count was associated with the bifidogenic response to treatment ($P < 0.001$)	
$n = 40$	Double-blind, parallel-group, DRR study	sc-FOS at dose of 2.5, 5.0, 7.5, and 10 g/d or placebo for 7 days	DRR between the sc-FOS and fecal bifidobacteria counts	• DRR bifidogenic effect from dose of 2.5 g/d	Bouhnik et al., 2006

$n = 80$	DRR study of NDCH at higher doses than 10 g/d	Prebiotic at dose of 10, 15, and 20 g/d vs. placebo for 7 days: • Lactulose • lc inulin • IMOS	Fecal bacterial count at d0 and d8	Increase of bifidobacteria • Lactulose at 20 g/d ($P < 0.05$) • lc inulin at 15 g/d ($P < 0.01$) and 20 g/d ($P < 0.05$) • No linear DRR • A low baseline bifidobacteria count was associated with the bifidogenic response to treatment	Bouhnik et al., submitted (a)
$n = 50$	Randomized, double-blind, controlled study	Prebiotic for 15 days: • sc-FOS 10 g/d • lc inulin 10 g/d	Fecal bacterial count at d0, d8, and d15	• At d8, bifidobacteria counts were higher in sc-FOS than in lc inulin group ($P = 0.04$). • At d15, bifidobacteria counts increased in both groups ($P < 0.01$)	Bouhnik et al., submitted (b)

Bouhnik et al., 2004). Experimental data suggested that the importance of bifidogenic effects of sc-FOS and inulin could be related to their chain length. *In vitro* studies reported a difference in fermentation profile according to the chain length (Hidaka, 1986; Wang and Gibson, 1993). Moreover, a study performed in rats found that modifications in the fructan chain length could modulate the composition of the intestinal microflora (Kleessen, 2001). Therefore, a head-to-head comparison of sc-FOS and long-chain (lc) inulin (without small molecules) was performed in a randomized control trial including 50 volunteers (Bouhnik et al., submitted (b)). Bifidobacteria counts increased in both groups ($P < 0.01$), but the effects appeared quickly in sc-FOS group, probably because the fermentation was slower in lc inulin group.

3.2.1.2 Effect on Other Intestinal Bacteria

Even if the effects of fructans on the human gut microbiota were mainly investigated to search for a selective stimulation of bifidobacterial growth, other bacteria have been studied, such as lactobacilli, eubacteria, enterobacteria, enterococci (Kleessen, 2001; Apajalahti et al., 2002; Macfarlane et al., 2006; Louis et al., 2007). It has also been reported that FOS increased the colonization and translocation of *Salmonella* in an animal model (Ten Bruggencate et al., 2004). This was not observed, however, in human volunteers on a regular diet (Scholtens et al., 2006).

In another study, Kleessen et al. (2001) investigated changes in bacterial species in human flora associated rats fed on diets containing various mixtures of short- and long-chain fructose polymers. Bacteria were enumerated using FISH (fluorescent *in situ* hybridization) with group-specific probes. They showed that a mix of FOS and lc inulin or inulin alone enhanced the numbers of the clostridial cluster XIVa group, which was unaffected by FOS alone.

A recent human study using analysis by temporal temperature-gradient gel electrophoresis (TTGE) and fluorescent *in situ* hybridization also showed changes in the diversity and composition of dominant bacterial communities in response to dietary supplementation with hormone-related compounds combined with sc-FOS (Clavel et al., 2005). Overall, different groups of bacteria may be stimulated by fructans (Louis et al., 2007). For instance, fructan consumption may stimulate growth of *Roseburia inulinivorans*, which is a butyrate-producing inulin degrader belonging to clostridial cluster XIVa. The increased production of butyric acid from FOS, therefore, may be attributed in part to direct stimulation of butyrate-producing species (Manderson et al., 2005). It has also been recently shown that two distinct mechanisms of metabolic cross-feeding between *Bifidobacterium adolescentis* and butyrate-forming bacteria may operate in gut ecosystems, one due to consumption of fermentation end products (lactate and acetate) and the other due to cross-feeding of partial breakdown products from complex substrates (Belenguer et al., 2006).

3.2.1.3 Limits

Human studies performed to investigate the effects of fructans on facal microflora present two main limits. The first one is that the initial level of bifidobacteria may have an impact on the microbiological results, as we recently demonstrated that a low baseline bifidobacteria count was an independent factor significantly associated with an increased count after treatment (Bouhnik et al., 2004). The second is the variability and the specificity in bacteria counting methods. This is a major point because proving the selective stimulation of growth and/or activity of bacteria by prebiotics is contentious and difficult to fulfill (Gibson et al., 2004). In recent years, the development of molecular methods helped to overcome limitations of microbiological plating methods (Tannock, 2002; Gibson et al., 2004; Mai and Morris, 2004; Zoetendal et al., 2004; Egert et al., 2006). Based on 16S rDNA sequence similarities, these methods indeed allow for gender and even species-specific bacteria counts (Matsuki et al., 2004). The main molecular methods are FISH, denaturing gradient gel electrophoresis (DGGE), quantitative dot-blot hybridization, restriction fragment length polymorphism (RFLP), and large-scale rDNA sequencing. Although these methods provide an advanced tool for accurate microbiota characterization, some shortcomings can be underlined: some predominant species—including bifidobacteria—may not be detected due to imperfect DNA denaturation (Wilson and Blitchington, 1996; Suau et al., 1999). Moreover, these methods are still limited by the relative paucity of sequenced gene fragments and the use of fecal biota as a surrogate for the entire gut microflora. Overall, a combination of conventional and molecular microflora analysis tools will help to better define the complexity of human microbiota and the effects of prebiotic candidates on it (Mai and Morris, 2004).

3.2.2 Effects of Fructans on Intestinal Functions

3.2.2.1 Stool Weight

In adults, nondigestible oligosaccharides may increase stool weight through an increase in bacterial biomass (Gibson and Roberfroid, 1995; Cummings et al., 2001; Cummings and Macfarlane, 2002; Marteau and Boutron-Ruault, 2002), leading to a reduction in transit time (Cummings et al., 1992; Spiller, 2003). This effect depends on the dose ingested and the polymerization degree of the oligosaccharide (Cummings et al., 2001; Marteau and Seksik, 2004). Another property of nondigestible oligosaccharides that may contribute to their effect of transit time is their gasogenic effect, which is supposed to trigger the acceleration of transit (AFSSA, 2005). In two studies (Gibson and Roberfroid, 1995; Den Hond et al., 2000), the increase in stool wet weight corresponded to 1.5 to 2.0 g per gram of FOS or lc inulin fed. This is less than that seen with nonstarchy polysaccharide sources, such as wheat bran (5.4 g) or fruit and vegetables (4.7 g), but similar to that produced by more rapidly fermented polysaccharides (soluble fibers), such as pectin, guar gum, and acacia gum (Cummings, 1993; Cherbut et al., 2003b). Three other human experiments did not

show any increase in stool weight after ingestion of fructans (Ikeda et al., 1994; Alles et al., 1996; Bouhnik et al., 1997), but in none of these studies was the subjects' diets controlled, which would tend to mask a small effect.

3.2.2.2 Fermentation and Production of Short-Chain Fatty Acids

A major metabolic function of colonic microflora is the fermentation of nondigestible dietary residue, such as fructans. Fermentation of carbohydrates is a major source of energy in the colon. The metabolic end point is generation of short-chain fatty acids (SCFA: acetate, propionate, and butyrate; Salminen et al., 1998). All these fatty acids have important functions in host physiology, but butyrate seems to be the most interesting. First, its oxidation makes up for more than 70 percent of the oxygen consumption by the human colonic tissue, indicating that butyrate is the prime energy substrate of the colonocyte (Cummings and Macfarlane, 2002). Acetate and propionate are found in portal blood and are eventually metabolized by the liver (propionate) or peripheral tissues, particularly muscle (acetate). Acetate and propionate might also play a role as modulators of glucose metabolism: Absorption of these short-chain fatty acids would result in lower glycemic responses to oral glucose or standard meal—a response consistent with an ameliorated sensitivity to insulin. In fact, foods with a high proportion of nondigestible carbohydrates all have a low glycemic index (Thorburn et al., 1993; Englyst et al., 1999).

Each prebiotic may be characterized by its fermentation profile, for example, the relative proportion of acetate, propionate, and butyrate resulting from its fermentation. Among fructans, sc-FOS presents a high level of butyrate production during bacterial fermentation, as shown both *in vitro* (Wang and Gibson, 1993; Luo et al., 1996) and *in vivo* in animals (Le Blay et al., 1999; Pierre et al., 1999) and humans (Boutron-Rouault et al., 2005).

SCFAs are also able to modulate intestinal and colonic motility (Cherbut et al., 1997; Fich et al., 1998). They may stimulate contraction in the terminal ileum and shorten ileal emptying, which could protect the ileal mucosa against the potentially harmful effects of reflux of the colonic contents (Cherbut et al., 1996, 1997). Mechanisms of action of SCFA on gastrointestinal motility may involve systemic humoral and neural pathways (Cherbut et al., 1998) as well as reflexes and myogenic responses (Cherbut et al., 1996).

Colonic microorganisms also play a part in vitamin synthesis and in absorption of calcium, magnesium, and iron. Absorption of ions in the cecum is improved by carbohydrate fermentation and production of SCFAs (Guarner and Malagelada, 2003). Much research in experimental animals has shown positive effects of nondigestible oligosaccharides on calcium, magnesium, iron, and zinc absorption (Scholz-Ahrens et al., 2001). The mechanism underlying these positive effects is most likely related to increased solubility of these minerals in the cecum and the colon as a consequence of increased microbial fermentation and lower luminal pH.

3.2.2.3 Epithelial Cell Growth and Differentiation

Possibly, the most important role of SCFAs on colonic physiology is their trophic effect on the intestinal epithelium. The rate of production of crypt cells is reduced in the colon of rats bred in germ-free environments, and their crypts contain fewer cells than do those of rats colonized by conventional flora, suggesting that intraluminal bacteria affect cell proliferation in the colon (Guarner and Malagelada, 2003). Differentiation of epithelial cells is greatly affected by interaction with resident microorganisms. All three major SCFAs stimulate epithelial cell proliferation and differentiation in the large and small bowel *in vivo*. However, among the SCFAs produced by fermentation, butyrate has specific biological activities in the colon. Butyrate stimulates proliferation and differentiation in normal epithelial cell lines and has the opposite effects in transformed cell *in vitro*. Moreover, butyrate promotes reversion of cells from neoplastic to nonneoplastic phenotypes (Guarner and Malagelada, 2003). In pig also, FOS has been shown to stimulate SCFA production: a test diet containing FOS (10 percent) *ad libitum* for 10 days led to a significant increase in the concentration of SCFA, especially for *n*-butyrate (Tsukahara et al., 2003). sc-FOS, which presents a high level of butyrate production during bacterial fermentation (Tsukahara et al., 2003), thus may modulate cell proliferation in a beneficial manner.

3.2.2.4 Immunity

The intestinal mucosa is the main interface between the immune system and the external environment. Thus, it is not surprising that gut-associated lymphoid tissues contain the largest pool of immunocompetent cells in the human body. The colonic microflora, especially bifidobacteria, has been reported to exert a high influence on the immune system of the host, such as mitogenic or adjuvant activities, promotion of macrophages, stimulation of antibody production, and antitumor effects (Salminen et al., 1998; Bornet et al., 2002).

In children, a controlled study showed that a preparation of cereals containing a mixture of inulin and FOS (0.2 g/kg of body weight) increased vaccinal immunoglobulin G (IgG) levels 10 weeks after immunization of the infant against measles (Firmansyah et al., 2001). The level of positive reaction with adequate IgG response was 96 percent in children receiving the prebiotic compared with 88 percent in the control infants. There was no difference in the levels of antimeasles IgM. A recent study (Bakker-Zierikzee et al., 2006) also demonstrated a significant increase in fecal secretory IgA in infants who received a formula enriched with a mix of GOS and FOS (0.6 g/100 mL) compared to a standard group. This mix of prebiotics, which stimulates bifidobacteria and leads to a fermentation profile close to the one found in breastfed children, was often studied and appeared as beneficial for infants (Moro et al., 2003; Boehm et al., 2004; Decsi et al., 2005; Knol et al., 2005).

Atopic diseases, such as atopic eczema, allergic rhinitis, and asthma, are increasing in Western societies, demanding rapid comprehension and prevention. Several

promising studies have been conducted with probiotics (Kalliomaki et al., 2003; Ishida et al., 2005; Weston et al., 2005; Ishida et al., 2006), but the potential effects of prebiotics in children on atopic eczema, either therapeutic or preventive, are little known (AFSSA, 2003). Similarly, no studies are available demonstrating a significant effect of a prebiotic on allergic conditions in adults (AFSSA, 2005). However, in patients with atopic eczema a correlation was shown between the amount of bifidobacteria and the severity of atopic eczema (Bunselmeyer, 2006) and recent studies proved the efficiency of consumption of synbiotics, such as *Lactobacillus casei* subsp. *casei* with dextran (Ogawa et al., 2006), on the prevention and treatment of allergic reactions in adults (pollen allergy) or children (atopic dermatitis) (Passeron et al., 2006). On the contrary, two studies reported allergic reactions after consumption of foods containing inulin (Salminen et al., 1998; Gay-Crosier et al., 2000).

3.2.3 The Barrier Effect

Resident bacteria are a crucial line of resistance to colonization by exogenous microbes and, therefore, are highly relevant in protecting the internal medium of the host against pathogenic organisms and toxic substances (Cherbut, 2003). It is probably through their effects on the colonic flora that prebiotics are able to reinforce the intestinal barrier as it has been demonstrated that inulin and FOS modify the profile of bacterial biofilms associated with the intestinal mucosa (Cummings and Macfarlane, 2002). Studies in animal models implanted or not with human flora suggested favorable effects of inulin and FOS on intestinal mucosa, e.g., increase of the thickness of the mucin layer and of the number of mucus-containing cells (Hoebler et al., 2002; Kleessen et al., 2003), and modification of the distribution between neutral, acidic, and sulfated mucins in favor of sulfated mucins, possibly more protective (Fontaine et al., 1996). A clinical study conducted in humans failed to show a change in mucin expression (Meijer et al., 2000). Gaudier et al. (2004) suggested that the effects of fructans on mucins could be mediated by the production of butyrate because this SCFA increases the production of certain mucin genes (MUC3) (Gaudier et al., 2004). As mentioned by Fowler et al. (2003), mucins are highly heterogeneous among individuals, so that the effect of fructans could be different depending on the subject.

However, prebiotics also could have deleterious effects on the intestinal barrier. A study found that inulin and FOS increased the hepato-splenic translocation of salmonella *in vivo* in rats (Ten Bruggencate et al., 2004). In healthy humans, a recent placebo-controlled cross-over study found that FOS consumption (20 g/day over a 2-week period) doubled fecal mucin excretion indicating mucosal irritation (Ten Bruggencate et al., 2006). These results have to be balanced by the fact that overall observed effects were more moderate than those in rats and that the dose ingested was relatively high, especially as it was added in a liquid food (lemonade), leading to increased flatulence and intestinal bloating. Overall, the effect of fructans on the intestinal barrier should be further studied in well-designed clinical trials in humans.

3.3 EFFECTS OF FRUCTANS ON GASTROINTESTINAL DISEASE

3.3.1 Infectious Diarrhea

The efficacy of probiotics in prevention of acute diarrhea in children and in adults has been recently demonstrated in a meta-analysis; even though most pronounced on antibiotic-associated diarrhea, a significant effect was also observed in nonantibiotic-associated diarrhea and nontravelers' diarrhea (Sazawal et al., 2006).

Prebiotics—either alone or in addition to a probiotic—have been less extensively studied in this situation. The main studies carried out to assess the effects of fructans in the prevention or treatments of acute diarrhea are reported in Table 3.3.

In infants, a mix of a probiotic and oligofructose decreased duration of acute diarrhea compared to control (Ahmas et al., 2000; Agustina et al., 2007). Oligofructose has been recently found effective in preventing intestinal disturbances in very young children (Waligora-Dupriet et al., 2007). In infants living in a community with a high burden of gastrointestinal and other infections, oligofructose failed to show any benefit for prevention of diarrhea (Duggan et al., 2003).

In adults, oligofructose significantly decreased the relapse of diarrhea associated with *Clostridium difficile* (Lewis et al., 2005). In a randomized, double-blind, controlled study, 244 healthy subjects, traveling to high- and medium-risk destinations for travelers' diarrhea, consumed FOS at 10 g/day during 2 weeks before the trip and during the 2-week trip (Cummings et al., 2001). If there were no significant differences in the primary end points of bowel frequency or consistency between the two groups, as recorded in bowel habit diaries, subjects taking FOS experienced less severe attacks of diarrhea than subjects in the placebo group. These results are indicative of a benefit of prebiotics, but not conclusive.

3.3.2 Inflammatory Bowel Disease

The enthusiasm with which probiotics have been used in inflammatory bowel disease (IBD) and their apparent benefits have led to the suggestion that prebiotics might also be useful (Sartor, 2004). Reports of animal studies are quite numerous, and overall they show a benefit in reducing symptoms, including inflammation, with appropriate increases in bifidobacteria or lactobacilli, and in some reports, in concentrations of butyrate in the gut. These effects are seen across a wide range of models of IBD, and with varying oligofructose (Cherbut et al., 2003a; Moreau et al., 2003).

The main studies carried out to assess the effects of fructans in the prevention or treatments of IBD are reported in Table 3.4.

Two controlled studies that evaluated prebiotics in association with mesalazine or probiotic in ulcerative colitis gave contradictory results (Furrie et al., 2005; Casellas et al., 2007).

In a small open-label trial in humans, 10 patients with active ileocolonic Crohn's disease were given 15 g FOS daily for 3 weeks (Lindsay et al., 2006). A significant

Table 3.3 Main Randomized Controlled Clinical Studies Using Fructan Prebiotic In Prevention Or Treatment Of Acute Diarrhea

Subjects	Study Design	Prebiotic Type, Consumption	Method/end Points	Main Results	Ref.
Indonesian well-nourished male infants, aged 3–12 months, Acute diarrhea with moderate dehydration $n = 58$	Randomized double-blind clinical trial	Special infant formula containing probiotic, prebiotic, and supplements[a] vs. control	Duration of diarrhea	Diarrhea significantly shorter in the study group than in the control group (1.63 vs. 2.45 days; $p < 0.05$).	Agustina et al., 2007
Infants in a community with a high burden of gastrointestinal and other infections $n = 282$	Randomized controlled trial 1	Infant cereal supplemented with oligofructose (0.55 g/15 g cereal) or not supplemented	Duration of diarrhea	Mean (± SD) days of diarrhea were 10.3 ± 9.6 in the nonsupplemented cereal group and 9.8 ± 11.0 in the prebiotic-supplemented cereal group ($P = 0.66$).	Duggan et al., 2003
Infants in a community with a high burden of gastrointestinal and other infections $n = 349$	Randomized controlled trial 2	Zinc (1 mg/15 g cereal) added to both oligofructose-supplemented and nonsupplemented cereals	Duration of diarrhea	Mean days of diarrhea were 10.3 ± 8.9 in the group consuming cereal fortified only with zinc and 9.5 ± 8.9 in the group consuming cereal containing both zinc and prebiotics ($P = 0.35$).	
Consecutive inpatients with *C. difficile*-associated diarrhea $n = 142$	Randomly allocation	Oligofructose or placebo for 30 days in addition to specific antibiotic treatment	Prevention of further diarrhea	Relapse of diarrhea was observed in 34% with placebo and 8% with oligofructose ($P < .001$).	Lewis et al., 2005
Healthy subjects, traveling to high- and medium-risk destinations for travelers' diarrhea $n = 244$	Randomized, double-blind, placebo-controlled study	10 g FOS or placebo daily for 2 weeks before travel	Prevention of further diarrhea	11.2% in FOS group and 19.5% in placebo ($p = 0.08$) Diarrhea severity score was lower in FOS than in placebo groups ($P < 0.05$)	Cummings et al., 2001

[a] Special antidiarrhea infant formula containing probiotic (*Lactobacillus rhamnosus* LMG P-22799; 5×10^8 colony-forming units/100 mL), prebiotic (inulin 0.15 g/100 mL), fiber (soy polysaccharides: 0.2 g/100 mL), and iron + zinc.

Table 3.4 Main Randomized Controlled Clinical Studies Using Fructan Prebiotic In Patients with Inflammatory Bowel Disease

Subjects	Study Design	Prebiotic Type, Consumption	Method/End Points	Main Results	Ref.
Subjects with active ulcerative colitis Mesalazine (3 g/day) *n* = 21	Randomized, placebo-controlled pilot trial	Oligofructose-enriched inulin (*n* = 10) or placebo (*n* = 9) 12 g/day, p.o. 2 weeks	Activity score Fecal calprotectin	• Rachmilewitz score decreased in both groups ($P < 0.05$) • Reduction of calprotectin in oligofructose-enriched inulin group ($P < 0.05$)	Casellas et al., 2007
Subjects with active ulcerative colitis *n* = 18	Randomized, placebo-controlled pilot trial	Synbiotic[a] or placebo bid 2 weeks	Clinical activity index (CAI) Intestinal biopsies Inflammatory markers	• CAI (NS) • IB ($P = 0.06$) trends in favor of symbiotic • Human betadefensin, tumor necrosis factor-α, interleukin-1α β ($P < 0.01$)	Furrie et al., 2005
Patients with active ileocolonic Crohn's disease *n* = 10	Small open-label trial	scFOS 15 g/d for 3 weeks	Activity index Intestinal biopsies	• FOS induced a significant reduction in the Harvey Bradshaw index ($p < 0.01$) • The percentage of IL-10 positive dendritic cells increased from 30 to 53% ($p = 0.06$)	Lindsay et al., 2006
Stable asymptomatic pouchitis *n* = 24	Randomized double-blind cross-over study	Enteral supplementation of inulin or placebo (24 g/d) for 3 weeks	Pouchitis disease activity index Intestinal flora	Compared with placebo, inulin: • Increased butyrate concentrations • Lowered pH • Decreased numbers of *Bacteroides fragilis* • Diminished concentrations of secondary bile acids in feces • Decreased inflammation endoscopically and histologically	Welters et al., 2002

[a] Synbiotic combined a probiotic, *Bifidobacterium longum*, isolated from healthy rectal epithelium, and a prebiotic (Synergy 1), a preferential inulin-oligofructose growth substrate for the probiotic strain. Test patients were given 2×10^{11} freeze-dried viable *B. longum* in a gelatin capsule and a sachet containing 6 g of prebiotic fructo-oligosaccharide/inulin mix (Synergy 1; Orafti, Tienen, Belgium) consisting of a probiotic, *B. longum* W11, and the sc-FOS or biotic.

reduction in the Harvey Bradshaw index of disease activity was observed, and fecal bifidobacteria increased from $\log_{10}$ 8.8 to $\log_{10}$ 9.4 cells per gram dry feces. The proportion of dendritic cells expressing Toll-like receptors TLR2 and TLR4 also increased ($p < 0.001$).

Patients with pouchitis do well with probiotics, and one successful study has been reported in which prebiotics were used for this condition (Welters et al., 2002). In a randomized double-blind cross-over study, 24 patients with stable asymptomatic pouchitis were given 24 g of inulin or placebo daily, for 3 weeks each. At the end of the prebiotic period, results showed that there was a reduction in the endoscopic and histological pouchitis disease activity index (PDAI) score, together with lower gut pH, and reductions in fecal *Bacteroides fragilis* and secondary bile acids. Butyrate concentrations were increased, while symptom scores were low initially, and were essentially unchanged.

The link between intestinal microflora and IBD is now well established and the use of prebiotics, therefore, might be a promising therapeutic strategy for ameliorating chronic intestinal inflammation (Andoh and Fujiyama, 2006; Ewaschuk and Dieleman, 2006).

3.3.3 Irritable Bowel Syndrome

There are currently no published full papers of randomized controlled trials (RCT) concerning the use of prebiotics in irritable bowel syndrome (IBS). A number of studies using probiotics have been carried out with varying benefits, but the pathogenesis of IBS may preclude the use of prebiotics in this condition. In fact, there are several recent reports of low-grade mucosal inflammation in IBS with increased mucosal T lymphocytes in both unselected diarrhea-predominant IBS (Chadwick et al., 2002) as well as those whose IBS begins with an acute episode of bacterial gastroenteritis (Spiller, 2003; Marshall et al., 2007). These results suggest that IBS could be a subclinical inflammatory bowel disease and an intervention on intestinal bacteria could improve symptoms.

The main studies carried out to assess the effects of fructans in the prevention or treatments of IBS are reported in Table 3.5. Only two open-label multicenter studies evaluated the effects of prebiotic in constipation-predominant IBS, with interesting results in term of digestive comfort and stool frequency (Colecchia et al., 2006; Dughera et al., 2007).

3.3.4 Colonic Tumors

Intestinal bacteria could play a part in initiation of colon cancer through production of carcinogens, cocarcinogens, or procarcinogens. In healthy people, diets rich in fat and meat, but poor in vegetables, increase the fecal excretion of *N*-nitroso compounds, a group of genotoxic substances that are known initiators and promoters of colon cancer (Guarner and Malagelada, 2003). Another group of carcinogens of dietary origin is the heterocyclic aromatic amines that are formed in meat when it is cooked. Some intestinal microorganisms strongly increase damage to DNA in colon cells induced by heterocyclic amines, whereas other intestinal bacteria can uptake

Table 3.5 Main Clinical Studies Using Fructan Prebiotic In Patients With Irritable Bowel Syndrome

Subjects	Study Design	Prebiotic Type, Consumption	Method/End Points	Main Results	Ref.
Patients with constipation-type IBS $n = 636$ (250 men, 386 women)	Open-label, prospective, uncontrolled, multicenter trial	Synbiotic[a] at a dose of 3 g/d for at least 36 days	Roma II criteria	• In the more severe symptoms classes (moderate-severe), symptom frequency dropped significantly for bloating and abdominal pain • Stool frequency significantly increased	Colecchia et al., 2006
Patients with constipation-type IBS $n = 129$	Open-label, prospective, uncontrolled, multicenter trial	Synbiotic zir fos®[b] 3 g/d for 3 months	Rome II criteria at T0, T1, and T3	• Total symptom frequency reduction was observed at T1 and T3 vs. T0 ($p < 0.0001$) • An increase of stool frequency ($p < 0.0001$)	Dughera et al., 2007

[a] Synbiotic consisting of a probiotic, *Bifidobacterium longum* W11, and the scFOS or biotic.
[b] A synbiotic drug (zir fos Alfa Wassermann, Alanno Scalo, Pescara, Italy) constituted by a probiotic, *B. longum* W11 (5×109 cfu), and by a prebiotic short-chain oligosaccharide, Fos-Actilight® (2.5 g).

and detoxify such compounds (Wollowski et al., 2001). Bacteria of the *Bacteroides* and *Clostridium* genera increase the incidence and growth rate of colonic tumors induced in animals, whereas other genera, such as *Lactobacillus* and *Bifidobacterium* prevent carcinogenesis (Pool-Zobel, 2005). Although the evidence is not conclusive, colonic flora seems to be a major environmental factor that modulates risk of colonic cancer in humans (Guarner and Malagelada, 2003).

Numerous studies have shown that inulin-type fructans prevent chemically induced preneoplastic lesions, aberrant crypt foci (ACF), and tumors in the colon of rats and mice (Pierre et al., 1999, 2001; Wollowski et al., 2001; Pool-Zobel et al., 2002, 2005). Contradictory studies have been shown to enhance adenoma growth in mice (Pajari et al., 2003; Misikangas et al., 2005, 2008).

In humans, several trials have been carried out to examine possible effects of prebiotics on colonic carcinogenesis. These trials used fecal butyrate concentration, fecal bile acids, and rectal crypt cell proliferation as promising surrogate markers for the risk of colorectal carcinogenesis (Rafter, 2002). Fecal bacterial enzymatic activities, such as β-glucuronidase, have been extensively studied as they may play a role in the metabolic activation of procarcinogens and deconjugation processes in the colonic lumen (Goldin, 1990). Some trials have been performed in healthy volunteers, with the aim to modify some potential marker of colon carcinogenesis.

Fecal bacterial β-glucuronidase activity is increased in patients on a high meat diet, and this enzyme could act to raise the amount of substances, such as carcinogens, within the colonic lumen (Reddy et al., 1998). In a previous study, we demonstrated that sc-FOS ingestion led to a significant decrease in β-glucuronidase activity (Bouhnik et al., 1996). In a recent RCT, 15-day consumption of 10g/day sc-FOS in healthy subjects has been shown to reduce the activity of β-glucuronidase in fecal samples, whereas consumption of 15 g/day lc inulin over the same period did not change enzymatic activities (Bouhnik et al., 2007). Similar results were found by Kleessen et al., who did not demonstrate changes in β-glucuronidase activity following lc inulin consumption for 19 days at doses ranging from 20 to 40 g/day (Kleessen et al., 1997).

Three interventional studies using fructans or synbiotics in patients with polyps or cancer have been published (Boutron-Rouault et al., 2005; Rafter et al., 2007; Roller et al., 2007) (Table 3.6). In one of them, the effect of a 3-month consumption of 10 g/day sc-FOS on these markers was assessed in subjects with large (>10 mm) or small adenomas (<10 mm), or in healthy subjects (Boutron-Rouault et al., 2005). The butyrate concentration, which was initially significantly lower in subjects with adenomas compared to healthy subjects, significantly increased to the level found in healthy subjects after the 3-month sc-FOS consumption. If there is little doubt that butyrate may exert an effect on colon cancer development, exact mechanisms by which butyrate acts remain unclear. Variable effects could indeed be obtained according to the *in vivo* or *in vitro* environments, the timing of butyrate administration in relation to the stage of cancer development, the amount of butyrate administered, as well as an interaction with dietary fat (Lupton, 2004). For example, prebiotics may be protective against the early stages of polyp formation, but not at the stage of transition of polyp to a carcinoma, and low amounts of butyrate may stimulate cell proliferation

while high amounts may inhibit it (Lupton, 2004). In the study performed by Rafter et al. (2007), the synbiotic intervention resulted in significant alterations in the composition of the colonic bacterial ecosystem, which presumably have consequences for the metabolic activity of this organ. These results also provide indirect evidence that some of the consequences of the synbiotic intervention might be decreased exposure of the epithelium to cytotoxic and genotoxic agents, decreased colonic cell proliferation, and improved mucosa structure. These exciting results suggest that synbiotics may represent a feasible means of chemoprevention of colon cancer in humans.

3.4 EFFECTS OF FRUCTANS ON METABOLISM OF MINERALS AND VITAMINS

Demonstrating an effect of a given food factor on mineral bioavailability in humans is difficult for methodological reasons (Guéguen and Pointillart, 2000; Scholz-Ahrens et al., 2001). The choice of relevant biological markers is essential. Abrams et al. (1994) showed that calcium absorption can be correctly measured using either the chemical balance or the dual-stable-isotope methods. But the site (serum or urines samples) as well as the timing (e.g., urine collected less or more than 24 hours after tracer administration) for markers measurement chosen can also induce various interpretations of results as observed on trials dealing with nondigestible oligosaccharides (Coudray and Fairweather-Tait, 1998).

3.4.1 Fructans and Calcium Absorption

A review on calcium (Ca) consumption in France revealed that a large part of the population consumes less than two-thirds of the recommended dietary allowance (RDA), the critical threshold for defining groups at risk (Guéguen, 1996). Therefore, there is real public health interest in studying the impact of prebiotics, such as fructans, on Ca absorption, especially for prevention of osteoporosis.

In adolescents, van den Heuvel et al. (1998) found that 15 g/day inulin, FOS, or GOS had no effect on the intestinal absorption using the dual-stable-isotope method. Griffin et al. (2003) showed that the main determinant of the effect of fructans in preadolescents was "Ca absorption during the placebo period." In fact, individuals with lower Ca absorption during the placebo period had the greatest increase in Ca absorption. Regarding the nature of the prebiotic tested, a study in adolescent girls demonstrated that 8 g/day of a mixture of inulin and oligofructose significantly increased Ca absorption while 8 g/day of oligofructose alone had no effect (Griffin et al., 2002), confirming previous findings in animals (Coudray et al., 2003; Kruger et al., 2003).

In young men, Coudray et al. showed that 40 g inulin per day increased Ca absorption using the chemical balance (Coudray et al., 1997). In postmenopausal women, Holloway et al. (2003) tested a mixture of inulin and oligofructose for 6 weeks (Holloway et al., 2003), showing no effect of prebiotics on Ca absorption, but a great variation in individual responses to the treatment. Interestingly, the efficacy

Table 3.6 Main Randomized Controlled Clinical Studies Using Fructan Prebiotic For Effect In Patients With Colonic Polyps And/Or Cancer

Subjects	Study Design	Prebiotic Type, Consumption	Method/end Points	Main Results	Ref.
Colon cancer with "curative resection" (n = 34) Polypectomized patients (n = 40)	Randomized double-blind, placebo-controlled trial	Encapsulated bacteria[a] and 10 g of inulin enriched with oligofructose (SYN group) or placebo once daily	Modulation of immune functions	• In the cancer group, SYN resulted in an increased capacity of PBMC to produce interferon-gamma ($P < 0.05$) • In the polyp group, IL-2 secretion by activated PBMC[b] increased ($P < 0.05$)	Roller et al., 2007
Colon cancer (n = 37 Polypectomized (n = 43)	Randomized, double-blind, placebo-controlled trial (phase 2 study)	Synbiotic food composed of the prebiotic SYN1—a mixture of short-chain and long-chain inulin (SYN1)—and probiotics LGG and BB12[c] vs. placebo for 12 weeks	Reduction in cancer risk biomarkers in stools and intestinal mucosa	In all patients: • Increase of *Bifidobacterium* and *Lactobacillus* • Decrease of *Clostridium perfringens* • Reduction in colorectal proliferation and the capacity of fecal water to induce necrosis in colonic cells In polypectomized patients: • Improvement of epithelial barrier function • Decreased exposure to genotoxins • Prevention of an increased secretion of IL-2 by peripheral blood mononuclear cells In cancer patients: • Increase in interferon-gamma production	Rafter et al., 2007

Subjects with small colorectal adenoma(s) (n = 26) With large adenoma(s) (n = 18) With no adenoma (n = 30)	Interventional study	sc-FOS 10 g/d for 3 month	Reduction in cancer risk biomarkers in stools	The mean fecal butyrate: • Was lower at baseline concentration in the adenoma groups than in the adenoma-free group • Was increased in this group after 3-mo ingestion of sc-FOS (P = 0.02) In subjects without adenoma: • sc-FOS ingestion was associated with a decrease in fecal lithocholic acid (P = 0.02) and an increase in cholic acid (P = 0.02), chenodeoxycholic acid (P = 0.04), total primary bile acids (P = 0.03), and ursodeoxycholic acid (P = 0.05)	Boutron-Rouault et al., 2005

[a] 1 × 10(10) colony-forming units of *Lactobacillus rhamnosus* GG (LGG) and 1 × 10(10) colony-forming units of *Bifidobacterium lactis* Bb12.
[b] Peripheral blood mononuclear cells.
[c] Synbiotic preparation-oligofructose-enriched inulin (SYN1) + *L. rhamnosus* GG (LGG) and *B. lactis* Bb12.

of the treatment seemed to be higher in women with lower initial bone density of the spine (Coxam, 2005). In addition, genetic factors (such as vitamin D receptor gene polymorphism) may also be associated with differences in sensitivity to the effects of fructans (Abrams et al., 2005).

A physiological retro-control mechanism provides good intestinal regulation of Ca absorption, thanks to the calcium-binding protein. As it is highly regulated, a high impact of fructans on this absorption is not expected. Overall, the increase of Ca absorption under fructans would only have weak amplitude and few long-term nutritional consequences.

3.4.2 Fructans and Absorption of Magnesium, Copper, Selenium, and Zinc

Positive effects of fructans on magnesium (Mg) absorption were naturally expected in humans because, in contrast to Ca, Mg absorption occurs mainly passively and is not regulated depending on intakes and requirements. First results from animal models indicated stimulant effects of fructans on Mg absorption (Rémésy et al., 2002). This has been confirmed for sc-FOS and Mg absorption in a recent study in humans: An increase of 11 percent of relative magnesium absorption was observed after administration of 7 to 10 g/day sc-FOS during 5 weeks in postmenopausal women (Tahiri et al., 2001). Even on Mg, only a weak effect (increase by 10 to 20 percent) can be induced by FOS. However, about 20 percent of the population has Mg intakes lower than two-thirds of RDA. This property of FOS, therefore, could have an impact, even if small, on subjects with insufficient food Mg intakes.

A randomized double-blind, placebo-controlled trial showed that feeding 10 g of FOS per day for 5 weeks increased the absorption of copper in healthy postmenopausal women (Ducros et al., 2005). However, no effects were seen in relation to zinc and selenium uptake. This selectivity would suggest that factors other than simple acidification of luminal contents were involved.

3.4.3 Fructans and Isoflavone Metabolism

Fructans may have a beneficial effect on the metabolism of isoflavones, which have been shown to prevent postovariectomy bone loss in rats and mice (Tokunaga, 2004; Coxam, 2005). In ovariectomized mice (Ohta et al., 2002) or rats (Mathey et al., 2004), two experimental models for postmenopausal osteoporosis, oligofructose (sc-FOS) consumption has been shown to increase the bone-sparing effect of isoflavones by improving equol production. However, opposite results were reported in the study of Zafar et al. (2004) as isoflavones enhanced Ca absorption without synergy from inulin, and inulin decreased equol production in rats. In humans, a 2-month intervention trial on 39 postmenopausal women showed that addition of prebiotics (sc-FOS) or probiotics, by partially modulating the bioavailability of soy isoflavones, improved parameters of bone turnover (Coxam, 2005).

3.4.4 Fructans and Vitamin Production

As mentioned by Gibson and Roberfroid (1995), bifidobacteria produce vitamins, mainly from the B-complex (Gibson and Roberfroid, 1995). In an *in vitro* study, Noda et al. (1994) emphasized the fact that bifidobacteria strains, such as *B. bifidum*, produced biotin (vitamin B_8) extracellularly. Folic acid and vitamin K are also produced by bifidobacteria. Therefore, it could be thought that consumption of prebiotics, thanks to their stimulation on bifidobacteria growth, could have beneficial effects for subjects deficient in vitamin K or B. However, quantities of vitamins produced by bifidobacteria are very limited compared to dietary allowance (RDA), suggesting that prebiotic stimulation of bifidobacteria is not sufficient to exert significant effects on vitamin status.

3.4.5 Fructans and Absorptive-Productive Functions

Although the number of studies on the effect of nondigestible oligosaccharides on mineral metabolism in humans is limited, so far, positive effects on Ca absorption seem to occur under conditions of increased Ca requirements (e.g., adolescence and postmenopause). The extent of the effect seems to be specific for the type of carbohydrate. Contradictory results on the effect of prebiotics in the literature may be due to differences in the experimental design. Several experimental conditions promoted the stimulation of Ca absorption and retention by nondigestible oligosaccharides, such as high dietary Ca, an optimum dose of prebiotics, sufficient duration of administration, and the age of subjects (Scholz-Ahrens et al., 2001).

Despite the belief that Ca absorption is thought to occur in the proximal gut in humans, a colonic phase may exist. Ellegard et al. (1997) showed that neither inulin nor FOS when fed to ileostomy subjects had any effect on ileostomy excretion of Ca, Mg, zinc, or iron. Because prebiotic carbohydrates pass through the small bowel unchanged, but are fermented in the cecum or colon, a large bowel effect on absorption is possible (Macfarlane et al., 2006).

3.4.6 Metabolic Parameters and Satiety

Recent research has been reported concerning the effect of fructans on satiety and control of energy intake. The available data suggest a beneficial effect of inulin and FOS in modulating energy balance in humans consuming diet *ad libitum*. In a recent pilot study (Cani et al., 2006), 10 healthy volunteers were included in two 2-week phases during which they received twice a day either 8 g oligofructose or 8 g placebo (maltodextrin), with each phase separated by a 2-week wash-out period. It appeared that oligofructose treatment increases satiety following breakfast and dinner and reduces hunger and prospective food consumption following dinner. However, total energy intake per day was only 5 percent lower during the oligofructose than the placebo periods, what should not have a high impact on the body mass index of subjects.

3.5 DIGESTIVE TOLERANCE OF FRUCTANS

Fermentation of NDCHs in the colon by the microflora produces gases (H_2, CH_4, and CO_2), which may cause flatulence, abdominal pain, or osmotic diarrhea. It appears that digestive tolerance thresholds for prebiotics are clearly influenced by the chemical nature of the prebiotic, the administered dose, and individual factors (Marteau and Seksik, 2004). The individual factors include visceral sensitivity and differences in bacterial profile of the colonic flora (Cherbut, 2003); it has been seen that populations of lactate-utilizing bacteria in subjects reporting the highest number of symptoms of discomfort following consumption of FOS were different from subjects reporting no disturbance (Cherbut, 2003). Digestive tolerance is also influenced by the type of food (differing mainly between solid and liquid food) and the way of consumption (isolated consumption outside meal times favors symptoms) (Absolonne et al., 1995). Overall, it is important to note that digestive tolerance thresholds for NDCHs are significantly higher than efficient doses, which supports the interest in prebiotics as a safe and beneficial modulation of gut microflora.

Besides the evaluation of digestive tolerance of fructans, an increased interest in their impact on the quality of life of subjects is to be noted. In a recent study, it appeared that the regular consumption of sc-FOS at moderate doses (5 g/day over 6 weeks) can improve digestive comfort and daily quality of life in a working and nonmedically treated population suffering from minor functional bowel disorders (Paineau et al., 2008). This was the first study to assess the effects of prebiotics on quality of life with the use of relevant evaluation methods. A quality-of-life questionnaire was completed at the start and end of the treatment period to assess potential effects on well-being and social performance. At the end of the consumption period, the intensity of digestive disorders decreased by 43.6 percent in the sc-FOS group versus a 13.8 percent increase in the placebo group ($P = 0.026$). Expressed as change in quality of life (improvement, worsening, or unchanged), daily activities were significantly improved in the sc-FOS group ($P = 0.022$).

3.6 CONCLUSIONS

Prebiotics are widely available food ingredients that may exert a number of beneficial effects on human health. Most of these effects are mediated through their bifidogenic properties. Promising effects include a benefit in different situations in gastroenterology, such as infectious diarrhea, IBS, IBD, and colonic carcinogenesis. Objectives of future studies must investigate mechanisms in humans to define the optimal consumption of prebiotics. Well-controlled clinical trials in humans are needed especially in IBS, IBD, and prevention of colonic polyps, which are all major and increasing health problems in industrialized countries.

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CHAPTER 4

Galacto-Oligosaccharides

Arjen Nauta, Astrid M. Bakker-Zierikzee, and Margriet H. C. Schoterman

CONTENTS

4.1 INTRODUCTION

The interest in functional foods or food ingredients that exert a beneficial effect on human well-being and health is expanding. This is clearly illustrated by the increasing use of prebiotics. Prebiotics are defined as "nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity

of one or a limited number of bacteria in the colon and thus improve health."[1] There are several classes of prebiotic oligosaccharides of which galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS), and inulin are most applied. GOS have attracted particular attention because they have certain similarities to oligosaccharides occurring in human breast milk and modulate the microbial population (microbiota) in the gut. Thus, they affect different gastrointestinal activities and have the potential to influence inflammatory and immunological processes.[2] Enzymatically produced GOS (also named transgalacto-oligosaccharides, transgalactosylated oligosaccharides, trans-GOS, TOS, or oligogalactosyl-lactose) have been shown to have similar beneficial prebiotic effects as human milk oligosaccharides.[3]

4.2 MANUFACTURING

GOS are one of the most commonly produced prebiotic oligosaccharides worldwide. They can be obtained through the enzymatic conversion of lactose (milk sugar) by the enzyme β-galactosidase (EC 3.2.1.23). Lactose is a disaccharide that consists of β-D-galactose and β-D-glucose bonded through a β1–4 glycosidic linkage and is usually purified from cow's milk whey.[4] The β-galactosidase can mediate both the hydrolysis and polymerization of β-linked sugars. Normally, the enzyme forms an active intermediate with lactose and reacts with water to catalyze the hydrolysis of this β-galactoside. The monosaccharides galactose and glucose are liberated. Under the specific conditions used in the commercial production process, however, the enzyme reacts with the available lactose to form an oligosaccharide liberating a glucose molecule (Figure 4.1).

The consecutive trans-galactosylation reactions during the production process, with lactose or the formed oligosaccharides of different chain length as a donor, gives rise to heterogeneous mixtures of (β-linked, β) GOS with varying chain length and linkages (Figure 4.2a). A general structure of the resulting GOS is shown in Figure 4.2b: A chain of variable numbers of galactose units, with a lactose moiety at the reducing end.

4.3 COMPOSITION

The amount and type of GOS produced depends on several factors, such as the enzyme, lactose concentration and source, type of process, process conditions, and medium composition. Although, in principle, almost all glycosidic linkages can be formed during the production of GOS, β(1–4) and β(1–6) are the most abundant.[4] The trisaccharides β(1–4)-galactosyl-lactose (4′-galacto-oligosaccharide) and β(1–6)-galactosyl-lactose (6′-galacto-oligosaccharide), present in commercial products, are also found in human milk.[3,6] There are more similarities between commercially available GOS and oligosaccharides occurring in human milk. Like human milk oligosaccharides, commercial GOS contain a high amount of galactose and carry lactose at their reducing end.[7]

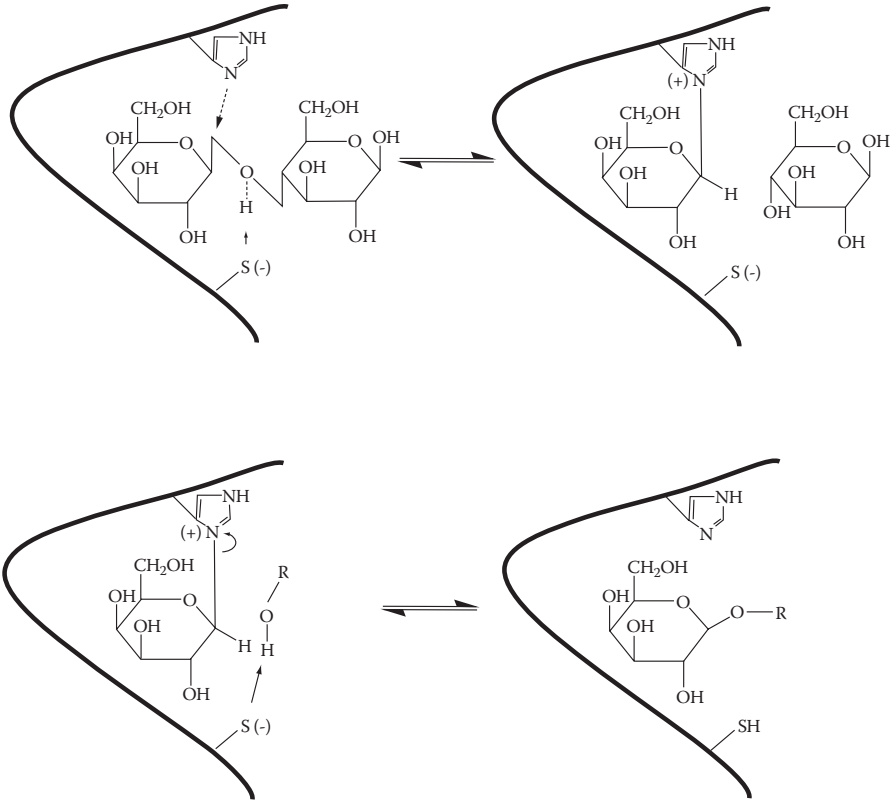

Figure 4.1 Proposed mechanism of the *trans*-galactosylation reaction by β-galactosidase.[5] OH–R represents a lactose or (galacto)-oligosaccharide of variable chain length.

Vivinal® GOS is a commercially available GOS (FrieslandCampina Domo, Zwolle, the Netherlands). The saccharides in Vivinal GOS vary in chain length from disaccharides (DP2) to octasaccharides (DP8). The type of linkage between the monomer units is mainly 1→4 Gal (55 percent in the trisaccharide fraction and 72 percent in the higher oligosaccharide fraction). 1→6 Gal linkages occur 3 to 4 percent. Other linkages including 1→2 Glc, 1→3 Glc, 1→4 Glc, 1→6 Glc, 1→2 Gal, and 1→3 Gal can also be present.[8–10]

4.4 APPLICATIONS

GOS have a safe history of use in food and infant nutrition and are applied in various kinds of products. Products containing GOS were first launched in Japan in the late 1980s. The first GOS-containing product in Europe was launched in 1997 with a Dutch fermented milk product. The use of GOS is increasing gradually in various applications worldwide. Because of their high solubility and stability (e.g.,

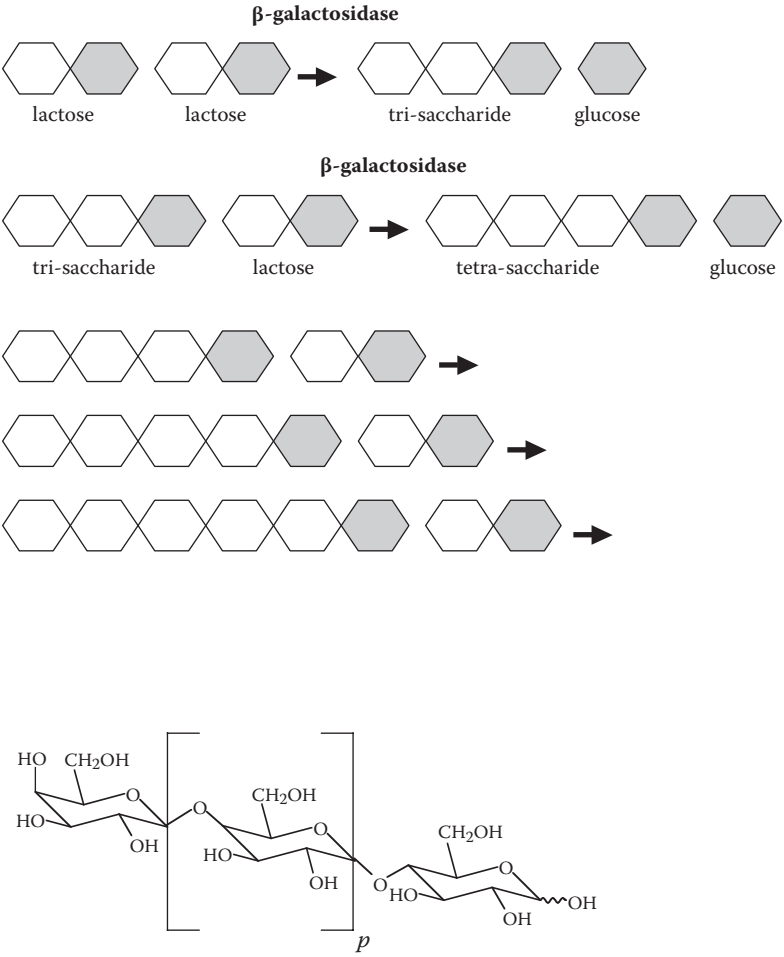

Figure 4.2 (Top) Schematic representation of the consecutive trans-galactosylation reaction that give rise to the formation of the GOS mixture. (Bottom) Structure of GOS (p = 0 to 6). (From Nauta and Schoterman, 2009. With permission.[10])

under pasteurization and sterilization conditions and in acid environments), GOS are particularly suitable for use in acid products, such as fruit juices and yogurts, and heat-treated products, such as bakery products. At present, GOS is applied in a wide range of commercial products, including dairy products, bakery products, breakfast cereals, beverages, and snack bars. More specialized applications include its use in infant nutrition, functional foods, and clinical nutrition. The dosage of GOS varies per product. Infant nutrition contains up to 0.8 g GOS/100 mL product. Current functional foods contain up to 5 g GOS per 100 g food.

The first product for a specific target group was introduced in the early 1990s, when an infant formula containing GOS was launched. GOS are increasingly

applied as ingredients for infant formula to mimic the biological functions of human milk oligosaccharides. For more than a decade, over 90 percent of infant formulas in Japan have been supplemented with nondigestible oligosaccharides (NDOs) as growth-promoting factors for bifidobacteria.[11] In Europe, mainly GOS or a combination of GOS and long-chain (lc)FOS is applied in infant formulas, follow-on formulas, and growing-up milks. According to the European Union (EU) directive 2006/141/EC on Infant Formulae and Follow-on Formulae, GOS and FOS can be added to infant nutrition in all member states of the EU in amounts up to 0.8 g/100 mL.[12] In the United States, Vivinal GOS has the self-affirmed GRAS (generally recognized as safe) status for use in food as well as term infant formulas at a maximum proposed concentration of 0.8 g/100 mL infant formula.

GOS is also increasingly incorporated in synbiotic formulations that consist of both pre- and probiotics. Many probiotics (live microorganisms that confer a health benefit on the host) are applied for their demonstrated health benefits, such as antipathogen activity and immune stimulation in the gastrointestinal tract (GIT). GOS is used to advantage in enhancing the survivability, colonization, and/or functionality of the probiotics.

4.5 PHYSIOLOGICAL EFFECTS

4.5.1 Digestibility

The salivary and digestive enzymes and the acidic conditions of the stomach have virtually no effect on acid-stable GOS. This makes GOS highly resistant to digestion and absorption during transit through the stomach and small intestine.[13–15] An important structural element of GOS with respect to its stability is the β-glycosidically bound galactose.[16] As the human intestine lacks dedicated enzymes able to hydrolyze the β-glycosidic linkages (with lactose as an exception), GOS molecules are protected from digestion. As a result, these NDOs, which can also be labeled dietary fiber, reach the colon fairly intact and are completely fermented by health-promoting members of the gut microbiota (Figure 4.3).

4.5.2 Gut Health and Well-Being

Today, it is well established that the composition and activity of the microbiota significantly contribute to the health and well-being of the host.[17] After birth, the human GIT exists in symbiosis with the intestinal microbiota, composed of a large number and variety of bacteria. It supports the host by, among others, the production of essential micronutrients, the fermentation of nondigestible dietary fiber, and the removal of harmful compounds.[18–20] It also constitutes the first line of defense, by competing with opportunistic and pathogenic members of the microbiota for space, nutrients, and receptors on intestinal cells. The microbiota and their metabolic products (mainly short-chain fatty acids, SCFAs) also have an important trophic effect on the intestinal epithelium, stimulating epithelial cell proliferation and differentiation

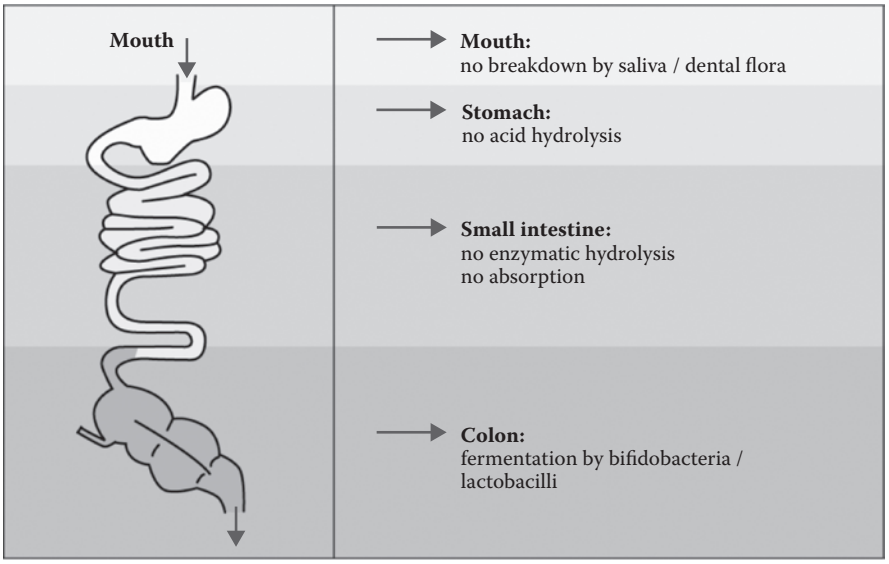

Figure 4.3 The digestibility of GOS. (From Nauta and Schoterman, 2009. With permission.[10])

of the small and large bowel. Some members of the microbiota play a role in the modulation of mucosal and systemic immune functions with an impact extending that of the intestine.

A healthy microbiota is considered one that is predominantly carbohydrate fermenting (saccharolytic) and that is comprised of significant numbers of bifidobacteria and lactobacilli. Both species have been linked to increased resistance to infections and diarrheal disease, stimulation of immune system activity, protection against colon cancer, and the synthesis of various vitamins. The products of the saccharolytic fermentation, principally SCFAs, have a positive impact on colon physiology.[21] The metabolism of peptides and proteins (putrefaction) by other anaerobes also produces SCFAs, but, in addition, generates potentially toxic substances (e.g., biogenic amines and sulfides, ammonia, phenols, thiols, and indols) that can increase the risk of colon cancer.

An important factor influencing the composition of the microbiota is nutrition, as exemplified by the differences between breastfed and standard formula-fed infants. Whereas breastfed infants have a microbiota dominated by bifidobacteria (up to 95 percent), standard formula-fed infants have a more complex (and less stable) flora, which more resembles the adult gut.[19] The latter also have higher fecal levels of potentially harmful bacterial metabolic by-products.[22] These differences are, most likely, due to the supply of human milk oligosaccharides (HMOs) present in breast milk.[19]

GOS have been shown to positively influence both the composition and activity of the microbiota. Through their effect on the microbiota, GOS also affect the activity of the immune system. In addition, GOS have various other effects that positively

influence host health and well-being as discussed below; an overview is shown in Figure 4.4.

4.5.2.1 Bifidogenic Activity

The bifidobacteria-stimulating (bifidogenic) activity and the positive impact on lactobacilli of GOS are well established. Many studies with healthy adult subjects demonstrated increased numbers of bifidobacteria and/or lactobacilli in the feces upon the consumption of GOS.[24–29] The bifidogenic activity has also been clearly demonstrated with GOS-supplemented and GOS/1cFOS-supplemented (containing 90 percent GOS and 10 percent 1cFOS) formulas in term and preterm infants.[30–36] In many of the published studies in infants, a GOS/lcFOS mixture, containing 90 percent GOS and 10 percent lcFOS, was used.

For term infants, various clinical trials with GOS-supplemented infant formula have been published. In general, the supplementation was shown to elicit a dose-related bifidogenic response and increase in bifidobacterial predominance.[30–32,35] The microbial diversity and composition of the microbiota of GOS/lcFOS-supplemented infants was shown to closely resemble that of breastfed infants, also at the level of the different *Bifidobacterium* species.[30-32,35-38] In contrast, standard formula groups harbor a more adultlike microbiota. At the end of a 6-week study, it was found that the bifidobacteria and lactobacilli accounted for 80 percent of the fecal microbiota in breastfed and GOS/lcFOS-supplemented groups while the percentage was only 50 percent in the standard formula group. The supplementation also gives rise to such stool characteristics as pH, SCFA profiles, and consistency that more resemble those of breastfed infants.[36,39] Administration of infant formulas containing GOS/lcFOS to preterm infants gave similar results with increased fecal bifidobacteria and softer stool consistency.[7] In addition, the number of pathogens in the fecal samples of

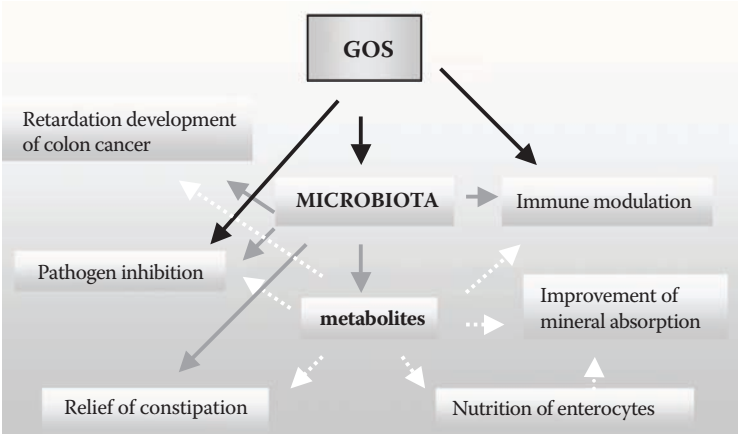

Figure 4.4 Schematic showing the various beneficial effects of GOS. (Adapted from Ouwehand, A.C. et al, 2005.[23])

infants fed supplemented formula was lower as compared to the standard formula.[40] Supplemental GOS/lcFOS also has the ability to alter fecal microbiota in the weaning period.[41]

An alternative approach to influence the colonization of the neonatal gut by GOS/lcFOS has also been tested.[42] As the maternal microbiota plays an important role in the first colonization at birth, pregnant woman were supplemented with GOS/lcFOS. Although the proportions of bifidobacteria were significantly increased in the maternal gut, no direct effect on the bacterial transfer between mother and child was observed. Probably, this was due to a masked effect of the HMOs as the infants were breastfed. An alternative approach could be the targeting of the vaginal microflora prior to delivery.[43]

A random controlled trial (RCT) of GOS and the prevention of antibiotic-associated diarrhea (AAD) showed that, in children who had received amoxicillin for bronchitis (resulting in significantly reduced fecal bifidobacteria concentrations and increased numbers of *Escherichia coli*), the administration of GOS positively influenced bifidobacteria concentrations.[44] GOS has been shown to have a synergistic effect on the bifidogenic activity of probiotics.[45] The increase in the amount of bifidobacteria in school-aged children was significantly greater after the ingestion of GOS combined with *Lactobacillus rhamnosus* GG (LGG) as compared to the ingestion of LGG alone.

4.5.2.2 Inhibition of Pathogens

Some members of the microbiota are considered potentially harmful (pathogenic) in view of their involvement in toxin production or activation of carcinogenesis, feeding intolerance, inflammatory responses, mucosal invasion, and infections. As GOS are able to selectively manipulate the intestinal microbiota in the lumen and at the mucosal surface, they indirectly result in the displacement of less-desirable members of the microbiota.[46, 47] In addition, the metabolism of GOS by the specific members of the microbiota results in the production of antagonistic agents (e.g., diacetyl, hydrogen peroxide), antimicrobial peptides,[48,49] and SCFAs. The last reduce the luminal pH in the colon to levels below those at which the pathogens can effectively multiply.

GOS have also been shown to have a more direct inhibitory effect on pathogens as they competitively prevent bacterial adherence. GOS resemble the receptor sites coating the intestinal epithelial cells to which pathogens adhere for initiation of the infection process.[50] As a result, they can act as "molecular receptor decoys" or "anti-adhesives" that competitively inhibit bacterial adherence by mimicking the host cell receptors.[50–52] GOS were shown to impair the adherence of an enteropathogenic *E. coli* (EPEC) strain on HEp-2 and Caco-2 cells by 65 and 70 percent, respectively, in a dose-dependent manner.[52] In addition, the average number of bacteria per microcolony was significantly reduced (over 70 percent) when GOS were present. GOS were also shown to strongly inhibit the attachment of another EPEC and *Salmonella typhimurium* to HT29 adenocarcinoma cells *in vitro*.[51]

4.5.2.3 Gastrointestinal Diseases

The pathogenesis of GIT diseases (e.g., inflammatory bowel disease, IBD) is associated with an imbalance in the intestinal microbiota. Both genetic predisposition and alterations in the mucosal microbial communities and overexposure to luminal bacterial products are thought to be involved in the development of these conditions. IBD is a chronic inflammatory condition of the GIT that manifests as ulcerative colitis (UC) or Crohn's disease (CD). CD, affecting the small intestine, has been linked tentatively to mycobacterial inhabitants. UC, concentrated in the large intestine, has been associated with sulfate-reducing bacteria and their metabolic product sulfite causing destruction of colon cells. In addition, changes in gut microbiota include a relative deficiency of bifidobacteria. Manipulation of the microbiota seems to represent a way to prevent and treat GIT diseases. Numerous studies with various prebiotics, in general, show a benefit in reducing IBD activity and increasing bifidobacteria and lactobacilli and concentrations of butyrate in the gut.[53] The administration also resulted in immune modulation as the proportion of dendritic cells (DC) expressing Toll-like receptor 2 (TLR2) and TLR4 was shown to be increased.[54] The ability of GOS to selectively increase bifidobacteria and lactobacilli should, in principle, allow correction of the observed microbial imbalances.[55]

4.5.2.4 Retardation of the Development of Colon Cancer

GOS can retard several fermentation-related processes that are associated with the development of colon cancer. GOS give rise to a reduction in the activity of several genotoxic bacterial enzymes (such as β-glucuronidase, β-glucosidase, arylsulfatase, azoreductase, nitrate reductase) involved in the formation of toxic and carcinogenic compounds.[56] In human microflora-associated rats, administration of GOS lowered cecal pH and reduced the activities of β-glucuronidase and nitrate reductase.[56] The formation of secondary bile acids is also positively correlated with an increased colon cancer risk. Because of the reduction in the colonic pH upon GOS fermentation, the formation of these compounds is inhibited.[57] In a study with healthy subjects, a decrease in the concentration of other harmful compounds, ammonia, *p*-cresol, and indoles in the feces was observed after the consumption of GOS.[24,27] GOS also suppress the production of phenols in the intestinal tract and the accumulation of phenols in the serum.[58] The latter is important for patients with renal failure as the accumulation of phenols in their serum has toxic effects.

GOS has been shown to be protective against the development of induced colorectal tumors in rats.[59] Rats were fed diets with either a low or a high dose of GOS and a low, medium, or high amount of fat. A high dosage of GOS resulted in a significant reduction in the multiplicity of adenomas, carcinomas, and the total number of tumors. The incidence and size of the tumors were also reduced, irrespective of the amount of fat in the diet of the rats.

4.5.2.5 Stimulation of the Absorption of Minerals

The human diet should contain sufficient amounts of minerals (e.g., calcium, magnesium, phosphorus, iron) as they play an important role in physiological processes. The dietary intake or bioavailability of the minerals, however, is not always sufficient to meet the requirements, especially in certain target groups. Several studies have shown that GOS can be used to stimulate the absorption of various minerals.[60–62] Most studied is the absorption of calcium, as it is required as a structural component of bones, and also plays an important role in blood coagulation and muscle contraction. The effect of GOS (Vivinal GOS) on calcium absorption was demonstrated in postmenopausal woman in a double-blind randomized cross-over study.[63] The consumption of a GOS-supplemented yogurt drink increased the absorption of calcium. This effect was not accompanied by an elevated urinary calcium excretion, indicating that GOS also increases the uptake of calcium by the bones and/or inhibits bone resorption. This was shown in a study with rats that were given a diet containing 5 percent (w/V) GOS for a period of 30 days. In addition to the increased absorption of calcium, GOS were shown to result in higher bone ash weight and calcium content in femur and tibia, indicative of the prevention of bone mineral loss.[29] Other studies in rats showed similar results on calcium absorption and bone calcium content.[60,61] The bioavailability of magnesium and phosphorus is also positively influenced by GOS as was demonstrated in magnesium-deficient rats.[64]

Several mechanisms for the stimulation of mineral absorption by GOS fermentation have been proposed.[65] The SCFAs produced result in a reduction in pH, which can lead to an increase in the solubility and, thus, the absorption of minerals.[60,62] The SCFAs lactate and butyrate also promote the proliferation of enterocytes. The resulting enlargement of the absorption surface could also positively influence mineral absorption.[65]

4.5.2.6 Alleviation of Constipation

A problem frequently encountered among pregnant women and elderly individuals is constipation. Several human studies have demonstrated that the consumption of GOS can alleviate constipation in persons who are constipated or who have a predisposition to this condition. Healthy adults with a tendency for constipation were shown to benefit from the consumption of GOS.[66] Their defecation significantly improved as manifested by an increased stool frequency and softer feces. Other studies demonstrated similar beneficial effects in elderly subjects suffering from constipation.[67,68] A study with infant formula supplemented with the probiotic *B. longum* BL999 and a GOS/lcFOS mixture showed that children receiving this synbiotic treatment had less constipation as compared to the control.[69]

A number of mechanisms are thought to be involved in the improvement of bowel movement by GOS consumption. The stimulation of bacterial growth could result in an increase in bacterial biomass and fecal weight.[70] The SCFAs that are sub-

sequently produced could stimulate intestinal peristalsis and increase fecal moisture with osmotic pressure.[71]

4.5.3 Immune Modulation

The intestinal epithelial cells, as part of the gut-associated lymphoid tissue (GALT), play a crucial role in signaling and mediating innate immune responses. Epithelial cells also produce essential signals for the induction of memory pathways of the adaptive immune system. The adaptive immune system exists of B cells, producing antibodies against proteins (humoral immunity) and T cells removing antigens and viral infected cells (cellular immunity). These immune responses develop in specialized lymphoid structures, predominantly found in the ileum of the small intestine, the Peyer's patches (PP).

The GALT receives signals from the microbiota or food antigens and induces a state of nonresponsiveness, so-called mucosal tolerance.[72] When pathogenic bacteria invade the intestinal mucosa, however, it should elicit strong humoral and cellular immune responses. The composition and/or the activity of the microbiota influence the maturation and modulation of the immune system activity.[73] This is clearly illustrated in germ-free animals that are shown to have an immature and poorly developed immune system.[74] The absence of a normal microbiota can also result in an increased antigen transport across the gut mucosa.[75] The communication between GALT and the microbiota is based on rapid recognition of specific bacterial products through pattern recognition receptors (PRR) like the membrane-associated TLRs. These receptors are essential for discriminating potential pathogens from the beneficial members of the gut microbiota and, thus, for immune homeostasis both in the gut and systemically.[72]

4.5.3.1 Immune Activity

HMOs have been shown to influence the immune system not only through the intestinal flora, but also by direct interaction with immune cells.[76] With its effect on the microbiota, GOS indirectly influence mucosal and systemic immune activity.[77] In addition, the increased production of SCFAs by GOS fermentation contributes to the maintenance of a noninflammatory environment in the intestine as several of these SCFAs have been shown to modulate immune responses.[78–81] Butyrate has also been shown to inhibit NF-κβ activation in intestinal epithelial cells under proinflammatory conditions[78,79] and has also been shown to inhibit T-cell activation.[80] Similar findings have also been reported for acetate and propionate.[81]

The GOS/lcFOS mixture also has a (partially) microbiota-independent effect on the immune response.[82] It increased the proportion of fecal bifidobacteria and lactobacilli and enhanced, vaccine-specific, delayed-type hypersensitivity (DTH) responses dose dependently. FOS/inulin induced similar effects on the gut microbiota. However, FOS/inulin scFOS/lcFOS did not enhance DTH responses, indicating that an increase in the proportions of bifidobacteria and lactobacilli is not sufficient for the observed immunomodulatory effect.

As the gut microbiota is established, the capacity of the GALT to produce immunoglobulin A (IgA)-secreting cells increases. Secretory IgA (sIgA) plays an important (immune exclusion) role in the defense of the GIT. Maternal breast milk is full of sIgA antibodies against her intestinal microflora (enteromammaric link). As a result, a major percentage of the fecal microbes are coated with IgA, thereby preventing the induction of immune reactions against commensal residents and attachment to and subsequent translocation through mucosal membranes.[83,84] The level of sIgA antibody is also associated with increased neutralization and clearance of viruses. Formula-fed infants who lack the transfer of protective maternal sIgA from breast milk can benefit from strategies to support maturation of (humoral) immunity and endogenous production of sIgA. In an intervention study, infants fed on a formula supplemented with a GOS/lcFOS mixture showed a trend toward higher fecal sIgA levels compared with the standard formula-fed infants.[85] In contrast, infants fed on a probiotic (*B. lactis* BB12) formula showed a highly variable fecal sIgA concentration with no statistically significant differences compared with the standard formula group. A recent double-blind, randomized, placebo-controlled study also demonstrated higher concentrations of fecal sIgA after consumption of GOS/lcFOS-supplemented infant formula, suggesting a positive effect on mucosal immunity.[86]

4.5.3.2 Allergy

In both eczema and food allergy, there is evidence of an inflammatory response in the GIT.[87] In addition, the permeability of the intestinal mucosa is increased in allergic disease, which can allow the systemic absorption of antigens, bypassing antigen-presenting cells and thus producing systemic hyperresponsiveness.[88] Infants with early onset allergic disease are also at risk of other allergic manifestations, a phenomenon described as "the allergic march." Atopic dermatitis (AD) is usually the first manifestation of allergy during early infancy. AD is associated with delayed maturation of Th1 immune responses during early infancy with raised total IgE and IgE to dietary antigens in the serum.

A promising approach in high-risk infants seems to be prevention of allergic diseases by dietary supplementation of pre- and/or probiotics. This has been shown to enhance mucosal barrier function, participate in degradation of protein antigens, promote early immune system maturation toward nonallergy, and alleviate symptoms of eczema.[87,89–91] Breastfeeding has been reported to lower the incidence of atopy-related disorders,[92–94] an effect that was also shown for a GOS/lcFOS mixture (Figure 4.5).[95] In a murine type I allergy model, the allergic reaction following sensitization with ovalbumin was attenuated in animals fed with dietary GOS/lcFOS.[96] The supplemented infant formulation reduced the cumulative incidence of AD in high-risk infants by altering immune development. The supplementation was shown to induce beneficial total serum antibody profiles (reduced IgE levels), specifically modulating the immune response toward food allergens, while leaving vaccination responses intact.[97–99] It was shown that total IgE, IgG_1, IgG_2, IgG_3, but not IgG_4, levels decrease after 6 months of treatment. Dietary supplementation of a combina-

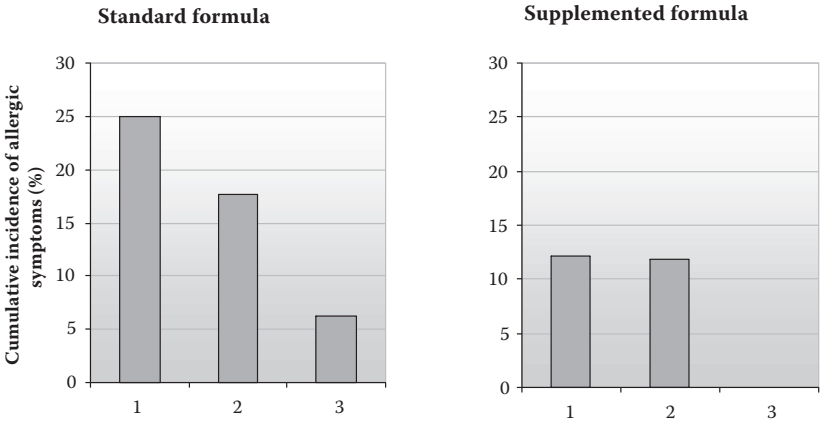

Figure 4.5 (a) Incidence of allergic symptoms in infants fed a standard formula; (b) GOS/lcFOS-supplemented formula. 1, atopic dermatitis; 2, bronchial symptoms; 3, acute allergic cutaneous reactions. (Adapted from Moro et al., 2006.[95])

tion of four probiotic strains and GOS on allergic diseases in allergy-prone infants significantly reduced eczema and IgE-associated eczema.[100]

Effects of GOS/lcFOS on allergic asthma have also been reported.[101] Experimentally induced asthmatic mice were fed GOS/lcFOS. The supplement was shown to inhibit airway hyperresponsiveness and the number of inflammatory cells in bronchoalveolar lavage. Allergen-specific IgE levels were decreased. The authors hypothesized that GOS/lcFOS treatment increases Th1 over Th2 type responses. The use of GOS/lcFOS in dietary products might provide an opportunity to stimulate the adaptive immune response in a Th1 direction and subsequently inhibit infections and Th2-related immune disorders in humans, for instance, allergies.

4.5.3.3 Infections

The mixture GOS/lcFOS not only has a protective effect against allergic manifestations, but also against infections. It was shown to reduce the incidence of infectious episodes during the first 6 months of life.[102] Infants who received prebiotic-supplemented (GOS/lcFOS) hypoallergenic formula had fewer episodes of physician-diagnosed overall and upper respiratory tract infections. Blind follow-up continued until two years of age and showed that the observed protective effect lasted beyond the intervention period. A study with infant formula supplemented with the probiotic *B. longum* BL999 and GOS/lcFOS also showed that children receiving this synbiotic treatment had a nonsignificant tendency toward fewer airway infections as compared to the control.[69]

4.6 CONCLUSIONS

For GOS, it has been convincingly demonstrated that they beneficially affect the gut microbiota toward a more healthy composition and activity. As a result, GOS is increasingly applied to support health and well-being and protect specific target groups at risk for certain diseases. GOS have also been shown to induce supportive mucosal and systemic immunomodulatory effects. Whether these are mediated through microbiota-related and/or (partially) microbiota-independent routes has to be revealed. Insight in the underlying mechanisms will enable us to exploit the prebiotic characteristics of these oligosaccharides to their full extend.

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CHAPTER 5

Functional Disaccharides
Lactulose, Lactitol, and Lactose

Andrew Szilagyi

CONTENTS

5.1 INTRODUCTION

This chapter discusses three disaccharides: lactulose, lactitol, and lactose. The first two are derived from the parent compound lactose and are shown in Figure 5.1. Each may be perceived as having prebiotic properties; however, the definition has been evolving and at this time it is more restricted. *In vivo* effects must be proven and physiological and microbiological effects should reach more distal parts of the colon.[1,2] As a result only lactulose is currently accepted as a probable prebiotic.[2] It is nevertheless recognized that different disaccharide compounds may, under *in vitro*

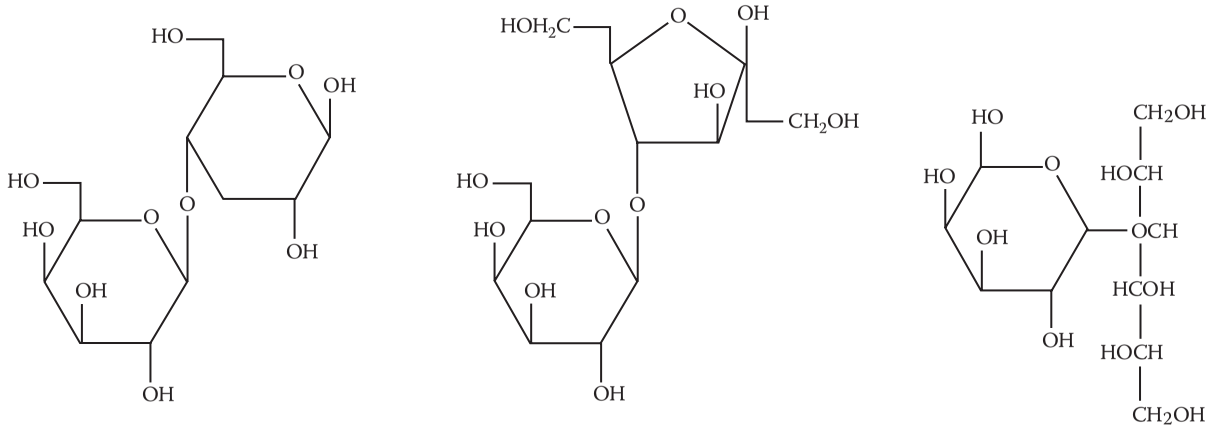

Figure 5.1 Structural similarities and differences among lactose, lactulose, and lactitol are shown in a two-dimensional conformation. Actual molecules have chair configurations as depicted by X-ray crystallography. The basic galactose molecule is bound to glucose, fructose, or sorbitol in lactose, lactulose, and lactitol, respectively. The drawings are based on References 5, 7, and 8.

controlled conditions, exert microfloral changes and result in various amounts of short chain fatty acid (SCFA) production in comparison with controls.[3] These criteria have been translated into a Prebiotic Index (PI).

The PI is a description of the relative efficiency of particular molecule(s) to increase absolute numbers of bifidobacteria and lactobacilli while subtracting any expansive effects on bacteroides and clostridia. Increases in the lactic acid bacteria are desirable, whereas increases in the other two may not be. The magnitude of bacterial change depends on the numbers present in the host colon prior to introduction of the compound.[2] In this schema, lactulose compared with control has a PI of 4.66 (control 1.23) and lactose 5.75 (control 1.02).[3] By comparison, the bimolecular fructo-oligosaccharide is listed to have a PI of 7.64. Lactitol or any of the sugar alcohols are not listed. Whether the length of time a particular prebiotic is ingested alters effects is unclear. For example, are there quantitative or even qualitative differences between ingestion of a particular prebiotic for 1, 6, 12 months, or half a lifetime?

In addition, short chain fatty acids produced from disaccharides may have independent antiinflammatory effects. It has been known for some time that butyrate is the preferred nutrient of colonocytes and exerts antiinflammatory and possible antineoplastic effects. Recently, similar attributes of cytokine inhibition and antiinflammatory effects for acetate and propionate were reported in an *in vitro* model.[4]

As discussed, for each disaccharide, a number of well-defined human health benefits are attributed to them. Therefore, with the exception of lactulose, which is recognized as a prebiotic, perhaps a new definition needs to be developed for lactitol and lactose, or the concept of prebiotic needs to be again reassessed. All three certainly should be considered functional food components.

5.2 LACTULOSE

The disaccharide lactulose was first manufactured from lactose in 1930,[6] and although not a natural product, it forms in small amounts by noncatalytic isomerization when milk is heat-treated. The product 4-*O*-β-d-galactopyranosyl-d-fructose has the identical empirical formula as for lactose ($C_{12}H_{22}O_{11}$). Industrial production of the sugar requires alkali hydroxides and boric acid.[7] However, biological manufacture of lactulose is also possible through transgalactosylation from lactose using β-galactosidase from *Aspergillus oryzae* and from *Pyrococcus furiosus*.[8] The end product is available as a liquid syrup or in crystalline form.

5.2.1 Physiological and Prebiotic Effects of Lactulose

Lactulose is not digested by human intestinal enzymes and, therefore, reaches the lower bowel making it available for bacterial metabolism. A very small amount is absorbed in healthy subjects through tight junctions between cells. Approximately 0.4 to 2 percent may be absorbed in this way under normal conditions.[9] As membrane permeability changes, more disaccharide is absorbed via this route and excreted intact into urine.

In the colon, bacteria metabolize it into hydrogen, methane, and carbon dioxide, mainly lactic acid and acetic acid, lowering the pH, especially in the cecum.[9,10] *In vitro* some propionate and butyrate are also produced.[3] In the naïve subject, large amounts of undigested lactulose induce symptoms of cramps, gas, bloating, and diarrhea.[7,9] The mechanism is thought to be largely due to osmotic forces increasing small intestinal volume and transit with resultant high flow across the ileocecal valve and possibly overwhelming the reserve capacity of the colon. It has also been shown that small amounts (10 to 15 g twice a day) may induce tonic contractions of the colon leading to the known anticonstipating effect.[11,12]

This compound shares with lactose the ability to auto adapt to continued ingestion.[13,14] The definition of colonic adaptation refers to reduction of measured exhaled breath hydrogen, improvement of the outlined symptoms, and a statistically significant increase in measured fecal β-galactosidase.[14] In human volunteers, adaptation is achieved by consuming 20 g of lactulose twice daily for 8 days.

Lactulose is unusual among prebiotics because it has been in medicinal use since the 1950s, predating the labeling and definition of the food additive by Gibson and Roberfroid.[15] Although it was recognized early that many of the effects of lactulose may work through its influence on microflora, especially bifidobacteria,[7] historically it was sold and prescribed as a drug. As such, it still requires a doctor's prescription in most countries. However, by the late twentieth century, lactulose was available as a food additive in Italy, Japan, and the Netherlands.[7]

Lactulose, therefore, is the first true prebiotic recognized for its effects on the gut flora. The effects of the sugar may be divided into physiological and those related to selective promotion of specific gut bacteria. However, it is difficult to separate these two effects, with few exceptions, because as more is learned about host/bacterial communications, the more putative medicinal and bacterial effects merge.

A marked effect on bifidobacteria of this substance has been shown in numerous human trials.[7] Addition of lactulose to bottle-fed infants raised bifidobacteria levels to that found in breastfed infants.[16] Large daily dose intake of 20 to 60 g[17,18] or small amounts of 5 g twice a day for 6 weeks[19] both induce bifidobacteria in human volunteers. The magnitude of bifidogenic effect is influenced by the initial bacterial counts. The lower the initial levels, the higher the postconsumption expansion.[20] In addition to total bifidobacteria, the species *Bifidobacterium adolescentis* is specifically increased after 18 weeks of lactulose ingestion in healthy volunteers.[21]

The effects of lactulose also result in the decrease of a number of enzymes including β-glucuronidase,[17,22] an enzyme considered pathogenically relevant to colorectal carcinogenesis.[23,24] Furthermore, lactulose reduces colonic fermentation of amino acids in human volunteers.[25] Reduction of bacterial proteolysis is considered therapeutic for hepatic encephalopathy (see below) and possibly beneficial for some intestinal diseases. The same study also revealed that over a 4-week course, intestinal permeability decreased, but gastric emptying and oral cecal transit were unaffected.[25] Other postulated effects are discussed in the context of specific medical use.

5.2.2 Medical Uses of Lactulose

Actual and potential medical uses of lactulose have been described over the past 50 years. As stated, understanding of which specific function or physiological or indirect effect through bacterial action is somewhat blurred by the lack of detailed knowledge of host/microbial/microbial interactions in the intervening years. The conditions and putative mechanisms are listed in Table 5.1.

As a drug, the disaccharide was initially prescribed for constipation[7,9] and is still used for this purpose, particularly in elderly patients. The traditional explanation for the drug effect is the induction of osmotic diarrhea. In a study by Jouet et al.,[26] a 40-g single lactulose dose added to a meal increased both small intestinal and colonic motility, raising a possibility that a small dose had effects on gut transit. Subsequently, it was shown by the same group that lactulose has tonic effects on the colon.[11,12] The mechanism is not yet clear, but may involve release of peptide YY[27,28] and perhaps other gut peptides.

Table 5.1 Indications for Which Lactulose Is Firmly Established and for Which Further Research May Confirm Benefit

Established Indication	Comments	Refs.
Constipation	Osmotic effect, direct motility	7, 11, 12
Hepatic encephalopathy	Osmotic effect, ammonia trapping by pH; reduced ammonia production, altered bacterial metabolism	7, 9, 10, 29–34
Diagnostic uses	Measurement of intestinal permeability; estimate of oral cecal transit; evaluation of intestinal bacterial overgrowth; these last two are controversial to an extent	77–81
Potential Indications		
Reduced bacterial carrier states	Shown for *Shigella*; some controversy for *Salmonella* after acceptance as indication	7, 35, 36
Metabolic effects	Controversial human studies in dyslipidemias and diabetes; some human studies showing enhanced mineral absorption and one study on improvement of lactose intolerance	42–47, 49, 50, 53
Reduced bacterial translocation	Mainly based on animal data, human study for urinary tract infections and obstructive jaundice, proposed for prevention of complications of chronic liver disease	57–60
Antiendotoxin effect	Animal models and *in vitro* evidence for reduced TNF-α production	37–41
Anticolorectal cancer	Some human studies on bile acids and reduced carcinogenic bacterial enzymes attenuation of carcinogens in animal models	64–66
Therapy in IBD	Prevention in animal model, minimal in humans	73–75

Note: Potential mechanisms are listed as shown. TNF-α, tumor necrosis factor alpha; IBD, inflammatory bowel disease.

Another early indication for lactulose therapy was the amelioration of hepatic encephalopathy (PSE).[7–10] Although the precise cause of the progressive clouding of sensorium with advancing liver functional deterioration is likely multifactorial, the role played by ammonia produced in the gut is preeminent in pathogenesis, and methods to reduce its formation are the key to successful therapy.[29] Early reports comparing nonabsorbable antibiotic with lactulose showed equivalence in low-grade PSE.[30]

The benefit of this treatment in subclinical PSE, which can only be diagnosed with psychometric tests, has now been shown.[31] Physiological studies where lactulose was incubated *in vitro* with stool from healthy volunteers revealed that it inhibits short-chain fatty acids produced from protein via a marked drop in pH,[32] reduced ammonia concentrations, and increased nitrogen excretion.[22,33] In addition, acetate and lactic acid with reduction of pH also trap nonlipid-soluble ammonia in the colon.[34]

Lactulose has also been used to reduce the rate of *Salmonella* carriage in chronic carriers and apparently this was also an early indication recognized in some countries.[7] Similarly, the carrier rate of *Shigella* was reported to be reduced.[35] However, a rat model of the effects of lactulose on infection showed that while colonization was reduced with *Salmonella,* translocation into the host was increased.[36] Currently, this indication for lactulose is not used in North America.

Other clinical situations exist in which lactulose may potentially help. Oral lactulose was given to patients in a nonrandomized controlled study in the pericholecystectomy operative period and was found to reduce postoperative sepsis in patients with obstructive jaundice.[37] Both a reduction in circulating endotoxin and tumor necrosis factor-α (TNF-α), a key cytokine induced by endotoxin, has been shown with lactulose in animal models.[38,39] These effects could be also attributed to the bifidogenic impact of lactulose. In at least one model, contamination with galactose, limiting hepatotoxicity of galactosamine, may have been more relevant.[40] However, another *in vitro* study found that lactulose directly inhibits TNF-α.[41] The hypothesis raised was that, in patients with biliary obstruction, intestinal permeability is increased, which allows the disaccharide to be absorbed in larger amounts, leading to TNF-α inhibition, which then attenuates endotoxin effects.

Lactulose is also postulated to affect several metabolic processes. First, it was reported in a human study that after a week of treatment of dyslipidemic patients, there was a 17 percent decrease in serum cholesterol lasting for at least 4 weeks after discontinuation.[42] In a small-animal model, reduction of serum cholesterol and the lithogenic index (a marker for gallstone formation risk) was found to be more effective with a combination of lactulose and lignin, than with the latter substance alone.[43] More recent studies on lipids conflict with these earlier reports. De Preter et al.[25] did not find that long-term lactulose changed serum lipids in healthy volunteers. In another human study, Vogt et al.[44] also failed to show an effect of 4 weeks of lactulose on serum cholesterol in healthy men. They did find a 10 percent decrease of serum triglycerides in this partial randomized cross-over trial.[44] Opposite effects of lactulose were observed by Jenkins et al.,[45] again in healthy volunteers. After 2 weeks consumption of 18 to 25 g of lactulose, fasting total and low density lipoprotein associated serum cholesterol were higher by 9 percent.[45] The authors felt that rapid fermentation of lactulose raised acetate levels contributing to lipid metabolism.

Whether or not lactulose has a different impact on patients with dyslipidemia compared to healthy subjects has not been addressed.

Lactulose may have hypoglycemic effects in individuals with diabetes.[7] A plausible explanation was provided in an animal model showing that the disaccharide reduced glucose absorption in an isolated jejunal loop by 40 percent.[46] In a small study of 10 obese subjects, a biscuit prepared with fiber and lactulose blunted glucose and insulin response to regular meals.[47] However, because commercial lactulose syrup contains small amounts of absorbable sugar impurities, these can adversely affect glycemic control. In fact, this is a known cautionary warning for use. Although usually well tolerated, there is at least one report of a severe disruption of glycemic control in a diabetic cirrhotic patient on lactulose.[48]

Mineral absorption, particularly calcium and magnesium, have been shown to be enhanced by ingestion of lactulose. In a recent double-blind, randomized trial confined to healthy men, a dose effect of lactulose was found on the absorption of both minerals using a stable isotope method.[49] An earlier study on postmenopausal women also found a dose–response absorption of calcium.[50] The same enhancing absorptive effect on calcium was shown using a dog model.[51] Mechanisms by which lactulose and other prebiotics or probiotics and combinations of the two may enhance mineral absorption are reviewed by Scholz-Ahreins et al.[52]

As discussed above, long-term ingestion of lactulose can lead to amelioration of symptoms and reduction of breath hydrogen measurements.[14] Adaptation to lactose is discussed more in Section 5.4. However, it was reported that 10 g twice a day of lactulose over 3 weeks led to improvement of response to lactose challenge as shown by a reduction in breath hydrogen and symptoms with increased fecal β-galactosidase.[53] Interestingly, in a single subject, adaptation to lactose with dairy foods did not result in adaptation to a lactulose challenge.[54]

The ability of lactulose to alter bacterial translocations has led to research in other areas of prevention of infections as described above for obstructive jaundice. The likely mechanism of reduced transfer of bacteria to mesenteric lymph nodes across intestinal epithelium is through a bacterial effect on intestinal permeability.[55] However, conflicting information exists on the subject. It was already alluded to above that lactulose increased *Salmonella* translocation in a rat model.[36] Demirogullari et al.[56] reported that in 3-day starved rats, lactulose and lactitol both enhanced coliform translocations from the cecum. Alternatively, De Preter et al.[25] found decreased intestinal permeability. In support of reduced permeability, some earlier publications reported prophylactic effects against urinary and respiratory tract infections in elderly patients using lactulose.[7,57] Although no further trials were found for this indication of lactulose, the concept remains of interest.[58]

The other important area of research remains that of cirrhosis, where many of the complications are attributed to such bacterial translocations.[59] In this context, Zhang et al.[60] showed in the carbon tetrachloride rat model of cirrhosis, lactulose prevented bacterial translocation into mesenteric lymph nodes and small bowel overgrowth compared with placebo. The postulated mechanism is enhanced intestinal transit and improved permeability.[60] The subject of bacterial translocations is not settled and the type of disease may determine outcome of studies.

Modification of complex pathogenic diseases like colorectal cancer and inflammatory bowel disease (IBD) represents an interesting area of research. These two diseases are classic examples where host and environmental factors both participate in disease formation. The particular role of environment in these cases leads to some alterations in the colonic milieu.

In the case of cancer, bacterial or dietary effects on host signaling pathways leads to genetic alterations in the colonic epithelium and carcinogenesis.[61,62] In addition, secondary bile salt formation through bacterial 7-α-hydroxylase has been postulated to contribute.[63] Lactulose affects bacterial enzymes and participates in inhibiting conversion of bile acids. Van Berge Henegouwen et al.[64] found that a high dose, 60 g per day for 12 weeks, in patients with adenomas decreased the secondary bile acid deoxycholate. This result was attributed to lowering of fecal pH and increased transit in the colon.[64] The specific pathogenic role of bile acids in carcinogenesis is still unsettled. However, a rat model of cancer using dimethyl hydrazine equally induced colon tumors in rats given lactulose or placebo.[65] On the other hand, *B. longum* in combination with lactulose did prevent aberrant crypt foci (early marker of polyp formation) in rats given azoxymethane, a colonic carcinogen.[66] Further research is of great interest in this area.

The other complex diseases of IBD, Crohn's disease, and ulcerative colitis also fit well into the host–microbial interaction model. In these, the current pathogenic paradigm is thought to be related to a genetically dysregulated, inappropriate inflammatory response to commensal bacterial flora that penetrate the host via a leaky gut.[67] However, there are disturbances in the microflora as well.[68] Whether these precede or are concomitant with disease is not yet clear. With either possibility, intestinal membrane alterations are certainly involved and this may be more pronounced with Crohn's disease.[69] There is also evidence that both IBD forms are associated with deficiencies of either lactobacilli or bifidobacteria.[70,71]

Because lactulose is associated with a bifidogenic effect, as well as possibly an antiendotoxin effect, either directly or indirectly as described above, Liao et al.[72] postulated that lactulose may be of benefit in IBD. Indeed, in the interleukin-10 (IL-10) knockout model of enterocolitis, Madsen et al.[73] found that either rectally administered lactobacilli or oral lactulose did attenuate colitis. Based on the concept of colonic adaptation, a study was carried out to test whether lactulose could lead to adaptation in patients with both forms of IBD compared with healthy controls.[74] While controls adapted to lactulose challenge after a 3-week, 10-g twice a day dosage, patients with IBD did not. In fact, patients with Crohn's disease fared worse than patients with ulcerative colitis. Perhaps because of a leaky intestinal membrane, lactulose may not have adequately reached the colon, failing to exert prebiotic effects. Whether a longer interval would improve results was recently evaluated in another clinical study by Hafer et al.[75] Patients with either Crohn's disease or ulcerative colitis were given 10 g daily lactulose along with standard therapy for 4 months. While there were no significant clinical changes, patients with ulcerative colitis reported an improvement in quality of life, but patients with Crohn's did not. Since it was shown in a pilot study that a 3-week, daily ingestion of fructo-oligosaccharide did significantly increase bifidobacteria counts and improved clinical state,[76] the observations

of the failure of lactulose suggest that short-circuiting of the colon through a leaky gut (especially in Crohn's disease) is a plausible explanation. As such, disaccharide prebiotics like lactulose may be less useful in IBD. Further studies in IBD are warranted to clarify these issues.

Other important indications for the use of lactulose take advantage of the fact that under normal conditions most of the sugar spills into the colon and can be used to detect bacterial metabolism. As such, lactulose, which is universally malabsorbed in most conditions, may be used to assess small bowel transit time[77,78] and bacterial overgrowth.[79] However, these two techniques are becoming more controversial. Because of the very small amount absorbed and excreted into the urine, lactulose is also used to assess intestinal permeability by comparing the ratio excreted to an amount excreted of another sugar, either mannitol[80] or rhamnose.[81] These tests are not discussed here.

5.2.3 Safety Issues with Lactulose

Lactulose is considered generally safe as attested to by its long clinical use.[7] There are few serious problems. These are outlined in Table 5.2. One of the more important warnings is to suspend use if more than two loose bowel movements occur because rarely hypernatremia can develop.[82,83] A more recent important safety issue has been raised in patients taking anticoagulant medications. Lactulose may enhance the effects of these drugs by reducing bacterial populations that produce vitamin K.[84] Several potential problems may rarely emerge in patients using lactulose with diabetes and dyslipidemia as outlined above. However, the clinical relevance of these latter potential problems needs further study. Lactulose may aggravate gastro-esophageal reflux through its possible effects on upper gastrointestinal motility as outlined above[11,12,27,28] and in a recent study published in abstract form only.[85] Finally, there have been a few cases of pneumatosis intestinalis (air in the bowel wall) associated with use of lactulose.[86]

Table 5.2 Safety Issues with Lactulose

Side Effect	Comments	Refs.
Overdose	More than two loose bowel movements; may provoke hypernatremia	82, 83
Enhanced anticoagulation	Reduced microbial populations producing vitamin K	84
Possible Problems		
Aggravation of dyslipidemia	Rapid metabolism, acetate induces lipid synthesis	45
Aggravation of diabetes	Variation of other sugar contaminants; may affect glycemic control	48
Aggravation of gastrointestinal reflux	Release of peptide YY slows gastric emptying	28, 85
Pneumatosis intestinalis	Tracking of air in the intestinal wall through retained gases with insufficient bacteria to metabolize hydrogen	86

In summary, lactulose is one of the first prebiotics produced, predating the nutritional definition. As a result, it was and is used predominantly as medication. Its original indications for constipation and hepatic encephalopathy remain the major uses of this disaccharide. However, as this review shows there are many potential indications for which lactulose could be used. Further studies will need to be carried out before such other indications are accepted.

5.3 LACTITOL

Lactitol is also derived from lactose through hydrogenation of the parent compound.[87] This sugar alcohol is designated as 4-β-d-galactopyranosyl-d-glucitol or 1:4 B-galactosido-sorbitol.[88] It appears to be about 35 percent as sweet as sucrose, contains 2 to 3 kcals/g of compound and has better taste qualities than lactulose.[88] It is also neither hydrolyzed nor absorbed in the intestine, but spills into the large bowel where it is metabolized by bacteria.[88–90]

5.3.1 Physiological and Bacteriological Effects

The reaction to lactitol in humans is similar to reaction to lactulose, and the diarrheic effects are also putatively related to osmotic influences as in the case of lactulose. A study of young Japanese women calculated that a dose above 0.36 g/kg of a single ingested amount of lactitol would induce diarrhea. This amount was half that tolerated with a single ingestion of lactose.[91] Lactitol in the colon leads to the induction and release of the motility- and appetite-regulating peptide YY. However, the effect appears to be less in humans than in rats.[92] No data exist whether motility in the colon is as affected as for lactulose.[12,92]

The effects on microflora also resemble the effects of lactulose described in Section 5.2.[17] Ballongue et al.[17] reported a comparative double-blind, placebo-controlled trial in human volunteers of 20 g/day lactitol against lactulose. These authors found similar outcome with both, but the effects of the latter were more distinct and were of faster onset. In this case, both bifidobacteria and lactobacilli were found to be increased and bacteroides and clostridia species were decreased.[17] Fecal pH was reduced equally by both lactulose and lactitol. Short-chain fatty acids resulted in increased acetic acid, but only about half were found with lactulose. The proteolytic short-chain fatty acid valeric acid was decreased somewhat more by lactulose than by lactitol. In addition, a number of enzymes, azoreductase, 7α-dehydroxylase, β-glucuronidase, nitroreductase, and urease, were significantly reduced compared with placebo, but again lactulose was more efficient.

Conflicting *in vitro* studies found that some monosaccharides and disaccharides did increase carbohydrate-derived short-chain fatty acids.[3,93] In fact, in another *in vitro* fermentation system, it was found that both bacteroides and bifidobacteria were reduced but butyrate was increased by lactitol.[94] Similarly, it was shown in a rat model that lactitol in combination with polydextrose raised butyrate levels and induced secretion of mucosal IgA better than individual compounds.[95] In

a randomized clinical trial, combinations of sucrose and lactitol were evaluated at different doses for effect on fecal flora and short-chain fatty acids.[96] While total bacteria remained constant, at the highest intake of lactitol a significant increase in bifidobacteria was observed. In contrast to the larger dose mentioned above, at this dose both propionic and butyric acid were significantly increased without gastrointestinal symptoms. Production of butyrate is desirable as it is the preferred nutrient of colonocytes and may have antineoplastic effects.[97–99] These reports are conflicting and more consistent results are needed regarding lactitol.

5.3.2 Medical and Theoretical Uses of Lactitol

Indications and possible indications for the use of lactitol are listed in Table 5.3. Because lactitol is less sweet than lactulose, it is perceived to be better tolerated by patients. Effects on the motility of the bowel have not been as detailed as with lactulose. A comparison of lactulose with lactitol showed that lactulose significantly affected colonic (especially right side) motility compared with placebo. However, while lactitol did increase motility as well, it was not statistically significant compared with placebo.[100] Nevertheless, in a small clinical study in children with chronic constipation, both disaccharides worked equally well, resulting in statistically significant increases in the number of bowel movements.[101] Interestingly, diarrhea induced by lactitol in high doses may be reduced by addition of more fiber, like guar gum.[102]

The other important area of medicinal use for this sugar alcohol is for hepatic encephalopathy. As noted above, lactulose and lactitol were found to have equivalent physiological effects,[88] and lactitol was found to easily replace lactulose for clinical effect in a small but longitudinal study.[103] Patients preferred the better taste and more

Table 5.3 Uses of Lactitol Together with Possible Benefits

Indications	Comments	Refs.
Laxation	May increase motility, few trials better tolerance	100, 101
Hepatic encephalopathy	Comparative trials show equivalence, but questions regarding efficacy after 40 years of regular use	103–106
Possible indications		
Metabolic effects	Animal studies support but human study fails to show increased calcium absorption	106–108
	Attenuates elevation of triglycerides in a single human study	109
Inhibits bacterial translocation?	Single human study showing decreased endotoxin in chronic viral hepatitis	110
Possible antiparasite therapy	Animal and *in vitro* study showing interference with *Trypanosoma cruzi* cell cycle	112
Antidental caries effect	Some early evidence in laboratory animals, replaced clinically by xylitol	113–115

predictable effects. A recent trial of lactitol compared its effects with a new intraluminal acting antibiotic rifaximin in hepatic encephalopathy. The prospective 5- to 10-day trial showed equal efficacy with about 80 percent of patients improving. Although this trial supported the use of rifaximin over lactitol because of a greater rate of improvement with the former, it also supports the efficacy of lactitol in this condition.[104] Indeed, an earlier meta-analysis comparing lactitol, lactulose, or lactose in lactase-deficient cirrhotic patients confirmed equality of the two disaccharides.[105] However, in a Cochrane meta-analysis of 30 randomized controlled trials of lactulose or lactitol compared with placebo, no intervention or addition of antibiotics, it was concluded that not enough high-quality studies are available to prove whether disaccharides are better than placebo in hepatic encephalopathy. Antibiotics may be a better alternative.[106] However, disaccharides are still considered standard treatment in hepatic encephalopathy, but further trials will need to be carried out to prove the concept.

Other uses of lactitol follow the pattern outlined for lactulose. Metabolic effects particularly for mineral absorption like calcium have been published. Ammann[107] reported increased calcium absorption from the colon in rats gavaged with 2.5 g/kg of lactitol over a week. Another study using a rat model reported that 2 weeks of feeding lactitol resulted in enhanced magnesium absorption.[108] However, a month-long prospective human study failed to find any effect of 20 to 40 g/day of lactitol on calcium metabolism.[109]

A single cross-over study was reported in which a combination of polydextrose and lactitol substituted for sucrose and lactose in both an animal and clinical setting reduced elevation of triglycerides after consumption of chocolate.[110] The authors reasoned that less fat was absorbed due to physiological effects of the substitute sugars.

There is a clinical report of a prospective trial on the ingestion of lactitol of 15 to 45 g/day compared with standard diet for 3 weeks in patients with proven hepatitis B (most) or C and documented elevated endotoxin in the serum.[111] A significant increase in lactobacilli and bifidobacteria and a decrease in *Clostridium perfringens* were observed in treated patients. These microfloral changes were also associated with reduced endotoxin levels. This observation needs confirmation.

An unusual function for lactitol has been found as a possible therapeutic agent for the achalasia-like infectious disease caused by *Trypanosoma cruzi.* Acquired through a bug bite, it causes Chagas disease, which affects mainly the esophagus and heart. It is found largely in South America, but also on occasion in the southern United States. Recent research discovered that part of the organism's protective mechanism, against complement lysis, involves a lactose-binding site that attaches to parasite sialic acid in mucin.[112] Substitution by lactitol at the lactose/β-galactose accepting site inhibits sialic transfer and allows lysis to take place.[112,113] However, this feature is likely not relevant to the sugar's prebiotic potential because the disease is acquired by a hematogenous route and not gastrointestinal. Nevertheless, the leaky gut permeability that affects transcellular absorption of lactulose[9] could also affect the absorption of lactitol as a whole molecule and may prove to have benefit.

The ability of lactitol to prevent dental caries was demonstrated in rats,[114,115] some 20 years ago. However, from a practical point, xylitol has shown superior effects

and has replaced research trials in this area. The putative mechanism is related to increased salivary flow, but effects on oral microbes may also be involved.[116]

5.3.3 Safety Issues

In general, lactitol is considered safe. With the exception of a case of possible pneumatosis intestinalis related to lactitol use, no specific bad effects have been reported.[117] In rats there has been some evidence of testicular Leydig cell tumor genesis; however, it is doubtful that this risk is applicable to humans.[90,118] Whether or not caution expressed for lactulose use with anticoagulation medication[84] may be a problem remains to be seen, but it is reasonable to limit use in such situations.

In summary, lactitol derived from lactulose is a sugar alcohol with properties that are similar to lactulose with better tolerability. However, it has not been as extensively studied as other prebiotics. Although not evaluated for a PI, this sugar alcohol has been reported to produce short-chain fatty acids and may be exploited in clinical studies for antiinflammatory[119,120] and possible antineoplastic effects.[121]

5.4 LACTOSE

Lactose is composed of galactose and glucose as 4-*O*-β-d-galactopyranosyl-d-glucose. This disaccharide is unique among possible prebiotics in that it is naturally derived from mammalian sources. Because it is an integral component of milk, which is a complex food, specific effects of the disaccharide in nature are difficult to separate from other possible effects due to other components of milk.[122,123] From the point of view of the food industry, lactose is used as an additive both in foods and medications and as a parent compound for other possible prebiotics. These include the other two disaccharides discussed above as well as transglycosidation products.[124–127]

The second important attribute of lactose that makes it somewhat difficult to use clinically is that its digestion is genetically determined. Ability or inability to split lactose into its monosaccharide components in adulthood[128] divides the entire human race into digesters (lactase persistent [LP], a dominant genetic trait)[129] and maldigesters (lactase nonpersistent [LNP], a recessive genetic trait).[130]

This divide has intrigued scientists and anthropologists since the discovery of its genetic cause. There are three, not necessarily mutually exclusive, hypotheses given. The most readily accepted is the one by Simoons who postulated that ancient herding practices led to spotty geographic retention of intestinal lactase.[131] Another hypothesis of Anderson and Vullo is that LNP status in the world followed ancestral malaria-infested regions and the reduced consumption of dairy foods protected against this parasite by reducing riboflavin intake.[132]

The phenotypic divide also follows a distinct geographic global distribution. The predominant LP populations largely inhabit areas away from the equator while LNP populations live closer to the equator. There are some notable exceptions like the aboriginal populations of North America and lactose-tolerant Africans. The observation prompted Flatz and Rotthauwe to the third hypothesis

that northern LP populations retained the ability to digest lactose so they could eat more calcium-containing dairy foods and thereby compensate for less exposure to sunshine.[133]

Lactase in humans is found to be uninducable by long-term lactose ingestion.[134] The gene for lactase phlorizin hydrolase (LPH) is found on chromosome 2(2q21).[135] Perhaps it is uninducable because the mutation affecting phenotype is found in the promoter region some 19 to 22×10^3 base pairs away from the LPH locus. In European and ancestral populations from Europe, the predominant cytosine to thymidine substitution at C/T-13910 controls LPH at the transcription or to a lesser extent translational level.[136] In certain African and in some northern Chinese populations, the polymorphism controlling dominant absorption is found to be different from that in Europeans[137,138] and the original T-13910 haplotype may still be in an evolutionary flux.[139] In the recessive C/C genotype, intestinal LPH is downregulated in a spotty fashion starting at variable ages in different populations.[140,141] Lactose digesters are made up of homozygous normals and heterozygous mutants, which reduce intestinal lactase but still allow normal digestion under usual conditions. Lactose maldigestion can also be precipitated by diseases involving the proximal small bowel (like celiac disease) and colon[142] as well as by the aging process.[143] There is also the possibility that bacterial overgrowth can lead to lactose maldigestion,[144] and in elderly individuals the usual diagnostic tests may be unreliable.[143] Congenital lactase deficiency rarely occurs in infancy.

Nevertheless, the practical implications of this global phenotypic dichotomy is that, first, LNP populations consume lower quantities of dairy foods (the main source of lactose)[145] and, second, if LNP populations do consume lactose-containing foodstuffs, they either will get symptoms or they adapt and lactose exerts an effect on colonic flora.[146] It should be realized that some lactose (up to 8 percent) can spill into the lower intestine even in LP subjects and this is available for bacterial consumption.[147] It is therefore a very relevant question whether this unequal handling of lactose by LP and LNP populations, first, has any impact on human health. Second, any human study on dairy food effects where lactose may be implicated in causality may need to consider LP/LNP status.

Another possibility that may deserve exploration is whether other genetic traits are aligned preferentially with one or the other phenotype. Indeed, a north/south geographic association between LNP status and bitter taste was reported recently in Italians.[148] As a result of the scope of this chapter, these other nondairy food possible associations are not further discussed, but they are areas of future research.

5.4.1 Physiological and Bacteriological Effects

Because of the differential effects of lactose on LP and LNP populations, many clinical attributes may apply primarily to LNP status. Ill effects of lactose would more likely affect LP subjects. In LNP persons, introduction of lactose above the threshold for absorption in the small intestine could lead to symptoms of gas, bloating, cramps, and in more severe cases to diarrhea and even vomiting.[149] Precipitation of symptoms is caused by the same osmotic principles as for all other malabsorbed

carbohydrates and is modified by quantity, intrinsic orocecal transit time, which delivers a certain amount across the ileocecal valve per unit time,[150] and by drugs that prolong orocecal transit.[151] Symptoms of lactose maldigesters may also be exaggerated.[152] In Chinese participants (all LNP), intolerance or tolerance to lactose was evaluated and found not to be related to fecal microbes or β-galactosidase,[153] and was unrelated to alteration of oral cecal transit time.[154] As such, the mechanisms of intolerance deserve further research.

It has now been established that the threshold dose for lactose digestion for a single intake is between 6 and 10 g.[91,152,155–157] Above this threshold, lactose in LNP subjects spills into the colon and bacterial metabolism becomes dominant. It was found in a double-blind study comparing 3 day diet recall with response to a lactose challenge that the pretest average daily lactose intake correlated in a dose–response fashion with measured hydrogen response. A daily intake of greater than 20 g resulted in a sum of breath hydrogen that was significantly less than in subjects who consumed 1 to 10 g/day.[158] Between 11 and 19 g, breath hydrogen sum was less than the previous group, suggesting a dose effect. Regular lactose ingestion of 15 to 20 g/day may then be required to induce adaptation.

The adaptation to lactose has been observed in multiple epidemiological and clinical studies.[159–163] However, the formal description was clinically defined in a prospective study by Hertzler and Savaiano,[164] where under test conditions LNP participants were shown to virtually change to LP phenotype.[164] In the original description, the area under the curve for breath hydrogen was significantly reduced, symptoms of intolerance improved, and fecal β-galactosidase increased about threefold from baseline. Symptoms, especially gas and bloat, but also global effects, usually correlate with the magnitude of the hydrogen response.[151,158,165]

There has been some debate about whether improved symptoms found under laboratory conditions after adaptation are due to a placebo rather than a true effect.[166] Indeed, functional (no clear disease-related symptoms) explanations for symptoms of lactose intolerance are evident and severity may be overstated.[152] However, a placebo effect alone cannot explain all observations. If symptomatic improvement were uniquely a placebo effect, it should be observed with other tested carbohydrates. This is not the case with oligofructose and fructose itself.[167,168] In addition, symptoms of lactose intolerance after pregnancy increase, corresponding to exacerbation or unmasking of lactose maldigestion.[162]

The effects of lactose on fecal microflora are also unclear. Following lactose consumption, *in vitro* human fecal evaluation showed diminished hydrogen production.[169] An increase in fecal β-galactosidase was shown, and this is interpreted as either a population or metabolic expansion by bacteria. Because there is less hydrogen produced with adaptation, the suspicion of affected bacteria falls on lactic acid producers, although some 80 percent of colonic bacteria have been found to possess β-galactosidase.[170] The mechanism of adaptation is still not well defined. In a mouse model, it was demonstrated that a lactose catabolizing strain of *Lactococcus lactis* was able to digest orally fed lactose.[171] This may not be the case in clinical studies, where mere expansion of fecal microflora with lactic acid (and yogurt) producing bacteria do not necessarily lead to improved lactose digestion.[172,173] In addition,

initial prebiotic effects may be accompanied by increased bacterial β-galactosidase without a corresponding population expansion.[174]

Nevertheless, in an *in vitro* fecal fermentation system, both lactobacilli and bifidobacteria demonstrated increased lactose consumption.[175,176] In a model of the human colon, which can be used to measure segmental effects, a large increase in both bifidobacteria and short-chain fatty acids, propionate, and butyrate were observed. Although the predominant effect was in the cecal compartment, more distal compartments were also affected.[177] On the contrary, there is only a single existing *in vivo* study of microbial effects of lactose in LNP subjects.[178] This 6-day study used 15 g/day in Japanese subjects and reported a variety of bacteriological changes, including increased lactobacilli and a proportional increase in bifidobacteria.

5.4.2 Potential Medical Uses of Lactose

The first potential use is outlined above, namely, the autoinduction of colonic adaptation, which can improve symptoms of lactose intolerance (Table 5.4). Although not completely eliminated, continuous intake at reasonable doses diminishes symptoms markedly reducing the need for digestive aids.

The other area is the use of lactose for hepatic encephalopathy. After the introduction of lactulose for treatment of this condition, case reports appeared that suggested that, in LNP patients, lactose at 100 g/day could reverse clinical and electroencephalographic features of hepatic encephalopathy.[179] Two small controlled trials were conducted in Mexico where the population is predominantly LNP. In the

Table 5.4 Potential Uses of Lactose as a Prebiotic

Established Effects	Comments	Refs.
Improvement in lactose tolerance	Continued ingestion improves symptoms both psychologically and physiologically	159–164
Hepatic encephalopathy	Studies more than 25 years ago showed equivalent benefits with lactulose	179–183
Mineral absorption	Animal studies support enhanced calcium absorption in both intestine and colon, but minimal-to-no human trial support	184–190
Diagnostic aid for breast cancer	Some support that lactose consumption enhances nipple fluid secretion; further studies are needed to define appropriate population	191
Possible genetic food interaction	A dose differential impact of lactose on fecal microflora, between LNP/LP subjects may modify risks for some diseases; most plausible candidate at this time is colorectal cancer	194–197, 203, 204

Note: As noted in the text, both LNP and LP subjects spill lactose into the colon. However, beyond the single 6 to 10 g dose ingestion in LNP, which can still be digested, dose for dose the effect on colonic flora may have more impact on LNP subjects. From a practical point, any effect of lactose itself is, therefore, partly dependent on geno/phenotype.[91,147,153–157]

first trial, 10 patients were studied in a cross-over pattern and significant improvement in clinical, encephalographic changes, and blood ammonia levels were noted.[180] In the other prospective trial, lactose enemas achieved similar effects.[181] The *in vitro* effects on ammonia production were also found to be similar between lactulose and lactose.[182,183] These trials were never followed up. The use of lactose enemas theoretically should also work in LP populations because it would bypass the small bowel.

There are also studies evaluating the effects of lactose on mineral (mainly calcium) absorption. A number of early small-animal studies suggested that lactose enhanced calcium uptake in the small intestine.[184–186] Magnesium may not have been affected,[187] and in infants supplemented with lactose, calcium but not zinc absorption was improved.[188] In adults, the effects of lactose on calcium absorption were evaluated in LP and LNP subjects.[189] It was reported that lactose in LP but not LNP participants enhanced calcium absorption. A later study in only LP subjects, however, failed to show any increase in bioavailability of calcium.[190]

Lactose intake was found to enhance nipple aspirate fluid, a test used to aid in diagnosing breast cancer.[191] The place this test has in such a diagnostic role and whether both LP and LNP women may benefit need further evaluation.

The phenotypic/genotypic dichotomy may play a role in the modification of diseases distributed in a geographic pattern. There have been articles in the past suggesting that dairy food consumption and/or LP/LNP status may modify certain illnesses.[192,193] It is observed that the risks of some diseases (mostly "western" afflictions) can be mathematically defined based on national per capita yearly dairy food consumption or the size of the population with LNP status.[194,195] For example, the risk for prostate cancer, colorectal cancer, and ulcerative colitis was directly increased with increasing consumption of dairy foods. That for stomach cancer was decreased. In all cases except stomach cancer (a disease more frequent in eastern geographic distribution), LNP status was protective.[194,195] In four diseases; prostate, ovarian, breast, and colorectal cancer, patient-level meta-analyses were compared with geographic results. Although in the case of prostate, ovarian, and breast cancer existing meta-analyses generally concurred with population data, in the case of colorectal cancer meta-analyses overall supported a protective effect. These results are diametrically opposed to that expected with the population data. In the patient-level analyses for colorectal cancer, however, there is a discrepancy between cohort and case-control studies, with the latter being inconclusive.[123] The lack of agreement is generally attributed to methodological differences between the two types of studies.

However, another explanation may be relevant. If the data are evaluated by dividing the countries of origin into three regions of the world, such that studies from countries with relatively homogeneous LP (generally northwestern) and LNP (mainly Asian) populations, the inverse association between increasing intake of dairy foods and reduced risks of colorectal cancer rates is confirmed.[196] This fact is poignantly expressed in a study from China where lack of dairy food intake is associated with increased cancer rates.[197] Differences between cohort and case-control methodologies were not as widely discrepant as nondivided studies suggested. These protective effects are achieved at a marked difference in average dose intake

of dairy foods between North Americans and Western Europeans and Asians. In mixed LP/LNP populations (e.g., southern Europe and South America) all studies were case control and these showed a modest but statistically insignificant reduction in risk.[196]

One of the putative mechanisms by which dairy foods protect against colorectal cancer and its predecessor adenomatous polyps is calcium of which large doses are required for effect.[198–200] Because dietary calcium intake may be less in Asians,[201,202] it is postulated that the dairy food protective effect noted in LNP populations might relate to a prebiotic effect of lactose. As geographic split studies show, these two populations may also face different risks despite residing in the same geographic location; LNP, through a facilitated effect of lactose on microbial flora and LP through high calcium antiproliferative as well as some prebiotic effects.[147] It may be possible to arrive at a diluted outcome of protection of dairy foods against colorectal cancer when the phenotypic status is not factored in the analysis and maximum protective effects of each possible mechanism are not synchronized.[196]

It could also be argued alternatively that the cause(s) of colorectal cancer may be less prevalent in high LNP populated countries. Therefore, such populations need to take in less amounts to be protected. This may be relevant in explaining relative risk reduction.

However, similar inverse protective effects in different ethnic groups with a spectrum of LP/LNP distribution were reported from a study in Hawaii and southern California.[203] These are regions of relative homogeneous high risk for colorectal cancer.[204] Analysis of dairy food intake by ethnic groups shows that Japanese Americans in this region consume about 60 percent of the intake of white Americans.[205] Yet increasing dairy food intake in Japanese Americans (predominantly LNP) is also dose dependently protective.[203] This observation suggests, that protection may be achieved in a region of homogeneous risk for colorectal cancer with lower doses than that recorded for presumably predominantly LP white Americans. Part of the different risks for colorectal cancer in different ethnic groups[204,205] in these regions may relate to such genetic/nutrient interaction. Clearly, this hypothesis needs further exploration. However, proof of concept that regular lactose consumption has an impact on disease risks would affect how studies on diet are conducted. Moreover, the regular use of lactose-free and lactose digestive aids for lactose intolerance would need to be reassessed.

5.4.3 Safety Issues

There are many articles written about either health benefits or ill effects of milk and dairy food consumption. There are similarly discussions on the ill effects of lactose malabsorption, particularly its relationship to osteoporosis. It is, however, not within the scope of this chapter to review those effects. There are few specific disease links to lactose. One is a hypothesis that lactose increases atherosclerotic coronary heart disease, independent of dyslipidemia.[206] However, this complication would less likely affect LNP subjects for reasons outlined above.

5.5 CONCLUSION

As the parent compound of the other disaccharides outlined in this chapter, lactose has been investigated the least for prebiotic effects. However, as the review suggests there are features that may make investigation of lactose the most relevant to human conditions. It is naturally consumed in great quantities and is to our knowledge the only widespread genetically determined carbohydrate nutrient. The genetics of lactase and the imposed differential handling of the disaccharide by LP and LNP populations may cause effects beyond those currently recognized. Future studies should further explore the potential impact that lactose/lactase interactions may have on human health.

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CHAPTER 6

Natural Resistant Starches as Prebiotics and Synbiotics

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CONTENTS

6.1 INTRODUCTION

Resistant starch (RS) is defined as "the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals" (Englyst et al., 1996, 2007). Its inherent digestibility is influenced by a variety of physicochemical factors, including the type and ratio of starch polymers (for example, straight chain [amylose] versus branched chain [amylopectin]),—the precise architecture of these polymers within a starch granule, degree of amylose crystallinity, and starch source. In native starch granules, high-amylose starches (HAS) tend to be more resistant to enzymatic hydrolysis than high-amylopectin starches (Finocchiaro et al., 2009). Consequently, more of the HAS is expected to reach the large intestine where fermentation can occur. Thus, HAS and various physically modified products made

from such materials can be considered logical sources of natural RS that may serve as the basis of viable prebiotic ingredients.

Resistant starch has been classified principally on a structural basis and most (with the exception of RS4) could be considered a natural source of RS. The four classes are simply referred to as RS1, RS2, RS3, and RS4. RS1 is starch trapped in or by a food material (e.g., whole grains). RS2 is found in native or physically processed starch granules. High-amylose cornstarches (HACS) including Hi Maize® 260 are considered primarily RS2. Resistant starch 3 is formed when starch-containing foods are cooked and cooled such as in bread, cooked-and-chilled potatoes, or retrograded high-amylose corn. The resistant structure that is formed can be degraded by microbial fermentation, but is not hydrolyzed by human alimentary enzymes. Novelose® 330 starch is a retrograded RS3 generated from annealed, enzyme-treated HACS (approximately 56 percent RS3). Resistant starch 4 refers to chemically modified starches using standard starch chemical modification techniques, such as cross-linking, substitution, or a combination of the various chemistries (Finocchiaro et al., 2008).

The colon harbors significant populations of butyrate-producing bacteria, such as *Clostridium, Eubacterium,* and *Fusobacterium* (Pryde et al., 2002). Fermentation of a commercially available Hi maize RS2 was dominant in the proximal colon, but degradation of hydrothermally treated HACS was more dominant in the distal colon (Bird et al., 2007). Fecal output and large bowel digesta mass and concentrations and pools of individual and total short-chain fatty acids (SCFAs) were higher (by about two- to threefold; all $P < 0.05$) and digesta pH lower (by about 1 unit, all $P < 0.001$) in pigs fed either HACS or hydrothermally treated HACS compared to the controls.

In ruminants, SCFAs provide a high proportion of the total energy gained from the diet. In humans, the overall contribution of SCFAs toward the energy requirement is far lower, but they do play an important role in colonic health (Pryde et al., 2002). Butyrate and propionate are preferred energy sources for the colonic mucosa as these SCFAs are preferential substrates for the aerobic ATP formation of colonocytes. Thus, HACS may play a role in protection against colitis and colorectal cancer (Jacobasch et al., 1999). Acetate may support these mechanisms by activating capillary blood circulation. High-amylose cornstarch is a suitable substrate for most intestinal bacteria producing glucose and SCFAs. High-amylose cornstarch and other RSs are considered butyrogenic, as more reduced substrates tend to promote butyrate formation (Brouns et al., 2002). In a pig study, the intake of HACS increased fecal butyrate and SCFA concentrations more than did the low HACS diet whether pigs were supplemented with probiotic bacteria or not (Brown et al., 1997).

Cummings et al. (1996) reported that RS increased stool wet weight by 1.6 g/day per gram RS fed for potato, 1.7 for banana, 2.5 for wheat, and 2.7 for maize, but this was significantly less than bran nonstarch polysaccharides (NSP) at 4.9 g/g. Resistant starch 2 and RS3 are broken down in the human gut, probably in the colon, although in 26 percent of cases this breakdown was impaired (Cummings et al., 1996). Resistant starch decreased NSP breakdown and RS2 tended to prolong transit time. All forms of RS increased fecal total SCFA excretion.

6.2 RS AS PREBIOTICS

A prebiotic is "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," whereas synergistic combinations of pro- and prebiotics are called synbiotics (Gibson and Roberfroid, 2008). Nondigestible oligosaccharides (particularly inulin, its hydrolysis product oligofructose, (*trans*) galacto-oligosaccharides, and resistant starch) meet the criteria for prebiotic classification. These fibers have shown a positive impact on the intestinal microflora. Other indirect health effects of prebiotics, mediated by the intestinal microflora, may include prevention of diarrhea or obstipation, modulation of the metabolism of the intestinal flora, cancer prevention, positive effects on lipid metabolism, stimulation of mineral adsorption, and immunomodulatory properties.

In the large intestine, RS is fermented by intestinal bacteria to produce SCFAs, particularly butyrate (Binder and Ramakrishna, 1998; Cummings et al., 1996; Topping et al., 2003). Some data suggest that the colonic microflora may adapt to produce more butyrate if given time and the proper substrate (Silvi et al., 1999). The fermentation of these RS led to *in vitro* SCFAs levels (acetate, propionate, butyrate) of 2,000 to 2,500 μmol/g feces dry weight with butyrate contents of 30 to 60 mol% (Schmiedl et al., 2000).

In human studies, RS2 and RS3 from HACS results in selective colonic microflora activity as well as increased fecal butyrate concentrations (Brown et al., 1997, 1998; Wang et al., 2002; Jacobasch et al. 2006; Finocchiaro et al., 2009). Thus, RS can be considered a prebiotic because it promotes health of the host through fermentation.

Jacobasch et al. (2006) demonstrated that RS3 (Novelose® 330 starch) was well fermented in the cecum and proximal colon in rats, whereas the degradation of hydrothermally treated RS3 (hydrothermally treated Novelose) took place beyond the cecum and increased continuously through the colon to favor SCFA production in the distal colon. Corresponding to the high rate of hydrothermally treated RS3 fermentation in the distal colon, the SCFA concentrations in the feces and the growth of bacteria increased significantly, resulting in a nearly twofold increase in wet content. An exchange of 10 percent starch with a butyrogenic RS3 in the diet was proved to be sufficient to provide enough substrate for bacterial fermentation in the distal colon and rectum. As SCFA concentration increased, the pH decreased in the large bowel. Consumption of RS3 lowered the pH in the cecum and proximal colon to 6.5 to 6.6 from 7.5 (control diet) and intake of hydrothermally treated RS3 lowered the pH in the distal part of the colon to 6.3.

Wang et al. (2002) demonstrated that different amylomaize starches could generate desirable variation in gut microflora in mice. In this study the effects of HACS and modified (carboxymethylated and acetylated) HACS on the composition of colonic bacteria and the production of volatile fatty acids was investigated in mice. All starches tested showed the increases in indigenous bifidobacteria in mice fed although 40 percent unmodified HACS showed the highest numbers. High-amylose cornstarch increased *Lactobacillus* numbers in the mice colon and acetylated HACS

significantly reduced the population of coliforms. High-amylose cornstarches utilizing bifidobacteria reached their highest levels and butyrate levels were markedly increased when bifidobacteria with HACS or carboxymethylated HACS were simultaneously administered in mice. It appears that the starch type influenced the populations of indigenous *Lactobacillus, Bacteroides,* and coliforms in mice. However, in an *in vitro* model with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), none of the *Lactobacillus* stains tested showed any starch-degrading activity (Wang et al., 1999a).

It has been recognized that the mouse is a good animal model for studying the dietary impact on colonic bacteria. Despite some anatomical differences in the gastrointestinal tracts, the fecal bacteria populations of the major groups of bacteria were similar between mice and humans (Tannock, 1997).

Wang et al (1999b) reported from an *in vitro* study that only a few species of *Bifidobacterium* could degrade and utilize HACS (Table 6.1 and Table 6.2). The 38 types of human colonic bacteria were tested in an *in vitro* model for their capacity to utilize soluble starch, gelatinized amylopectin maize starch, and HACS granules (Table 6.1). It was demonstrated that only *Bifidobacterium* spp. could efficiently utilize HACS and *Bacteroides* spp. could not hydrolyze HACS. *Bifidobacterium* spp., *Bacteroides* spp., *Fusobacterium* spp., and strains of other bacteria could hydrolyze the gelatinized amylopectin maize starch.

Bifidobacterium bifidum and *B. pseudolongum* had higher specific growth rates in the autoclaved medium containing high-amylose maize starch granules and hydrolyzed 70 and 40 percent of the amylose, respectively (Table 6.2).

6.3 RS AND *BIFIDOBACTERIUM*

The study of Wang et al. (1999a, 1999b) indicated that both amylopectin maize starch and HACS granules were fermented by several colonic bacteria and that *Bifidobacterium* spp. may play an important role in the utilization of starches, particularly HACS. *Bifidobacterium* and *Bacteroides* had more cell-bound starch-degrading enzymes. It was proposed that the degrading enzymes produced by the *Bifidobacterium pseudolongum* FII 509500 and *Bifidobacterium bifidum* FII 509800 may include both alpha-amylase and alpha-glucosidase as indicated by a range of molecular weights of starch-degrading enzymes. There was no detectable degradation of the amylose by *Bacteroides vulgaris* or *Eubacterium limosum*.

Resistant starch 2 diets containing HACS increased fecal/cecal levels of bifidobacteria in rats (Le Leu et al., 2005) and in mice (Brown et al., 1998; Wang et al., 2002), indicating coutilization of starch and its metabolites with other bacteria because lactobacilli could not utilize RS2 directly. Le Leu et al. (2005) reported a significant interaction between dietary RS and supplemental bacteria to a genotoxic carcinogen in the colon and fecal pH ($P < 0.01$). Rats fed the moderate-RS diet in combination with *Bifidobacterium lactis* had a significantly greater acute apoptotic response to genotoxic carcinogen (AARGC) in the colon than those fed that diet without *B. lactis*. The moderate RS diet (10 percent Hi-maize) increased SCFA levels and numbers

Table 6.1 Bacterial Hydrolysis of Soluble Starch, Granular High-Amylose Maize Starch, and Amylopectin (Mean Diameter of Clear Zone, Mm)

Bacterial Strain	Soluble Starch	High Amylose	Amylopectin
Bifidobacterium infantis	20.5	7.5	26.3
Bifidobacterium adolescentis	18	7.5	21
Bifidobacterium bifidum	32.2	22.2	33.6
Bifidobacterium longum	24.6	16.7	26.2
Bifidobacterium pseudolongum	31.33	21.7	34.5
Bifidobacterium breve	30	16	30.5
Bacteroides fragilis	19.2	0	23
Bacteroides vulgatus	20.2	0	16.3
Bacteroides thetaiotaomicron	0	0	0
Bacteroides distasonis	0	0	0
Bacteroides ovatus	18	0	22.8
Fusobacterium mortiferum	0	0	20.7
Fusobacterium gonidiaformans	18.5	0	22.7
Fusobacterium necrogenes	14.5	0	19.7
Fusobacterium necrophorum	0	0	19
Lactobacillus viridescens	0	0	0
Lactobacillus fermentum	0	0	0
Lactobacillus casei	0	0	0
Lactobacillus acidophilus	0	0	0
Lactobacillus plantarum	0	0	0
Lactobacillus rhamnosus	0	0	0
Lactobacillus brevis	0	0	0
Lactobacillus salivarius	0	0	0
Streptococcus thermophilus	0	0	0
Streptococcus salivarius	19	0	0
Propionibacterium acnes	20	0	16
Propionibacterium freudenreichii	0	0	0
Eubacterium limosum	26		0
Staphylococcus aureus	0	0	0
Lactococcus lactis	0	0	0
Peptostreptococcus anaerobius	0	0	0
Enterococcus faecalis	0	0	0
Enterococcus hirae	0	0	0
Escherichia coli	0	0	0

Note: Results are expressed as the size of the cleared zone after growth on agar plates containing the starches. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to detect bacterial starch-degrading enzymes.

Source: Adapted from Wang et al., 1999.

Table 6.2 Concentration of Total Carbohydrate Residues after Bacterial Growth for 48 h in Autoclaved Basal Medium Containing Glucose, Amylopectin, and HACS) Granules (Mean Concentration, Mg/Ml, 6 SD in Medium Containing Glucose, Amylopectin, HACS)

	Glucose	Amylopectin	HACS
Bifidobacterium bifidum	4.05	5.30	6.96
Bifidobacterium pseudolongum	4.64	5.47	7.53
Bacteroides vulgatus	7.08	10.23	9.66
Bacteroides fragilis	7.12	7.86	8.86
Eubacterium limosum	5.39	9.97	10.99

Note: Total carbohydrates were measured by using the Dubois method and are the means of two determinations from four individual experiments. The initial total carbohydrate concentration was 12 mg/mL.
Source: Adapted from Wang et al., 1999.

of bifidobacteria and lactobacilli species and lowered pH levels and numbers of total coliforms as compared with the low-RS diet (no supplemented RS). The moderate-RS diet also increased cell proliferation and crypt column height. Bifidobacteria bind to HACS granules, which increases survival at pH 6.5, pH 3.5, and under bile salt conditions (Wang et al., 1999b).

Lesmes et al. (2008) studied the possible effects of RS3 crystalline polymorphism on RS fermentability by human gut microbiota and the SCFAs production *in vitro*. Human fecal pH-controlled batch cultures showed that RS induces an ecological shift in the colonic microbiota. Polymorph B promoted the growth of bifidobacteria in the proximal part of the colon and double their relative proportion in the microbiota in the distal colon while increasing butyrate production to levels of 0.79 m*M*. Among several bifidobacteria strains, the preparations obtained from normal and waxy cornstarches were the best substrates for growth of *B. breve* KN14, even compared with glucose (Wronkowska et al., 2008).

In pigs, HACS (85 percent amylose) and hydrothermally treated HACS increased fecal and proximal colonic lactobacilli and bifidobacteria numbers by 1 and 3 log units ($P < 0.05$) (Brown et al., 1997; Bird et al., 2007). One human study reported that RS2 increased fecal bifidobacteria measurements (Brown et al., 1998).

6.4 RS AS PREBIOTIC AND SYNBIOTIC

Probiotics are defined as live microorganisms that are administered in adequate amounts to help beneficial intestinal microflora grow (Topping et al., 2003). Prebiotics are nondigestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria. Roberfroid (1998) and Nakanishi et al. (2003) have proposed the term synbiotics or symbiotics, a combination of a probiotic and a prebiotic because synbiotics or symbiotics are more potent than either a probiotic or

prebiotic alone. Specifically, a more potent inhibition of azoxymethane (AOM)-induced aberrant crypt foci (ACF) was found in rats administrated both inulin (prebiotic) and *Bifidobacterium longum* (probiotic) than in rats administered either inulin or *B. longum* separately (Rowland et al., 1998). Probiotic bacteria may use prebiotic substrates as an energy source in the colon, which facilitates the growth of the probiotic bacteria while reducing pathogenic bacteria in the large intestine.

It has been reported that HACS acted as a prebiotic and a synbiotic in promoting the fecal excretion of probiotic organisms in pigs (Brown et al., 1997). High counts of bifidobacteria were found when pigs were fed the experimental HACS diet with the bacterial supplementation (Brown et al., 1997). No bifidobacteria were detected in the absence of the supplement (at a detection limit of 4 cfu/g). The high HACS diet resulted in significantly higher counts than did the low HACS diet. The high HACS diet increased average fecal concentrations and total fecal excretion by 0.79 $\log_{10}$ cfu/g wet wt and 0.97 $\log_{10}$ cfu/day higher, respectively (Table 6.3). Several mechanisms have been proposed for HACS action on increased fecal probiotic numbers (Brown et al., 1997): (1) RS may protect the bacteria from bactericidal materials, such as bile acids, free fatty acids, and other products, by acting as a diluent in the upper gut; (2) the bacteria may have been protected in the gastrointestinal tract by adhesion to undigested starch or through entry into the pits formed in the starch granules; and (3) the HACS could serve as a substrate for the bifidobacteria, although bifidobacteria do not metabolize starches efficiently. This is supported by the lack of difference in fecal starch excretion between pigs fed RS alone and those fed RS with probiotic.

Synbiotic effects of RS (20 or 30 g HACS/100 g diet) and two strains of *B. lactis,* which facilitated the apoptotic response to a genotoxic carcinogen (AARGC) in the colon have been reported in studies using rats (Le Leu et al., 2005) and mice (Wang et al., 2002). A dosage used in this study was based on the study of Le Leu et al. (2003), which reported that higher amounts (i.e., 20 or 30 g HACS/100 g diet) do have an effect and that the moderate amount of RS did not affect the AARGC. The synbiotic combination of RS with *B. lactis* enhanced the apoptotic response by 33 percent (Le Leu et al., 2005). This change may have biological significance since only a small change (approximately 2 percent) in the proportion of apoptotic cells in the crypt column may be enough to influence colorectal tumor development (Chang

Table 6.3 Fecal Concentrations and Daily Excretion of Bifidobacteria of Pigs Fed Either A Low Amylose or High Amylose (Amylomaize) Cornstarch with Live *Bifidobacterium Longum*

Starch Type in the Diet	Fecal Concentration, $\log_{10}$ cfu/g Wet wt	Fecal Excretion, $\log_{10}$ cfu/d
Low amylase	8.12	10.76
HACS	8.91	11.73
Difference	0.79	0.97
Statistical analysis, *P* value	$P < 0.01$	$P < 0.01$

Source: Adapted from Brown et al., 1997.

et al., 1997). It appears that ingested RS acts as a metabolic substrate to create an optimal environment for *B. lactis*. Thus, RS can enhance the apoptotic response to DNA damage initiated by carcinogens in the colon of rats, which may lead to a reduction of the colorectal cancer risk.

Human fecal pH-controlled batch cultures showed that RS induces an ecological shift in the colonic microbiota by inducing *Bifidobacterium* spp. (Lesmes et al., 2008). A possible mechanism by which the *B. lactis* in combination with RS enhanced AARGC may be through the immunomodulating properties of probiotic bacteria (Perdigón et al., 2003). *Lactobacillus* also activated different immune receptors and induced a different cytokine profile (such as tumor necrosis factor-α, interferon-α, and interleukin-10) that promote immune responses in BALB/c mice (Perdigón et al., 2001; Dogi et al., 2008). *Lactobacillus casei, L. delbrueckii* ssp. *bulgaricus,* and *L.acidophilus* enhanced the immunoglobulin G_1 (IgG_1) response favoring Th2 balance, while *L. acidophilus* also increased the IgG_{2a} response inducing Th1 balance (Perdigón et al., 2001). The main immune cells activated after oral *L. casei* administration were those of the innate immune response, with an increase in the specific markers of these cells (CD-206 and TLR-2), but with no changes in the number of T cells (Galdeano and Perdigón 2006).

RS was successfully used as symbiotic in ice cream containing 1 percent RS with free and encapsulated *L. casei* (Lc-01) and *B. lactis* (Bb-12) (Homayouni et al., 2008). Crittenden et al. (2001) screened 40 probiotic *Bifidobacterium* strains using an *in vitro* screening regimen to find that *B. lactis* Lafti B94 possesses all the required characteristics to complement HACS in a synbiotic yogurt. *Bifidobacterium lactis* Laftitrade mark B94 was genetically closely related to the *B. lactis* type strain (DSM 10140), and to the commercial strains *B. lactis* Bb-12 and *B. lactis* DS 920. These strains produced the same pulse field gel electrophoresis patterns when the chromosomal DNA was cut using a restriction enzyme. However, *B. lactis* Laftitrade B94 was the only one of these isolates that could hydrolyze and utilize HACS. It survived well in an *in vitro* gastrointestinal model, grew well at temperatures up to 45°C, and grew to a high cell yield in laboratory-scale fermentations. *B. lactis* Laftitrade B94 survived without substantial loss of viability in synbiotic yogurt containing HACS during storage at 4°C for 6 weeks. Thus, the strain appeared to possess technological properties suitable for yogurt manufacture.

6.5 COLONIC CELL HEALTH

Prebiotics may exert their cancer protective effects via modulation of fermentative events, possibly by increasing SCFA production or by altering gut microbiota toward a more beneficial composition. Butyrate and, to a lesser degree, propionate are substrates for the aerobic energy metabolism (Jacobasch et al., 1999). In normal cells, butyrate induces proliferation at the crypt base, while inhibiting proliferation at the crypt surface. In neoplastic cells, butyrate inhibits DNA synthesis and arrests cell growth in the G_1 phase of the cell cycle. Butyrate is associated with induction of

differentiation, suppression of proliferation, enhanced apoptosis, and reduced DNA damage (Le Leu et al., 2005, 2007a; Finocchiaro et al., 2008).

DNA damage and apoptosis have been used as biomarkers of colonic cell health in animal models (Chang et al., 1997). DNA damage is an early step in cancer initiation. Rats fed high-RS2 diets had less DNA damage in rats fed high-protein diets (Bird et al., 2000; Toden et al., 2003, 2005, 2006, 2007a). Rats were fed diets containing approximately 15, 25, or 35 percent of cooked beef or chicken, both with or without 20 percent HACS as a source of RS, for 4 weeks. Red meat induced greater colonic mucus layer thinning than white meat, but HACS was protective in both cases. Dietary RS protects against the meat-induced damage and also against loss of the mucus barrier, probably through increased butyrate production. Dietary RS also attenuated casein, soy, or whey protein-induced colonocyte DNA damage (Toden et al., 2007b). But DNA damage remained significantly higher in rats fed 25 percent soy compared with those fed 15 percent protein, indicating that proteins differ in their effects on these indices of colon health. Inclusion of 10 percent HACS was found to be sufficient to reduce colonocyte DNA damage, and to increase SCFA pools in the colon (Toden et al., 2007c).

In a study by Fässler et al. (2007), batch fermentation of RS-enhanced antigenotoxic activity and decreased DNA damage by 9 to 30 percent. This suggests that RS may offer protection for the colon against diet-induced assaults. Using an apoptosis model, Le Leu et al. (2005) have showed that rats fed RS2 from HACS had reduced incidence of neoplasms in the colon and small intestine. HACS (20 percent in diet) prevented dietary protein-induced colonocyte genetic damage in rats, possibly through the SCFA butyrate, a bacterial fermentation product of RS (Bajka et al., 2008).

Apoptosis is a marker of the body's ability to remove damaged cells. Apoptosis appears to be a better predictor of carcinogenesis than proliferation in induced carcinogenesis models (Le Leu et al., 2002). Enhanced apoptotic ability to remove cells with DNA damage is associated with a reduced risk of colorectal cancer. Prebiotics such as RS in the form of HACS (20 to 30 percent wt:wt) and oligosaccharides (5 to 10 percent wt:wt) (Le Leu et al., 2003; Hughes et al., 2001) as well as wheat bran were shown to stimulate the acute apoptotic response to a genotoxic carcinogen (AARGC) azoxymethane in the rat colon (Hu et al., 2002; Le Leu et al., 2002). The AARGC may eliminate DNA damaged cells that might otherwise progress to malignancy. Thus, AARGC may play a role in regulating mutational load in the colon and may have a protective effect at the early stages in the onset of cancer. In a study by Jacobasch et al. (1999), the RS-fed rats showed the improvement of the 2,4,6,-trinitrobenzene sulfonic acid (TNBS)-induced colonic inflammation as compared to the RS-free group.

Supplementation with SCFAs, such as butyrate and acetate, may protect against H_2O_2 insult by postponing menadione-induced ATP (adenosine tri-phosphate) depletion and delaying onset of cell death. SCFAs decrease vulnerability against a H_2O_2 insult by stimulating DNA repair and antioxidant defense systems. Butyrate protection against DNA damage may also be related to the protection against apoptosis (Abrahamse et al., 1999). Hass et al. (1997) have demonstrated that the absence of butyrate after the isolation of the colonic epithelium-induced apoptosis and that

addition of butyrate protected against the induction of apoptosis. Butyrylated starch also protected colonocyte DNA against dietary protein-induced damage in rats (Bajka et al., 2008).

The increased SCFA production decreases the luminal pH, which lowers the activity of 7-dehydroxylase. Consequently, the transformation of primary into secondary bile acids is inhibited, and in particular transformation of cholate and chenodeoxycholate into deoxycholate and lithocholate, respectively (Jacobasch et al., 1999). Deoxycholate inhibits butyrate-mediated cell proliferation in the lower third of the colonic crypts in a rat colitis model. The lower pH and higher butyrate concentration of the cecal and colonic contents significantly suppressed the formation of secondary bile acids in RS3-fed rats based on a study with Novelose® 330 starch (Jacobasch et al., 2006). The formation of secondary bile acids was inhibited more strongly by hypothermally treated-RS3 versus the untreated RS3 control.

Resistant starch may also have a positive impact on a mucus layer (Nofrarías et al., 2007). Mucin serves as a protective layer for the mucosa, restricting the adhesion and invasion of pathogenic bacteria. Healthy rats fed high-RS2 diets had a thicker mucus layer with reduced colonic permeability (Morita et al., 2004). Incorporating RS2 into high-protein diets prevented mucosal thinning typically observed when a high protein diet is fed (Toden et al., 2006). In rats exposed to liver injury via a gut-derived endotoxin, mucin weight was higher, with improved mucosal barrier function shown by lower endotoxin translocation (Morita et al., 2004). The colonic mucosa functions as a barrier, protecting the body from harmful agents in the colon. Novelose 330 starch-containing diet also increased large-bowel surface and crypt length in the proximal colon in rats (Jacobasch et al., 2007). Colonic RS can improve colonic cell health, which therefore contributes to stronger barrier function (Toden et al., 2006; Finocchiaro et al., 2008).

Short chain fatty acids promote colonic tissue growth increasing the absorptive area, and promoting colonic blood flow. Colon length was 0.5 to 0.9 m longer (19 to 35 percent) in pigs fed the high-RS diets relative to those fed the highly digestible starch diet ($P < 0.05$; Bird et al., 2007). Large bowel surface and crypt length increased in the proximal colon in rats fed the Novelose 330 starch-containing diet (Bauer-Marinovic et al., 2006). However, Kim et al. (2003) reported no changes in colon or cecum length in rats fed RS from corn or rice source.

Long-term intake of RS from raw potato starch also improved the colonic environment, reduced damage to colonocytes, improved mucosal integrity, and reduced colonic and systemic immune reactivity as indicated by reduced numbers of intraepithelial T cells and blood leukocytes, neutrophils, and lymphocytes, mainly T-helper lymphocytes (Nofrarías et al., 2007). A rice porridge, high in RS, appears to modify the porcine large bowel microflora favorably through lowering *Escherichia coli* and coliform numbers, mediated by SCFAs production (Topping et al., 2003). High-RS2 diets also increased mineral absorption (calcium, magnesium, zinc, iron, and copper absorption) in rats as a lower pH in the colon can help improved mineral absorption (Lopez et al., 2001).

6.6 IMMUNE FUNCTION TREATMENT OF ACUTE DIARRHEA

Resistant starch stimulates the growth of various bacterial genera, in particular, facultative anaerobic organisms. RS increases the counts of bifidobacteria, lactobacilli, eubacteria, bacteroides, enterobacteria, and streptococci (Kleesen et al., 1997; Degnan et al., 1997). The enhanced counts of lactobacilli inhibit the growth of pathogenic bacteria, such as certain *E. coli* strains or sulfur/sulfate-reducing anaerobic bacteria.

Due to prebiotic and symbiotic properties of RS, RS2 ingredients made from HACS have been proposed for adjunct therapy to oral rehydration solution (ORS) for acute diarrhea (Binder and Ramakrishna 1998). Consumption of RS assists in recovery from infectious diarrhea in humans and animals (Topping et al., 2003). In three studies in India, RS2 from HACS improved water retention for children, adolescents, and adults suffering cholera-like diarrhea or acute diarrhea (Ramakrishna et al., 2000, 2008; Raghupathy et al., 2006). In a study of Ramakrishna et al., (2008), 50 adult males with severe watery cholera-like diarrhea of less than 3-day duration and moderate to severe dehydration were randomized to receive hypo-osmolar oral rehydration solution (HO-ORS) with or without high-amylose maize starch 50 g/L (substituted for glucose, HACS-ORS). Compared to HO-ORS, HACS-ORS reduced diarrhea duration by 55 percent and significantly reduced fecal weight after the first 12 hours of ORS therapy in adults with cholera-like diarrhea. This study confirmed the previous finding that the addition of an RS to ORS (50 g HACS per liter of ORS) reduces fecal fluid loss and shortens the duration of diarrhea in 48 adolescents and adults with cholera (Ramakrishna et al., 2000). In young children (6 months to 3 years) with acute diarrhea, the addition of HACS to glucose ORS (standard World Health Organization ORS) significantly shortened duration of diarrhea compared with glucose ORS treatment (Raghupathy et al., 2006). Time to first formed stool was also significantly shorter in children receiving HACS-ORS (median, 18.25 hours) compared with children receiving glucose ORS (median, 21.50 hours) ($p < 0.05$). In the HACS group, there was a tendency to have a lower mean stool weight in first 24 hours ($p = 0.0752$) as well as a lower total diarrheal stool weight ($p = 0.0926$).

In children, specific classes of fecal bacteria were lower during acute diarrhea than during a normal period, indicating alterations in the bacterial flora during diarrhea (Balamurugan et al., 2008). Altered flora of anaerobic bacteria may be due to colonization of the intestine by pathogens and to rapid intestinal transit (Balamurugan et al., 2008). In children with acute diarrhea, the numbers of *Bacteroides-Prevotella-Porphyromonas* group, *E. rectale, L. acidophilus,* and *F. prauznitzii* groups were low as compared with their levels after recovery from diarrhea. Administration of amylose maize starch as an adjuvant therapy was associated with lower levels of *F. prauznitzii* at the time of recovery, indicating that HACS had an impact toward establishment of more desirable microflora during diarrhea (Balamurugan et al., 2008). RS also have a positive impact on IgA. Morita et al. (2004) reported that rats fed high-RS2 diets containing HACS had higher intestinal and fecal IgA.

6.7 INTERACTION WITH OTHER NUTRIENTS

Resistant starch (10 percent HACS) not only protected against intestinal carcinogenesis but also ameliorated the tumor-enhancing effects of dietary resistant protein (Morita et al., 2004; Le Leu et al., 2007b). Feeding resistant protein increased protein fermentation products, but this effect was reduced by adding RS to the diet. Intestinal neoplasms and colorectal adenocarcinomas were reduced by feeding RS ($p < 0.01$).

Govers et al. (1999) reported that wheat bran can shift the fermentation of RS further distally in pigs, thereby improving the luminal conditions in the distal colonic regions. Authors concluded that the combined consumption of RS and wheat bran may contribute to the dietary modulation of colon cancer risk. Psyllium (15 g psyllium/kg diet) delayed the fermentation rate of HACS diets (50 g/kg diet) in the cecum and shift the fermentation site of HACS toward the distal colon, leading to the higher butyrate concentration in the distal colon and feces (Morita et al., 1999). Resistant starch altered the colonic luminal environment by increasing the concentration of SCFAs including butyrate and lowering production of potentially toxic protein fermentation products.

6.8 RS INTAKE IN THE UNITED STATES

Recent National Health and Nutrition Examination Survey (1999–2002 NHNAES) indicated that Americans aged 1 year and older consumed approximately 4.9 g RS per day (Murphy et al., 2008). Resistant starch intake was highest for men aged 20 to 49 years whose daily mean intake level was 5.9 g. Adult women had a mean intake of 4.3 g; children aged 1 to 5 years, 3.7 g; and older children aged 6 to 11 years, 4.2 g. Top sources of RS were breads, cooked cereals/pastas, and vegetables (other than legumes) and these foods contributed 21, 19, and 19 percent of total RS intake, respectively. In 10 different European countries, the mean daily RS intake has been estimated at 4.1 g per person (a range of 3.2 to 5.7 g; Asp et al., 1996). Per capita daily dietary fiber intake in the United States has been estimated to be in the range of 16 to 18 g (Cho, unpublished data), which is far below recommended intake levels (adult men aged 19 to 50 years, 35 g/day; adult women 25 g/day; IOM, 2002). It is imperative to increase the dietary fiber intakes of the western population to meet recommended intake levels.

6.9 CONCLUSIONS

The results of this chapter suggest that RS may be used to selectively modify gut function and that increasing butyrate availability may improve colonic health. HACS and the various commercial ingredients derived from them have been the focus of most of the studies regarding prebiotic and symbiotic effects. Given the current interest in developing new sources of commercial RS, there is great potential to increase the RS intake as well through consumption of many different types of processed foods.

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CHAPTER 7

AGE, ALE, RAGE, and Disease *A Food Perspective*

Stig Bengmark

CONTENTS

7.1 MAILLARD PRODUCTS IMPROVE PALATABILITY, BUT …

Humans have known for thousands of years that heating the food we eat to higher temperatures will improve both its taste and smell. High temperature makes food proteins change structure—coagulate, aggregate, and produce crusts—information that modern food chemists, chefs, and cooks use every day to produce new delicious foods. The French biochemist Louis-Camille Maillard explored and published in 1912 a description of the chemical processes that occur in foods during heating,[1] an achievement for which he received the distinguished prize of the French Medical

Academy in 1914. The process has ever since been referred to as the Maillard reaction and its products collectively named Maillard products. During the process, so-called reducing sugars—fructose, glucose, glyceraldehyde, lactose, arabinose, and maltose—will bind to amino acids and nucleic acids, both DNA and RNA, peptides, and proteins, and produce compounds usually called Amadori products, which with time undergo complex changes: cyclization, dehydration, oxidation, condensation, cross-linking, and polymerization to form irreversible chemical products. In particular, reactive carbonyls, such as glyoxal and methylglyoxal, have been found to rapidly modify reactive side chains of proteins. Important amino acids, such as lysine (essential amino acid) and histidine (essential for children), are often involved. During the heating process, thousands of good-tasting and good-smelling volatile compounds are released in addition to significant amounts of pigments (melanoids) that often make the food or parts of the food brown or black, which is why sometimes the process is referred to as "browning." Common browning products are bread crusts and the roasted surface of fried meat and fish. All sorts of broths, irrespective of vegetable or animal origin, Chinese soy, Balsamico products, smoked foods are rich in brown/black Maillard products. But not all Maillard products are dark in color. White Maillard products also exist; common examples are diary products, especially cheese and powdered milk. It was suggested early on that the Maillard process might be negative to health, at least when its products are consumed in larger amounts, as these products will accumulate in the body, sometimes for the rest of life, but also because the process might reduce the supply of important and essential amino acids to the body.

7.2 HEATING, REDUCTION OF ANTIOXIDANTS, AND ACCUMULATION OF MAILLARD PRODUCTS

Most of the well-known plant antioxidants are inactivated at temperatures between 30°C and 100°C. Antioxidants in common food oils such as olive and rapeseed oil will start disappearing at temperatures around 30°C. Heating to higher temperatures, as almost always occurs with microwaving, eliminates almost all antioxidants. The production of Maillard products occurs much in parallel to reduction of the content of antioxidants in foods, and accelerates dramatically, almost exponentially, as the temperatures are elevated above 100°C.

Maillard products based on association of carbonyl groups in sugars and proteins have in more recent years been collectively called advanced glycation end products (AGEs). Similar products are often formed between reactive fatty acids and proteins, referred to as advanced lipoxidation end products (ALEs). A long list of such synthetic products are identified, and two to three previously unknown such compounds are added to the list each year. Commonly studied AGEs/ALEs are pentosidine, N^{ε}-carboxymethyl)lysin (CML) and N^{ε}-(carboxyethyl)lysin (CEL).

It is important to observe that the production of both AGEs and ALEs is not at all dependent on enzymes. The intensity in production increases, not only with the increase in temperature, but also with the length of storage at elevated temperatures.

Other industrial processes commonly used by the food industry, such as irradiation, ionization, microwaving, smoking, also significantly contribute to increased production of AGEs/ALEs. No foods seem to be excluded; industrial treatment of plant products (roasting, drying, "curing") will contribute to increased amounts of AGEs/ALEs in foods to the same extent as animal products. Fresh tobacco leaves, fresh coffee beans, fresh peanuts are extremely rich in powerful antioxidants, which totally disappear during the industrial process ("curing," roasting) and are replaced by larger amounts of AGEs/ALEs. As the temperature increases above 100°C, carcinogens, especially heterocyclic amines, are also produced, a production that also increases dramatically with higher temperatures.

AGEs/ALEs do not reach the body exclusively through the food we eat; these compounds are also produced spontaneously in the body, especially with elevated levels of sugars and fatty acids in body fluids and tissues. Accumulation in the body of late Maillard products—AGEs/ALEs—is generally regarded as irreversible; what is accumulated will stay more or less forever. The observation that these substances are found in larger amount has commonly been regarded as an expression of normal aging. However, it might not be so. Instead, it might depend mainly on lifestyle and thus in theory be preventable. Large to extreme increases in content of AGEs/ALEs are regularly observed in body fluids and tissues of patients with chronic diseases, particularly in diabetes and chronic renal diseases, especially so in those suffering complications such as patients with diabetes with reduced wound healing,[2] nephropathy,[3] and angiopathy.[4,5] Advanced accumulation of AGEs/ALEs in tissues often occurs as amyloid,[6] fibrillary tangles,[6,7] or similar deposits. Such structures were long regarded as degenerative but biologically inert structures. However, increasing evidence supports the conclusion that these structures are foci with very strong proinflammatory potential, capable of maintaining chronic inflammation at high level in the tissues.

7.3 INTRODUCTION OF MOLECULAR BIOLOGY CHANGED THE VIEW OF AGEs/ALEs

Early on, Maillard had suggested that accumulation in the body of AGEs/ALEs could significantly contribute to progression in diseases, such as diabetes and some chronic urogenital diseases, especially uremia. He created what he called "index of urogenital imperfection," which he used to document an association between degree of accumulation in the body of Maillard products and severity of disease, especially chronic renal disease. However, the time was not yet ripe for such thinking and the concept was rejected by scientists and clinicians of that time and would remain so for several decades. With the introduction of modern molecular biology and particularly so with the identification of specific receptors in the body for these substances, human medicine became more seriously interested. Although identification by American Ann Marie Schmidt in 1992 of a specific receptor for AGEs/ALEs (RAGE) seems to be the turning point,[8-11] it is only in the last few years that a wider interest in the concept has developed. Since the year 2000, several international

scientific organizations have demonstrated a significantly increased interest in the concept, and new societies have even been founded with the main goal to investigate the effects on health and well-being of AGEs/ALEs in foods. The New York Academy of Science appears to have taken the lead and a large number of scientific contributions about AGEs/ALEs are published each year in its annals. In excess of 5000 titles about AGE and ALE are registered on PubMed, in addition to another 14,000 titles about the glycated hemoglobin, HbA_{1c}.

Several methods are available for measurement of content of AGEs/ALEs in body fluids and tissues: immunohistochemistry with polyclonal or monoclonal antibodies, high-performance liquid chromatography (HPLC), and mass spectrography. A large proportion, but not all, of these substances are autofluorescing,[12,13] even if not visible to the human eye. Often studied substances such as CML and CEL have no fluorescing ability or any color. Despite that, measuring fluorescence is an excellent method especially for screening of individuals with suspected high levels of AGEs/ALEs in the body, but also for screening of foods suspected to be rich in these dysfunctioning proteins. The fluorescence has its maximum at wavelengths between 350 and 440 nm.[12]

7.4 RAGE: A RECEPTOR AND MASTER SWITCH—A KEY ACTOR IN INFLAMMATION

RAGE is a prominent member of what has been called the immunoglobulin superfamily of cell surface molecules. It is described as a "master switch" with the ability to coordinate the inflammatory reaction in the body. RAGE induces a long-lasting activation of the proinflammatory transcription factor NF-κβ and suppresses a series of endogenous autoregulatory functions.[14–17] Increased deposition of AGEs/ALEs in tissues is suggested as a key element in the development of metabolic syndrome.[18,19] AGE/ALE accumulation and subsequent activation of RAGE are reported to induce a significant downregulation of leptin in adipose cells.[20] Pronounced effects of RAGE activation are often observed on endothelial cells, where increased expression of a long row of molecules, such as VCAM-1, ICAM-1, E-selectin, eNOS, TGF-β, TNF-α, IL-6, PAI-1, and VEGF, are induced.[21] Strong RAGE-induced effects are often reported on immune cells, macrophages,[22] and dendritic cells,[23,24] as well as on smooth muscle, particularly in the walls of blood vessels, under the mucosa and in the skin,[25] and associated with subsequent reduction in regenerative capacity and function of the cells, increased blood pressure, and with development of chronic diseases or exacerbation of complications to chronic diseases.[26]

AGEs/ALEs accumulated in endothelial cells can be significantly reduced by control of intake of foods rich in these substances. The situation is different in tissues with low regenerative capacity and long life length, such as myelin- and collagen-rich structures, where the substances risk staying forever: brain, peripheral nerves, skeleton muscles, tendons, joints, skin, and eye, especially the lens. More recent research has demonstrated the existence of an endogenous soluble form of RAGE called sRAGE, which acts as a decoy for RAGE and prevents accumulation of RAGE

in body tissues,[27] and studies suggest that chronic diseases are associated not only with increased levels of RAGE in the body, but also, and probably as important, with low levels of sRAGE.

7.5 MANY PLAYERS IN THE INFLAMMATION ORCHESTRA

The largest part of the immune system, in contrast to what was earlier believed, is to be found in the gastrointestinal system (Figure 7.1), which explains why the food we eat has such a dominating influence on our well-being and health.[28] Apart from AGEs/ALEs, many other food-related factors influence the level of inflammation in the body and thus our health and well-being. Some evidence suggests that these factors are additive and that they collectively contribute to the sustained, long-lasting, but often discrete and unrecognized, exaggerated level of inflammation in the body, which is common to most chronic diseases. Among these factors are the following:

- *Low level of vitamin D in the body.* A strong correlation among the level of vitamin D in the body, the degree of inflammation, and the incidence of chronic diseases has been observed. Individuals living at higher latitudes, northern Scandinavia, Russia, and Canada, are reported to have generally lower levels of vitamin D in serum, especially during the winter season, which is associated with the observed higher incidence of coronary-vascular diseases in these regions and is suggested to contribute to the higher incidence of acute coronary events during the winter months in these countries.[29,30]
- *Low levels in the body of antioxidants, such as folic acid and glutathione, and increased levels of homocysteine.* Figure 7.2 illustrates the central role of folic acid and glutathione in prevention of accumulation of homocysteine in the body,[31] a substance regularly associated with increased levels of systemic inflammation and chronic diseases.

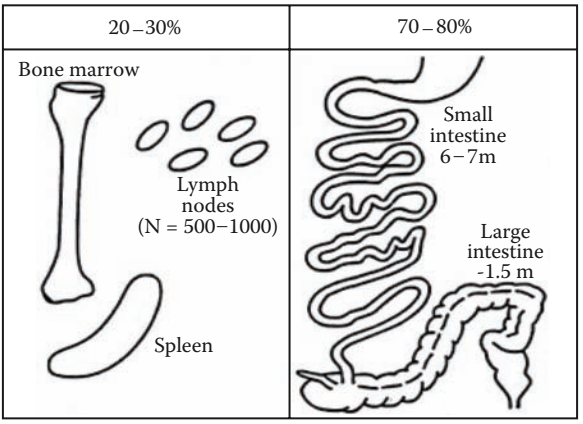

Figure 7.1 Distribution of the immune system within the body. (Adapted from Brandtzaeg P. et al., 1989.[28])

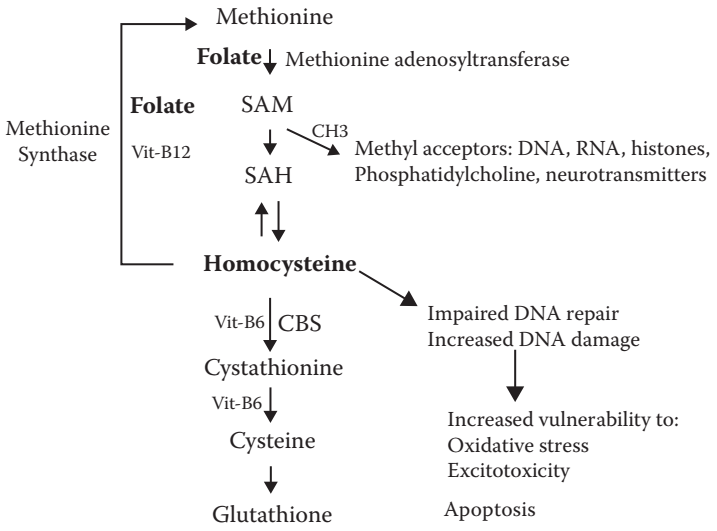

Figure 7.2 Mechanism through which homocysteine contributes to increased risks of chronic diseases with emphasis on the role of folic acid, vitamin B_6, and vitamin B_{12}. (Adapted from Mattsson, 2003.[31])

- *Impaired hormonal homeostasis.* Aging, as well as chronic diseases, is often accompanied by hormonal disturbances, and aging was recently referred to as a state of "hormonal chaos."[32] Hormonal disturbances accompanied by increased oxidative stress/increased release of free radicals, intracellular accumulation of "waste products," inhibition of apoptosis, disturbed repair mechanisms, reduced gene polymorphism, premature shortening of telomeres, reduced immune defense, and reduced resistance to disease are often observed in premature aging as well as in several chronic diseases.[32] 17β-Estradiol has been shown to induce a strong activation of RAGE mRNA in endothelial cells, an effect that is abolished by supply of an antiestrogen such as 4-OH tamoxiphen.[33,34] An impaired hormonal homeostasis is suggested to explain why chronic diseases are often aggravated during pregnancy, especially vascular and eye complications to diabetes.[34] Physical as well as mental stress contributes to activation of RAGE, and increased release of noradrenaline is reported to reduce immune defense and increase the sensibility to acquire infections with up to 4 logs.[35] Increased release of noradrenaline in the intestine will dramatically reduce the beneficial intestinal flora and increase the virulence of potentially pathogenic microorganisms, changes that most likely contribute to increased RAGE activation.[36,37] Permanently increased levels of noradrenaline are also observed in a chronic disease such as Alzheimer's disease and reported to correlate with the severity of disease.[38] Parathyroid hormones constitute another example of hormones deeply involved in the inflammatory process, and significant elevations in IL-6s is observed in hyperparathyroidism (up to 16 times), but also in other conditions with a high level of systemic inflammation, such as obesity.[29]

- *Angiotensin/rennin.* It is well documented that release of angiotensin is significantly associated with oxidative stress, increased levels of free fatty acids in serum, and with reduction in beta cell function in diabetes.[39–41] Recent studies demonstrate that blockage of the angiotensin receptor will reduce production and accumulation of AGE both *in vitro* and *in vivo.*[41]
- *Larger intake of glutenoids.* Glutenoids are increasingly regarded as proinflammatory in the body (Tlaskalová-Hogenová H, personal communication), even in the absence of intestinal changes.[42,43]
- *Low intake of plant antioxidants*
- *High intake of carbohydrates*
- *High intake of saturated and trans-fatty acids.* A strong association has repeatedly been documented between the average content of fat in food and the morbidity and/or mortality in chronic diseases in a country, as demonstrated for breast cancer in Figure 7.3,[44] but also reported for various other cancers and chronic diseases such as coronary heart disease[45,46] and diabetes.[47] As more than three-fourths of the consumed saturated fat is of bovine origin, similar curves are also reported that correlate amount of intake of dairy products to incidence of various chronic diseases.[48]

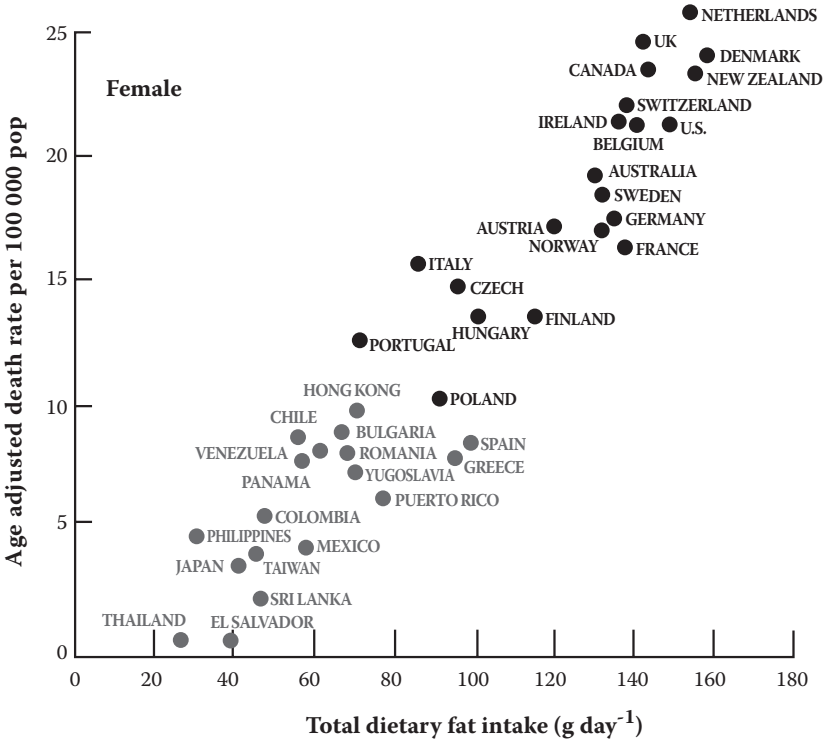

Figure 7.3 Mortality in breast cancer in a country related to the mean intake of saturated fat in the same country. (Adapted from Carroll, 1975.[44])

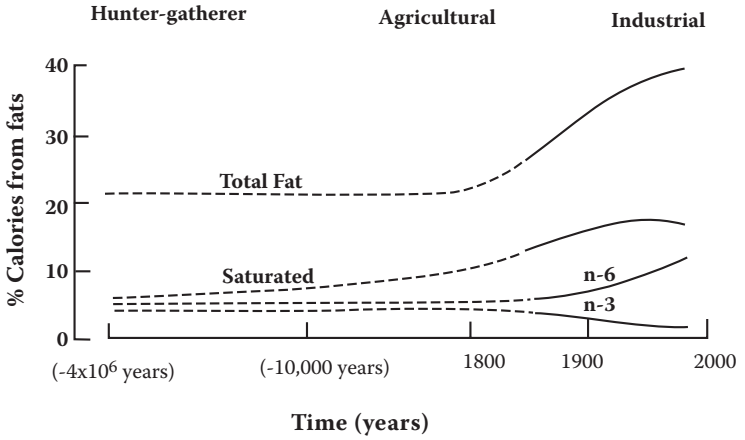

Figure 7.4 Changes in intake of fat in the Western world during the 40,000 years of human existence on Earth. (Adapted from Leaf and Weber, 1988.[49])

7.6 DRAMATIC ALTERATIONS IN FOOD HABITS

The incidence of most chronic diseases has dramatically increased during the last 150 years, much in parallel with a significantly altered intake of foods. The annual per person intake of saturated fat has doubled, the intake of omega-3 fatty acids has decreased by about 50 percent, and intake of omega-6 fatty acids more than doubled since the year 1850 (Figure 7.4).[49] During the same time period, the intake of refined sugar has increased from 0.5 kg to almost 50 kg per person per year. To this shall be added a recent and fast increase in intake of high-fructose corn syrup, mainly used in carbonated drinks and fast foods, an intake which today in the United States exceeds that of sucrose.[50] Much can be learned from studies in Japan, a country that has gone through identical changes in food habits in no more than 50 years and, during this time period, has seen a manifold increase in the incidence of several chronic diseases. The incidence of prostatic cancer, for example, has increased 25 times during this 50-year period, much in parallel with an increase in intake of industrially produced agricultural foods: egg 7 times, meat 9 times, and dairy products 20 times.[48]

The annual per cow production of milk has in the Western world during the last 150 years increased up to 50 times. In addition, modern milk is today heated to high temperature before it is delivered to the consumer. Although consumption of drinking milk has decreased significantly during the last 50 years in Western countries (United States: from 144 L in 1950 to 92 L per person per year in 2000), the consumption of cheese has instead quadrupled (from 4 kg in 1950 to 15 kg in the United States and 19 kg in the European Union per person and year in 2000), to a large extent due to extensive use of cheese products in fast foods: pizza, tacos, nachos, salads, fast-food sandwiches, and sauces for potatoes and vegetables. But it is in intake of powdered milk that the largest increases has occurred; powdered milk

is today used in most industrially produced foods as reconstituted milk, in bread and bakery products, chocolate, ice cream, and hundreds of other common foods, but also in baby formulas and clinical nutrition formulas.

Commonly, 10 to 20 percent, but sometimes up to 70 percent, of the amino acid lysine is reported to be modified during common industrial treatment of milk (sterilization, pasteurization, irradiation, etc.). Fructoselysine is the dominating modified molecule, but CML and pyrraline are also usually produced during processing of milk. The sugar content, level and time of elevated temperature, and storage time are the main factors behind increased production of AGEs/ALEs in milk products. Figure 7.5 demonstrates the influence of various industrial treatments on the content of the AGE furosine in various milk products including powdered milk.[51]

7.7 ANIMAL FEEDS HAVE CHANGED IN PARALLEL WITH HUMAN FOOD CHANGES

Not only human food but also animal feeds have undergone dramatic alterations during the twentieth century, from mainly forage-based feeds containing more starch-rich and fast-absorbed carbohydrates: corn, maize grains, barley, molasses,

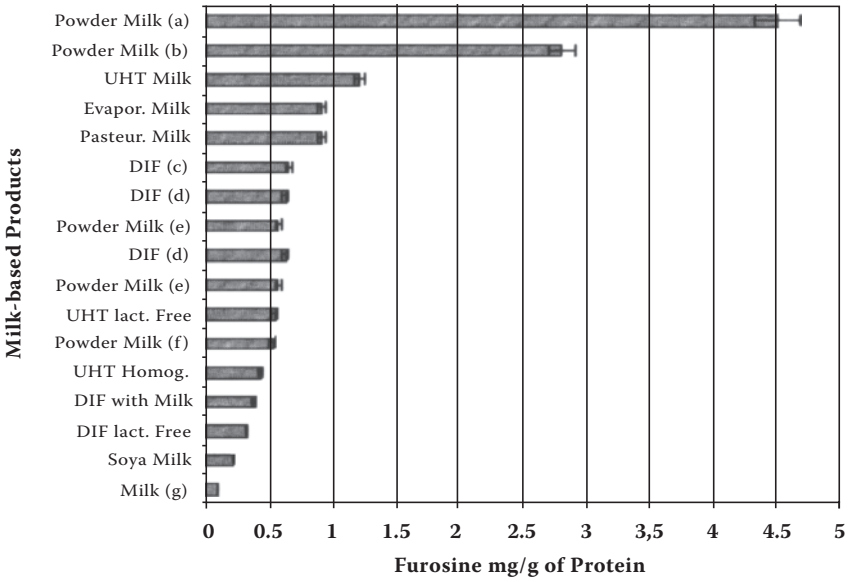

Figure 7.5 Relative furosine content in various milk-based products. Key: a, powdered milk kept for 2 years at room temperature; b, powdered milk kept for 1 year at room temperature; c, DIF with whey plus casein; d, DIF with hydrolyzed whey; e, powdered milk kept for 1 year at 4°C; f, fresh milk powder; g, raw (whole) bovine milk. DIF, dietetic infant formulas; UHT, ultra heat treatment. (Adapted from Baptista and Carvalho, 2004.[51])

and dextrose. Modern industry can produce a pig weighing 100 kg in less than half a year in contrast to about 2 years in the past and, as already mentioned, can drastically increase the cow's production of milk. However, as in humans, such "force-feeding" will most likely induce insulin resistance in animals and, if the animals were allowed to live long enough, manifest diabetes. Insulin resistance is also reported in intensively milk- and lactose-fed calves.[53] High levels of proinflammatory cytokines and various stress hormones are regularly registered in intensively fed animals. However, no information was found regarding whether or not such molecules can be transferred to humans by the food we eat. It is suggested that larger intake of hormone-rich foods, especially dairy products, might explain the reduced age of first menstruation in girls from approximately 17 years of age 200 years ago to the current of about 12 years, and be responsible for shorter menstruation periods and later menopause among Western women. About 80 percent of milk consumed today, much in contrast to the old days, comes from pregnant cows, and thus is rich in various hormones, especially sex hormones.[53] This is especially so for condensed products, such as butter, cheese, and most likely also powdered milk. As this problem is increasingly observed, "hormone-free" milk has become available in such countries as the United States.

7.8 DISEASES ASSOCIATED WITH HIGH TISSUE LEVELS OF AGEs/ALEs

Increased levels of various AGE/ALE substances in the body are reported in almost all chronic diseases from allergy and Alzheimer's disease to paradontosis, polycystic ovary syndrome, and various urogenital diseases, particularly uremia (Table 7.1). An association with dairy products is thus far reported in significantly fewer such conditions, but is reported in allergy,[84] coronary heart disease,[85,86] and diabetes,[87–89] Parkinson's disease,[90] and various cancers, such as breast,[48,91] prostatic,[92,93] testicular,[92] and ovarian[94,95] malignancies. Increasing evidence also suggests that reduced bone density and osteoporosis are not, as believed in the past, dependent on deficiency in minerals, but instead are a result of increased inflammation in the body, which explains the high incidence of osteoporosis in patients with chronic diseases. High levels of AGE/ALE in the body are also reported in patients with osteoporosis.[75,75] A recent American study reported reduced bone density in older women consuming more than three cola drinks per week compared to matched controls consuming similar amounts of other carbonated soft drinks.[96] This becomes especially interesting when considering that cola drinks, much in contrast to other soft drinks, are rich in AGE. Increased AGE/ALE levels are also reported in other disease conditions with obscure etiology, such as rupture of the Achilles' tendon and fibromyalgia.[54,72] The mouth reflects the health status of the body to a large extent, and paradontosis, frequently seen in patients with chronic diseases, is clearly associated with elevated inflammation in the body and elevated levels of AGE/ALE.[77] It would not be a surprise if the lowest levels of AGE/ALE are to be found in the group referred to as raw eaters, but this group has

Table 7.1 Diseases Associated with High Levels in the Body of AGEs/ALEs

	Ref.		Ref.
Achilles tendon rupture	54	Down's syndrome	70
Aging	55	Familiar amyloidotic polyneuropathy	71
Allergy	56	Fibromyalgia	72
Autoimmune diseases	57	Glaucoma	62
Alzheimer´s disease	58	Huntington's disease	73
Amyotrophic lateral sclerosis	59	Macular degeneration	62
Atherosclerosis	60	Liver cirrhosis	74
Cardiovascular disease	61	Osteoporosis	75, 76
Cataract	62	Paradontosis	77
Chronic endocrine disorders	63	Parkinson's disease	78, 79
Chronic lung diseases	64	Polycystic ovarial syndrome	80
Chronic renal diseases	65	Rheumatoid arthritis	81, 82
Creutzfeldt–Jakob's disease	66	Stroke	83
Cystic fibrosis	67	Uremia	21
Diabetes	68, 69		

attracted few studies and none with regard to the content of AGEs/ALEs. However, it has been demonstrated that vegans, much in contrast to meat eaters and lacto vegetarians, have significantly lower levels of AGEs/ALEs in the body. As a matter of fact, it has been shown that lacto vegetarians have even higher levels of AGEs/ALEs in the body than meat eaters,[97] which might be explained by a higher intake of dairy products, especially cheese, but might also be influenced by a higher intake of fructose. Significant health advantages are reported for vegans, when compared to the other groups: statistically significantly lower levels of proinflammatory molecules such as cytokines and acute phase proteins, lower systolic and diastolic blood pressure, lower total cholesterols, lower low-density lipoprotein (LDL)-cholesterols, lower fasting blood sugar and triglycerides, and lower incidence of chronic diseases, especially diabetes and complications to diabetes.

7.9 FOODS RICH IN AGEs/ALEs

So far, the information regarding AGE/ALE content in foods is incomplete. However, an international association has recently been formed with the goal of filling this gap. Leading universities around the world are building institutions for studies of nutragenomics; for example, on how various food ingredients affect our health. However, from existing information it is clear that dysfunctioning proteins are especially rich in foods that have been subjected to industrial processing. Table 7.2 provides guidance on foods expected to contain larger amounts of AGEs/ALEs.

Table 7.2 Foods Reported To Contain Larger Amounts of AGEs/ALEs

Dairy products, especially powdered milk	• Ice cream • Baby formulas • Clinical nutrition solutions **Cheese**: • Pizza • Tacos • Nachos • Salads • Fast-food sandwiches and sauces • Brown cheeses (Norwegian Brunost)
Grains, cereals, bakery products	• Toasted bread • Bread crusts • Crisp breads • Pretzel (500 kU/portion) • Rice Crispies (600 kU/portion) • Biscotti (1000 kU/portion)
Meat, poultry, and fish	Content increases as one goes from boiling to oven frying: • Boiling (1000 kU/serving) • Roasting (4300 kU/serving) • Broiling (5250 kU/serving) • Deep frying (6700 kU/serving) • Oven frying (9000 kU/serving); (see Goldberg T et al.[98])
Egg yolk powder, lecithin powder	
Coffee, especially dark roasted, dark hard-cured teas, roasted and salted peanuts, dark and sugar-rich alcoholic beverages, broth, Chinese soy, balsamic vinegar, smoked foods	

7.10 PREVENTION AND TREATMENT OF AGE/ALE ACCUMULATION

Several pharmaceuticals, especially those used for treatment of diabetes, are reported to reduce the content of AGEs/ALEs in the body, at least in short-lived tissues, that is, tissues with high turnover. Significant reduction in body content of AGE/ALE in comparison to controls (eating standard Western food) is observed in individuals who practiced caloric restriction (CR, they eat only two-thirds of what they would like to) for more than 2 years, which is also accompanied by significant health advantages compared to matched controls: lower blood pressure (102/61 ± 7 vs. 131/83 mm Hg), and lower levels of markers of inflammation, such as CRP (0.3 vs. 1.9 mg/L), TNF-α (0.8 vs. 1.5 pg/mL), and TGF-β (29.4 vs. 35.4 ng/mL).[99] Elevated RAGE and low sRAGE is reported in patients with active rheumatoid arthritis (RA), but patients with RA practicing CR for about 2 months are reported to have lower levels of pentosidine (an often measured AGE) in urine, as well as lower disease activity.[100]

Rich supply of vitamins, such as A, B, especially B_6 and B_{12}, C, D, E, and K as well as glutathione and folic acid, is often emphasized.[31,101,102] A long line of plant antioxidants, particularly those collectively defined as polyphenols, with documented up to 10 times stronger oxidation-quenching properties than conventional vitamins have been shown to have strong chemopreventive abilities, strong ability to prevent accumulation in the body of AGEs/ALEs, significant ability to reduce inflammation in the body, and to prevent reduction in organ function and premature aging.[103–105] Such plant antioxidants exist in nature in many thousands of different compounds, most likely hundreds of thousands; of flavonoids alone, more than 4,000 have been identified and of carotenoids almost 1,000. Table 7.3 summarizes some of the most well-known and studied such plant antioxidants. Supplementing histidine, taurine, carnetine, and carnosine has also been reported to have AGE/ALE-protecting abilities.[106,107] No vegetarian food with the exception of certain algae contains any taurine. This important amino acid is obtained only from eating animal-derived foods—meat, poultry, and fish.

7.11 INTESTINAL FLORA AND PROBIOTICS OF GREAT IMPORTANCE

Most of the above-mentioned substances will need assistance from microbial enzymes for their release from foods and absorption into the body. A rich intestinal flora is regarded necessary for release and absorption of various important

Table 7.3 Plant Antioxidants with Chemoprotective Effects on the Body; Reduction in Accumulation of AGEs/ALEs and Downregulation of the RAGE Receptor Function

- Aanthocyanins and hydroxycinnamic acids in **cherries**
- Epigallocatechin-3-gallate (EGCG) in **green tea**
- Chlorogenic acid and caffeic acid in **coffee beans** and **tobacco leaves**
- Capsaicin in **hot chili peppers**
- Chalcones in **apples**
- Daidzein and genistein in **soy beans**
- Euginol in **cloves**
- Gallic acid in **rhubarb**
- Hisperitin in **citrus fruits**
- Isothiocyanates in **cruciferous vegetables**
- Kaempferol in **white cabbage**
- Myricetin in **berries**
- Naringenin in **citrus fruits**
- Resveratrol and other procyanidin dimers in **red wine** and **virgin peanuts**
- Rutin and quercetin in **apples** and **onions**
- Various curcumenoids in main yellow pigments in **turmeric curry food**s

antioxidants. However, the increased intake of refined food and deficient intake of fresh fruits and vegetables among Westerners has led to a significant reduction in both density and diversity of the flora. This reduction is especially pronounced for strong fiber-fermenting lactic acid bacteria (LAB), such as *Lactobacillus plantarum* and *L. paracasei;* 75 percent of omnivorous Americans and 25 percent of vegetarians in the United States lack *L. plantarum*.[108] A more recent Scandinavan study found *L. plantarum* in only 52 percent and *L. paracasei* in only 17 percent of healthy individuals.[109] This information is particularly interesting as *L. plantarum* and *L. paracasei* belong to the small group of intestinal bacteria with ability to break down semiresistant fibers, such as inulin,[110] reduce inflammation, reduce infection, and eliminate pathogenic bacteria, such as *Clostridium difficile*.[111] Some LAB may well have the ability to eliminate AGEs/ALEs from foods, similar to what has been demonstrated for gluten[112] and heterocyclic amines.[113] *In vitro* studies have shown that fructoselysine, the dominating AGE in heated milk, can be effectively eliminated when incubated with fresh intestinal flora.[114]

7.12 FUTURE ASPECTS

Recent studies in the United States demonstrate an 83 percent reduction in rate of coronary heart disease,[115] a 91 percent reduction in diabetes in women,[116] and a 71 percent reduction in colon cancer in men[117] in patients adhering to what today is regarded as an "healthy lifestyle": no use of tobacco, moderate use of alcohol, regular physical exercise, and controlled eating. To these four factors should be added control of stress. Numerous studies demonstrate that both physical and mental stress increase the degree of inflammation in the body and activate RAGE.[118–120] It is likely that control of both intake and endogenous production of AGEs/ALEs might further add to a healthy lifestyle and further improve health and well-being. It is unfortunate that only a small fraction of us will give priority to issues related to active control of health and prevention of disease. A recent study in the United States suggests that only a small minority of 3 percent adhere to the four principles mentioned above.[121] Among these are mainly individuals who are otherwise fortunate in life, have a higher education, and a good financial status. Those who have low income and low level of education, including their children, are reported to be about 50 percent more unhealthy.

Too long have we ignored measures to control health by referring to the importance of genetic factors, which we thought we could not do much about. However, the message from numerous studies in monocygotic twins and in immigrants, especially Japanese and Italians, where one immigrated to the United States while the other remained in the home country is clear: lifestyle is significantly more important for health than genetic inheritance. The message from the winners of the 2006 Nobel prize in medicine and physiology is encouraging: it might well be possible to silence genes which might have a negative influence on health. Increasing evidence suggests that control of exaggerated systemic inflammation in the body is of the greatest importance for sustained health. For this, diet is a necessary, easily accessible, and most powerful tool.

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PART II

Sources of Probiotics

CHAPTER 8

Lactic Acid Bacteria and Plant Fibers
Treatment in Acute and Chronic Human Disease

Stig Bengmark

CONTENTS

8.1 WESTERN FOOD—THE THREAT TO HUMAN HEALTH

The modern Western diet is based on nutrients received from only a small number of plants; 80 percent of the nutrients come from 17 plants and 50 percent of the calories from 8 grains. Furthermore, the most Western food is extensively processed, which not only reduces the nutritional value of the food, but also increases the level of systemic inflammation in the body. Many nutrients and antioxidants do not sustain heating and drying; among them are the important amino acid glutamine and the master antioxidant glutathione. Furthermore, manipulation of food, especially heating, increases the content of unwanted proinflammatory ingredients. These include mutagens, oxidized fatty acids—trans-fatty acids—and dysfunctional and highly proinflammatory proteins, or Maillard products, which are most often advanced glycation and advanced lipoxidation end products; they are referred to as AGEs and ALEs (see Chapter 7). Among foods rich in AGEs and ALEs are dairy products especially powdered milk (frequently used in enteral nutrition and baby formulas, and in numerous foods such as ice cream), cheese, bakery products (bread crusts, crisp breads, pretzels, biscotti) and cereals (crisp rice), overheated (especially deep-fried and oven-fried) meat and poultry, as well as fish, drinks like coffee and cola, Chinese soy, balsamic products, and smoked foods in general (for further information, see Goldberg et al.[1,2]). The consumption of such foods, often the main constituents in fast foods, has increased dramatically in recent decades, much in parallel with the endemic of chronic diseases. The antiinflammatory effects of plant fibers and probiotic bacteria might not be strong enough to control chronically enhanced systemic inflammation, strongly associated with the global epidemic of chronic diseases.

8.2 DERANGED AND DYSFUNCTIONAL IMMUNE SYSTEM

Numerous chemical substances, additives to foods and pharmaceutical drugs, seem to derange the immune system. In the past, priority was not given to investigation of the eventual negative effects on the innate immune systems of consumed food

additives and pharmaceutical drugs. It is clear, even if not fully investigated, that a large number of chemicals have a strong negative influence on the immune system and the body's resistance to disease when consumed. As an example, it has long been known that antibiotics suppress various immune functions, especially macrophage activities, such as chemiluminescence response, chemotactic motility, and bactericidal and cytostatic ability.[3,4] Recent experience suggest that H_2-blockers, commonly used in many diseases and in critically ill patients, exhibit strong procoagulatory and proinflammatory effects. Ranitidine, as an example, has been shown in animal studies to enhance the inflammatory response and increase the extent of tissue injuries, especially in the liver.[5–7]

Several other factors increase the degree of systemic inflammation in the body:

- *Impaired hormonal homeostasis* increases oxidative stress/release of free radicals, increases intracellular accumulation of "waste products," inhibits apoptosis, disturbs repair mechanisms, reduces gene polymorphism, increases premature shortening of telomeres, and reduces immune defense and resistance to disease, changes often observed in premature aging and in various chronic diseases.[8]
- *Low level in the body of vitamin D* and subsequent secondary hyperparathyroidism.[9,10]
- *Low levels in the body of antioxidants,* such as folic acid and glutathione and increased levels of homocysteine.[11]
- *High levels in the body of estrogens*, especially 17β-estradiol, often induced by high consumption of hormone-rich dairy products.
- *High levels of angiotensin/rennin.*[12,13]
- *Larger intake of glutenoids.*[14,15]

The reason attempts to reduce inflammation with the use of probiotics have sometimes failed in the past might be that the proinflammatory pressure is simply too high due to underlying disease, but also due to consumption of too much of proinflammatory food and prescription drugs, all with inflammation-enhancing abilities. It is likely that in certain conditions additional measures are needed to achieve successful treatment with probiotics. Measures, such as reduced supply of proinflammatory foods, restriction in use of pharmaceuticals, and increased intake of plant foods rich in antiinflammatory vitamins and antioxidants, especially various polyphenols, might well be needed (see further below).

8.3 PLANT FIBERS REDUCE SYSTEMIC INFLAMMATION

Table 8.1 summarizes the content of fiber in some common plant-derived foods. It should be observed that various seeds, nuts, beans, and peas are especially rich in fiber, foods that no longer are eaten in the quantities they deserve. A common recommendation of minimum daily fiber intake is in the range of 30 to 35 g/day,[16,17] which roughly corresponds to about half a kilogram of fruits and vegetables, or, as often expressed, five to eight fresh fruits and vegetables per day. The recommendations for children above the age of 2 years are usually defined as age + 5 g/day.[18] No precise recommendation exists yet about intake of fiber under different conditions

Table 8.1 Content of Fiber in Common Plant-Derived Foods, g/100

Flax seeds	42	Cabbage	3.5
Sunflower seeds	21	Gooseberries	3.4
Passion fruit	16	Avocado	3.3
Soy flour	12	Fennel	3.3
Prunes	9	Savoy cabbage	3.2
Peanuts	8	Blueberries	3.1
Hazelnuts	6	Cauliflower	3.0
Blackberries	6	Bean sprouts	3.0
Green peas	6	Pears	2.8
Walnuts	5	Strawberries	2.4
Artichoke	5	Tomatoes	2.0
Black currents	5	Grapefruit	1.9
Onion	5	Orange	1.9
Beans	5	Apple	1.8
Brussels sprouts	4	Potato, cooked	1.4
Olives	4	Chili pepper	1.3/tsp
Kiwi	4	Turmeric	0.5/tsp
Raspberries	3.7		

of disease. The daily intake of dietary fiber is unsatisfactory in all Western countries, especially among people with a low level of education and low income. In the United States, for example, the estimated daily intake of fiber is approximately 14 to 15 g/day or about 50 percent of what is recommended, and far below the 60 to 80 g/day of substrate required to maintain a large bowel flora of 10^{14} microorganisms, which is known to be typical for a healthy and well-functioning human colon. Most Americans and Europeans have lost the ability to maintain a large proportion of what can be regarded as a natural flora.[19] A recent study in a northern European population found *Lactobacillus plantarum, L. rhamnosus,* and *L. paracasei* ssp. *paracasei* on the rectal mucosa of healthy humans in only 52, 26, and 17 percent, respectively.[20] The colonization rate with other, commonly milk-borne probiotic bacteria, such *L. casei, L. reuteri,* and *L. acidophilus* was in the same study only 2, 2, and 0 percent, respectively.

Commonly consumed cooked roots and other starchy vegetables; grains, consumed as bread, cereals, and porridge; and most fruit consumed in Western countries contain relatively little fiber, usually no more than 1 to 3 g/serving.[21] The largest amount of consumed plant fiber is provided by resistant starch (raw potato, unripe green banana, especially when allowed to cool after cooking, especially potato and whole-grain bread). However, the difference in intake between one person and another is several hundred percent (~8 to 40 g/day).[22] The second largest source of

fiber is nonstarch polysaccharides (~8 to 18 g/day). The third group of fiber is oligosaccharides (onions, artichoke, banana, cecoria), which although important to health, are today regrettably consumed in much too small quantities (~2 to 8 g/day).[22]

8.4 DIETARY FIBERS—FUNCTION AND DEFINITION

Dietary fiber is the collective name for pure fibers obtained from processing various plants. The term dietary fiber was coined some 50 years ago, and was then suggested to consist of cellulose, hemicellulose, and lignin,[23] all indigestible constituents of the cellular walls of plants. Some 20 years later, the concept was defined as "plant fibers and lignin, which are resistant to hydrolysis by the digestive enzymes of man."[24] A more recent definition by the American Association of Cereal Chemists (AACC) suggests that dietary fiber is "the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibers include polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation."[25] According to this definition, some noncarbohydrates like waxes, phytate cutin, saponins, suberin, and tannins, also are included in the concept, substances sometimes referred to as associated with nonstarch polysaccharide and lignin complex in plants. Of the many substances known, only a few have been properly investigated as dietary fibers and for health purposes, for example, as medical fibers.

Supplemented fibers are associated with several health benefits. The best documented physiological effects, in addition to providing energy and nutrients to the host and flora, are that they:

- Change in mucosal structure, increase mucosal growth, and improve mucosal function.
- Increase in intestinal flora, relieve constipation, reduce production of putrifactive gases, and provide resistance to invading microorganisms
- Reduce serum triglycerides, serum cholesterol, and very low density (VLD) lipoproteins
- Reduce the glycemic response to eating.
- Improve water and electrolyte balance and increase bioavailability and absorption of minerals, such as calcium, magnesium, iron, and zinc.

Consumption of medical fibers should always be regarded as a surrogate for not consuming enough fresh fruits and vegetables. There is no solid information to support that supplementation of medical fibers to healthy individuals eating a diet rich in fruits and vegetables is associated with additional health benefits. Medical fibers are mainly needed because the individual has lost the ability to consume enough fresh fruits and vegetables. This is often the situation in persons with severe allergies, in old and debilitated persons, and in persons with some gastrointestinal (GI) disorders,

such as short bowel syndrome and advanced diverticular disease. This is also most often the condition for critically ill patients, for whom enteral supply of concentrates of medical fibers has become a most valuable clinical tool. It must, however, always be remembered that bioactive fibers during the processing have lost their content of numerous important antioxidants and nutrients, some of which when possible should be separately supplemented, and whenever possible complemented by a supply of fresh fruits and vegetables.

8.5 DOCUMENTED HEALTH BENEFITS OF INCREASED FIBER CONSUMPTION

Significant information on beneficial effects from increased intake of plant fibers and prebiotics exists mainly for two large groups of diseases:

Blood glucose control/prevention of type 2 diabetes. Fiber is a slow-release system for delivery of glucose to the body. Sugar "entrapped" in plant cells is slowly released by fermentation and absorbed resulting in a controlled blood glucose and insulin response. It is well documented that the physical structure of starchy foods determines the glycemic index of that food. Fiber, regularly supplied to patients with diabetes, will significantly reduce the level of blood glucose and the need for insulin. Studies suggest that the most pronounced effects of fibers on glycemic index are obtained by water-soluble fibers. Guar gum is by far the most clinically used fiber and will, based on 15 different studies, induce a reduction in blood glucose to almost half (44 percent).[26]

Lipid control/prevention of coronary heart disease. Soluble fibers, such as pectin, guar gum, and betaglucans (oat) have repeatedly been shown to reduce blood cholesterol both in hypercholesterolemic and normocholesterolemic individuals, effects not found when nonsoluble fibers, such as cellulose and wheat bran, have been used. Common to water-soluble fibers is that they are gel forming. Soluble fibers are excellent substrates for production in the large intestine of short-chain fatty acids (SCFAs), known to reduce the level of cholesterol in the body. Studies both in animals and in humans suggest that it is especially propionic acid that is hypocholesterolemic.[27] A meta-analysis reports statistically significant protective effects against coronary heart disease in 14/16 studies.[28] In addition, fiber consumption is reported to reduce clotting and increase fibrinolysis, also important for prevention of building of arterial wall plaques and prevention of thrombosis formation.[29]

8.6 FIBERS COMMONLY USED IN CLINICAL NUTRITION

Substances, important to health—amino acids, such as arginine, glutamine, histidine, taurine, various sulfur and related amino acids, polyamins, omega-fatty acids, numerous vitamins, and antioxidants—are all to a great extent supplied to the body from plants. One cannot expect any significant amount of antioxidants to be delivered to the lower level of the GI tract, if not "hidden" in plant fibers. It is important

to remember that key nutrients, such as omega-3 fatty acids, glutamine, glutathione, and several other nutrients, are heat-sensitive and do not tolerate processing or storage to any great extent. Plant fibers that have been dried, heated, or microwaved cannot be expected to contain any large amounts of these key nutrients; they mainly come from unprocessed foods. It is highly desirable that, whenever possible, the supply of commercial nutrition formulas is complemented by a supply of fresh fruit and vegetable juices, produced as locally as possible. It is also desirable that several fibers are supplied in parallel, and that both soluble and nonsoluble fibers are used. For example, oat fibers are mainly metabolized in the proximal colon, whereas wheat fibers are known to be effective in the distal part of the colon, for example, the part of the colon where most cancers are localized. Oat has mainly shown sepsis-reducing effects while wheat has mainly been effective in cancer prevention. Among the fibers commonly used in clinical nutrition are discussed below.

8.6.1 Algal Fibers

Most of the algal fibers are resistant to hydrolysis by human endogenous digestive enzymes, but are fermented by colonic flora to various degrees. The soluble fibers consists in lamarans (a sort of β-glucan associated with mannitol residues), fucans (sulfated polymers associated with xylose, galactose, and glucoronic acid), and alginates (mannuronic and guluronic acid polymers). The insoluble algal polymers consist mainly of cellulose. Fermentation of alginates yields high levels of acetate (80 percent), while lamarans preferably yield butyrate (16 percent). It is most likely that algal fibers will be routinely used in clinical nutrition within a few years.

8.6.2 Fructans

Fructan starches and sucrose serve the plant as its energy reserve. These substances are also produced by bacteria and fungi. Fructans are said to enhance the tolerance of the plant to stressful conditions and make it possible for the plants to survive under harsh conditions, such as low temperature and draft. The most well known fructans are inulin (rich in chicory, artichoke, onions, banana) and phleins (rich in various grasses). Thus far, mainly inulin has been tried in human nutrition. Various oligosaccharides are reported to stimulate the flora and especially the growth of *Lactobacillus* and *Bifidobacterium* in the large intestine and to reduce the content of potentially pathogenic microorganisms (PPMs) in the intestine. Increase in the *Bifidobacterium* flora is regarded as especially favorable as bifidobacteria are known to produce important vitamins, among them thiamine, folic acid, nicotinic acid, pyridoxine, and vitamin B_{12}, which is of great importance for health. A fructan called neokestose, found in onion, is reported to have even better ability than inulin to promote growth of lactic acid bacteria (LAB).[30] Supplementation of fructans is also reported to reduce concentrations in serum of insulin, cholesterol, and triacylglycerol. It is also reported to promote absorption of calcium and other minerals. Other oligosaccharides, such as those extracted from peas and beans, especially soy bean

oligosaccharide (raffinose and stachyose) and pyrodextrin, produced by pyrolysis of maize and potato starch, are also reported to be beneficial for human health.

8.6.3 Glycomannans

Glycomannan, a glucose/mannose polymer derived from a plant called *Amorphophallus konjak*, has several English names, such as devil tongue, elephant yam, and umbrella arum. It has unique hydroscopic abilities and will swell and form a viscous gel on contact with water. Like other gels, this will delay gastric emptying and intestinal transit time. It has been shown to be effective in delaying absorption of digestible energy. It has thus far been used mainly in Japan and other Asian countries to treat diabetes, hypertension, and hypercholesterolemia. Dietary supply of konjak mannans has been shown to alter the flora and reduce tumorigenesis in experimental animals. It is also effective in controlling diarrhea in enteral nutrition, especially in elderly patients, and to increase the *Bifidobacterium* flora.

8.6.4 Oat Gum

Oat contains a series of interesting compounds, which is the reason an increasing part the world production of oat goes to the pharmaceutical and cosmetic industries. The amino acid pattern of oat is similar to that of human muscle (only that of buckwheat is more alike), and thus can be expected to deliver most of the amino acids needed to build muscles. Oat is rich in water-soluble fibers, β-glucans, known for their antiseptic properties. Oat is also rich in natural antioxidants, particularly ferulic acid, caffeic acid, hydrocinnamic acid, and tocopherols, and, before synthetic antioxidants, oat was available extensively and used to preserve foods: milk, milk powder, butter, ice cream, fish, bacon, sausages, and other food products sensitive to fat oxidation. Another ingredient richly available in oat is inositol hexaphosphate (phytic acid), a strong antioxidant, particularly known to enhance natural killer (NK) cell activity and to suppress tumor growth. Oat is also rich in polyunsaturated fats/polar lipids, such as phosphatidylcholine, known for its protective effects of mucosal and cellular surfaces.

8.6.5 Pectin

Pectin is also an interesting fiber, extensively used by the pharmaceutical and food industries. It has a unique ability to form gels and is commonly used as a carrier of pharmacologically active substances; it is common in baby foods. An important finding is that pectin is a very strong antioxidant against the three most dominating oxidation damages induced by peroxyl, superoxide, and hydroxyl radicals. These effects might explain why pectin has the capacity to stimulate the gut-associated immune system and to prevent disruption of the intestinal microflora. In experimental studies, pectins have shown strong protective and healing effects on gastric and on intestinal mucosa, not inferior to that observed with H_2-blockers, proton inhibitors, and surface-protection agents.[31,32] Pectin builds a protection layer in the

stomach and facilitates maintenance of gastric acidity, important for prevention of colonization of the stomach by pathogens. Pectin is also an excellent substrate for microbial fermentation.

8.7 LACTIC ACID BACTERIA IMPORTANT FOR FERMENTATION OF FIBERS

Not all fibers are easily fermented in the gut. Among the more fermentation-resistant fibers are wheat fibers, which usually are not digested until they reach the descending colon. Also oligofructans (inulin or phleins) are difficult to ferment, and only a small minority of LAB are able to do so. When the ability of 712 different LAB to ferment oligofructans was studied, only 16 of 712 were able to ferment the phleins and 8 of 712 inulin.[33] Apart from *Lactobacillus plantarum* only three other LAB species, *L. paracasei* subsp. *paracasei*, *L. brevis,* and *Pediococcus pentosaceus,* were able to ferment these semiresistant fibers. Another study investigated the ability of 28 different LAB to ferment pure fructo-oligosaccharides (FOS). All *L. plantarum*, *L. casei,* and *L. acidophilus* strains studied and most *Bifidobacterium* utilized FOS, in contrast to yogurt bacteria, such as *L. bulgaricus* and *Streptococcus thermophilus* and *Lactobacillus* strain *GG,* which were all unable to ferment these fibers.[34]

8.8 CLINICAL EXPERIENCE WITH SUPPLEMENTED PLANT FIBERS

8.8.1 Plant Fiber in Constipation

Chronic constipation is one of the most common disorders in Western countries. Its etiology remains unclear despite numerous clinical, pathophysiologic, and epidemiologic studies, but it is suggested that high intake of dairy products and intake of plant fibers plays a significant role in its pathogenesis. A randomized sample of 291 children with idiopathic chronic constipation was in a case control study compared with 1,602 healthy controls.[35] Constipation was clearly negatively correlated with low intake of cellulose and pentose fibers ($p < 0.001$). FOS may also have potential benefits in constipation because they exhibit many soluble dietary fiber-like properties. In a study, a total of 56 healthy infants, age 16 to 46 weeks (mean age 32 weeks) were randomly assigned to receive either 0.75 g FOS or placebo added to a serving of cereals for 28 days.[36] The mean number of stools per infant was 1.99 ± 0.62 per day in the FOS-supplemented group compared with 1.58 ± 0.66 in the control group ($P = 0.02$).

8.8.2 Plant Fiber to Prevent and Treat Diarrhea

In a large randomized study in acutely ill medical and surgical patients, all requiring enteral nutrition for a minimum of 5 days, supplementation of hydrolyzed guar gum was compared to fiber-free enteral nutrition. The incidence of diarrhea

was 9 percent with fiber supplementation, compared to 32 percent with fiber-free nutrition ($p > 0.05$).[37] One of the effects of certain fibers is that they increase the bioavailability and absorption of zinc, which is especially shown for oligosaccharides. Zinc supplementation was proved effective to lower both the incidence of diarrhea and the duration of diarrhea in a randomized study in 3- to 59-month-old children in Bangladesh.[38] In another study from Bangladesh, 250 g/L of green (unripe) banana (equivalent to two fruits) or 2 g pectin/kg food was supplemented to a rice diet in children suffering from persistent diarrhea.[39] The amounts of and frequency of stools, the duration of diarrhea, numbers of vomiting, use of oral rehydration, and amounts intravenous fluid solutions given were all significantly reduced with supplementation of both green banana and pure pectin. Recovery on the third day was seen in 59 percent in the green banana group and in 55 percent in the pectin group, compared to 15 percent in the rice-only control group.

8.8.3 Plant Fiber to Support Mineral Absorption

It is well accepted that nutrition is of great importance for bone health. Most of the interest has thus far focused on calcium and vitamin D. Much less interest has been paid to other important nutrients, such as protein, and especially to minerals, such as phosphorus, potassium, magnesium, and to vitamins, such as C and K. Recent studies suggests that increased intake of plant fibers, fruits, and vegetables is associated with an increased bone mineral density, including in elderly subjects, both women and men.[40,41] Of the pure fibers available, the effects of oligosaccharides have primarily been studied, and mainly in experimental animals. Calcium absorption, bone calcium content, bone mineral density, bone balance, and bone formation/bone absorption index are reported to significantly increase after 3 weeks of supplementation of a mixture of inulin and FOS.[41]

8.8.4 Plant Fiber to Control Weight

No major effects on body weight by supplementation of prebiotic fiber alone have thus far been reported. The effects of dietary fiber on subjective hunger ratings and weight losses were studied some 20 years ago in members of a weight loss club. Of 135 members, 108 completed the trial: 23 controls, 45 on ispaghula granulate, and 40 on bran sachets.[42] Both fiber preparations reduced hunger at all meals. The mean (± SD) weight reductions during the trial were 4.6 ± 2.7 kg for the controls, 4.2 ± 3.2 kg for the ispaghula group, and 4.6 ± 2.3 kg for the bran group ($p > 0.05$ for both groups). Although supply of dietary fiber immediately before meals did reduce the feeling of hunger, it did not provide any additional benefits to the weight reduction. A more recent cross-over study compared the effect on satiety of supplementation of 27 ± 0.6 g/day of fermentable fibers (pectin, betaglucan) with similar amounts of nonfermentable fiber (methylcellulose). The daily satiety was significantly more increased with nonfermentable (methylcellulose) than with fermentable fibers (betaglucan, pectin) ($p = 0.01$), but no differences were observed in daily energy intake or loss of body weight or body fat.[43]

8.8.5 Plant Fiber in Inflammatory Bowel Diseases

Although patients with both inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) are known to underconsume dietary fibers, there is little evidence that lack of dietary fiber plays a role in the pathogenesis of these diseases. The ability of maintaining remission in patients with ulcerative colitis (US) by a daily supply of 10 g of *Plantago ovata* seeds (also called psyllium or ispaghula husk) was compared with daily treatment with 500 mg of mesalamine and a combination of the two.[44] The 12 months of treatment failed to demonstrate any difference in clinical benefits between the three groups. Germinated barley foodstuff (GBF), a by-product from breweries, rich in hemicellulose and in glutamine, was tried in 39 patients with mild-to-moderate active UC.[45] Daily supply of 30 g reduced significantly the disease activity, increased concentration of SCFAs, and increased the numbers of *Bifidobacterium and Eubacterium* in stool. It may well be that the observed effect was due more to increased supply of glutamine and other antioxidants, such as various B vitamins than to the fiber per se as these compounds are known to be rich in by-products from breweries. Glutamine, as well as other antioxidants, are known to attenuate proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and to enhance release of heat shock proteins (HSP-72).[46] A controlled study using oat bran as fiber source was recently reported from a study in 22 patients + 10 controls with quiescent UC. Daily supply during 3 months of as much as 60 g of oat bran (equivalent to 20 g dietary fiber) resulted in a significant increase in fecal butyrate (average 36 percent), but also to a reduction in abdominal pain. All the treated patients tolerated well the large dose of fiber, and signs of relapse of disease were seen in none of the patients with colitis.[47] Butyrate has been shown to inhibit nuclear factor kappa B (NF-$\kappa\beta$) activation of lamina propria macrophages, and to reduce the number of neutrophils in crypts and surface epithelia, as well as the density of lamina propria lymphocytes/plasma cells in patients with ulcerative colitis[48]—findings correlating well with the observed decreased disease activity. In a study, 20 patients with ileal pouch-anal anastomosis received 24 g of inulin daily for 2 weeks. Significant reduction in inflammation was observed with endoscopy and histology. In addition, significant increase in fecal concentrations of butyrate and reductions in fecal pH, fecal content of secondary bile acids, and growth of *Bacteroides fragilis* were observed.[49]

8.8.6 Plant Fiber in Irritable Bowel Disease

Dysmotility disorders are increasingly common in Western societies. Some evidence suggests that various dysmotility disorders, gastroesophageal reflux problems, infant colic, and constipation are all food-related features, and often due to intolerance to cow's milk proteins.[50] IBS is a clinical diagnosis based on the occurrence of abdominal distension, abdominal cramps, often increased transit time, more frequent stools, and relief of pain on defecation. The prevalence of the syndrome varies between 7 and 22 percent, making IBS the most common functional GI disorder.[51]

Unfortunately, no effective pharmaceutical treatment exists or when existing is unacceptably toxic.[52] This has resulted in a need for additional modalities for the treatment of IBS. In this perspective, pre- and probiotics appear as attractive alternatives (see recent reviews[53,54]). Data from human intervention studies and especially results from recent animal studies clearly indicate that prebiotics have an impact on the immune system: Immune cells of the GALT (gut-associated lymphoid tissue) including Peyer's patches are primarily responsive to the oral administration of prebiotics.[55] However, a consequence of feeding the currently favored prebiotics (inulin, FOS, trans-galacto-oligosides, and lactulose) is increased gas production in the gut, which might preclude prebiotic use in diarrhea-predominant IBS, or where bloating or gas are prominent symptoms, but might allow their mild laxative properties to be useful in constipation-predominant IBS.[53] A few small open trials have been performed, but thus far no larger and randomized trial has been reported. However, a recent small open-label trial supplementing 15 g/day of a mixture of oligofructose (70 percent) and inulin (30 percent) reports significant reduction in disease activity (Harvey Bradshaw index fell from 9.8, SD 3.1 to 6.9 SD 3.4, $p = 0.01$) in parallel with a significant increase in fecal bifidobacteria concentration (from 8.8, SD 0.9 log10 to 9.4, SD 0.9 log10 cells/g dry feces $p = 0.001$). Also the interleukin 10 (IL-10) positive dendritic cells increased (from 30 to 53 percent, $p = 0.06$), and the percentage of dendritic cells expressing Toll-like receptor 2 (TLR2) and TLR4 increased from 1.7 to 36.8 percent, $p = 0.08$, and from 3.6 to 75.4 percent, $p = 0.001$),[56] respectively, which offers hope for the future.

Other dietary fibers have also been tried in various groups of abdominal pain. A recent Cochrane review was unable to find any evidence that fiber supplements, lactose-free diets, or *Lactobacillus* supplementation is effective in the management of children with recurrent abdominal pain.[57] However, a study in adult patients reports significant success with fibers other than the classical prebiotics. In one study, 188 adult patients with IBS were classified as having diarrhea-predominant, constipation-predominant, or changeable bowel habit type IBS and randomly assigned to groups receiving 30 g/day of wheat bran or 5 g/day of guar gum (PHGG).[58] After 4 weeks, patients were allowed to switch group, depending on their subjective evaluation of their symptoms. Both fiber and PHGG were effective in improving pain and bowel habits. Significantly more patients switched from fiber to PHGG (49.9 percent) than from PHGG to fiber (10.9 percent) at 4 weeks. Intention-to-treat analysis showed a significantly greater success in the PHGG group (60 percent) than in the fiber group (40 percent). In addition, significantly more patients in the PHGG group reported a greater subjective improvement than those in the fiber group. It was concluded that improvements in core IBS symptoms were observed with both bran and PHGG, but the latter was better tolerated and preferred by patients.[58]

The capsaicin (chili pepper) receptor (TRPV1) is known to play an important role in visceral pain and hypersensitivity states. It is of special interest that the numbers of TRPV1-immunoreactive fibers was found to be increased by 3.5 times in biopsies from patients with IBS compared with controls ($p < 0.0001$).[59] Substance P-immunoreactive fibers ($p = 0.01$), total nerve fibers (PGP 9.5) ($p = 0.002$), mast cells (c-kit) ($p = 0.02$), and lymphocytes (CD3) ($p = 0.03$) were also all significantly

increased in the IBS group. However, in multivariate regression analysis, only TRPV1-immunoreactive fibers ($p = 0.005$) and mast cells ($p = 0.008$) were significantly related to the abdominal pain score. The information of increased TRPV1 nerve fibers in IBS, in addition to the observed low-grade inflammatory response, makes TRPV1 nerve fibers an interesting new therapeutic target.[59]

8.8.7 Plant Fiber to Control Infections

In an effort to prevent nosocomial pneumonia and sepsis, patients with severe multiple trauma were treated with beta-1-3 polyglucose (glucan)—a component of cell walls of plants and microbes.[60] Pneumonia occurred in 2 of 21 glucan-treated and in 11 of 20 patients in the control group ($p < 0.01$). Infectious complications (pneumonia and/or general sepsis) occurred in 14 percent of the glucan-supplemented patients versus 65 percent in the control group ($p < 0.001$). Another study compared the effects of a high-protein formula enriched with fiber, but also arginine and antioxidants with a standard high-protein formula in early enteral nutrition in critically ill patients.[61] The supplemented group had, in comparison to nonsupplemented controls, a lower incidence of catheter-related sepsis (0.4 episodes/1,000 intensive care unit, ICU, days) than the control group (5.5 episodes/1000 ICU days) ($p < 0.001$), but no differences were observed between the groups in incidence of ventilator-associated pneumonia, surgical infection, bacteremia, urinary tract infections, mortality, and in long-term survival.[61]

8.9 PLANT FIBERS RICH IN ANTIOXIDANTS

LAB produce themselves and/or release from consumed plants a whole range of important vitamins and antioxidants. One important example is the essential B vitamin, folate, known to have a strong effect in reducing homocysteine and an ability to prevent some chronic diseases. Folate is synthesized by LABs, such as *Lactococcus lactis* and *Lactobacillus plantarum.* Other LABs, however, such as *L. gasseri,* are net consumers of folate. A recent publication describes successful transfer of five genes essential for folate biosynthesis from *Lactococcus lactis* to *Lactobacillus gasseri,* turning *L. gasseri* into a net producer of folate.[62] Anemia, iron deficiency, and folate deficiency are common among patients with both acute and chronic diseases, such as IBD.[63,64]

In a pediatric study of 43 patients and 46 controls, plasma total homocysteine (tHcy) concentrations were shown to be significantly higher in children with IBD than in control subjects ($p < 0.05$). Furthermore, the level of plasma tHcy levels correlated well with observed reductions in plasma 5-methyltetrahydrofolate ($p < 0.0005$).[65] A similar study in 108 adult patients with IBD and 74 adult healthy controls found significantly lower levels of folate ($p < 0.05$) in patients with both UC and Crohn's disease (CD).[66] Also in this study, the serum concentration of tHcy was significantly higher in both groups: UC 15.9 ± 10.3 mmol/l and CD 13.6 ± 6.5 compared to controls 9.6 ± 3.4 ($p < 0.05$).

The choice of fibers for medical use has probably not considered the content of vitamins and antioxidants as it should. Pectin has demonstrated high antioxidant ability, but most of the fibers generally used are not particularly rich in antioxidants. Numerous other plant fibers exist that should be considered as medical fibers and used either as replacement for or complements to other fibers in various enteral nutrition solutions. Plants with documented ability to boost resistance and decrease vulnerability to disease, often referred to as chemopreventive agents, are usually easily available, inexpensive to produce, rich in fibers, and have no or limited toxicity. Among the numerous agents with chemopreventive abilities are a whole series of phenolic and other compounds suggested to reduce the speed of aging and often documented to prevent degenerative malfunctions of organs: isothiocyanates in cruciferous vegetables, epigallocatechin-3-gallate (EGCG) in green tea, caffeic acid in coffee, capsaicin in hot chili peppers, chalcones in apples, euginol in cloves, gallic acid in rhubarb, hisperitin in citrus fruits, naringenin in citrus fruits, kaempferol in white cabbage, myricetin in berries, quercetin in apples and onions, resveratrol and other procyanidin dimers in red wine, and various curcumenoids found in turmeric curry foods, in addition to thousands of hitherto less explored or unexplored substances. Turmeric, dried and powdered roots of the plant *Curcuma longa*, is rich in natural antioxidants, and has proved to be a strong inhibitor of proinflammatory messengers, such as NF-κβ, cyclooxygenase-2 (COX-2), matrix mettaloproteinase-9 (MMP-9), inducible nitric oxide synthase (iNOS), TNF, IL-8, eotaxin, cell surface adhesion molecules, and antiapoptotic proteins.[67] (See further a recent review.[68])

Chili pepper, a herb with high content of flavonoids (>100 mg/100 g), has recently caught attention, especially since a specific receptor for its active substance, capsaicin, has been demonstrated and cloned.[69] The cloning of the vanilloid receptor 1 (TBRV1) has opened a floodgate for discoveries regarding the function of this complex molecule[70] and provided explanation for earlier observed clinical effects of intake of chili peppers. This receptor is associated with nociceptive afferent nerve fibers and broadly expressed, especially in brain, epidermis, and visceral cells. Old observations as well as recent studies suggest a great potential of antioxidant-rich chili fibers for control the immune cells, both innate and acquired,[71] of chronic diseases especially diabetes, both type 1 and 2,[72,73] hypertension,[74] and cancer,[75] as well as chronic pain conditions[76] and obesity.[77]

8.10 DIVERSITY IN MICROBIOTA FOR BARRIER FUNCTION

The gut mucosa and microbiota are intimately joined in the maintenance of a well-functioning barrier between the host and the external environment—see further two excellent reviews.[78,79] The barrier is suggested to be composed of three barriers in one: physical, innate immune, and adaptive immune. Emphasis has in the past focused mainly on the physical barrier, but tends in more recent years to switch to the importance of the innate immune mechanisms, particularly the role of antimicrobial peptides, such as defensins and more recently angiogenins.[80]

Several plant fibers (prebiotics) and a few LABs (probiotics) have documented significant effects in improving both the function of the innate immune system and the physical barrier and in increasing resistance to disease. The hope is that combined supply of these components will have synergistic, that is, more than additive, effects in boosting the immune system and enforcing the barrier functions. Products that combine pre- and probiotics are called synbiotics and treatment using the combination is termed synbiotic treatment.

The term "defense by diversity" was coined in 1999,[81] and seems applicable to synbiotic treatment. Natural foods supply both LAB and a great variety of plant fibers. A recent study concludes that combining several fibers has more than additive effects on the microbial ecosystem and immune responses,[82] and a recent review suggest that multispecies probiotics are superior to single-species probiotics to enhance growth, reduce antibiotic-associated diarrhea, prevent infections (*S. typhimurium*) and reduce pathogenic colonization (*Escherichia coli*).[83] The choice of pre- and probiotics must be based on scientific evidence (see below). This is especially important in the selection of LABs, as the majority of LABs have no or much limited effects on immune functions and outcome. It is important to remember in constructing synbiotic formulations that most of the LABs used by the food industry have no or limited ability to ferment bioactive fibers, such as inulin or phlein, no ability to adhere to human mucus, low antioxidant capacity, and most important do not survive the acidity of the stomach and bile acid content. Stronger bioactivities cannot be expected from LABs, such as yogurt bacteria, chosen mainly for their palatability. The LAB used in the synbiotic studies must be selected according to their bioactivity. Unfortunately, few studies have looked at the synergistic effects of simultaneous supply of LAB and fibers—synbiotics.

Although some studies have used various synbiotic compositions, only two such compositions have been produced after extensive preclinical studies:

1. *A one LAB/one fiber composition,* produced (Probi AB, Lund Sweden) by fermentation of oat meal with *L. plantarum* strain 299, containing 10^9 of LAB and approximately 10 g oat fiber.[84] In a few studies a commercial fruit juice, PRO VIVA™ containing 10^7 of a related *L. plantarum* strain called 299V (Skånemejerier, Malmö, Sweden), has also been tried.
2. *A four LAB/four fiber composition,* called Synbiotic 2000™, consisting in a mixture of 10^{10} (more recently also a Synbiotic Forte™ with 10^{11}) of each of four LAB: *Pediacoccus pentosaceus* 5-33:3, *Leuconostoc mesenteroides* 32-77:1, *Lactobacillus paracasei* subsp. *paracasei* 19, and *L. plantarum* 2362 and 2.5 g of each of the four fermentable fibers (prebiotics): betaglucan, inulin, pectin, and resistant starch (Medipharm AB, Kågeröd, Sweden and Des Moines, Iowa).

Lund University microbiologists Åsa Ljungh and Torkel Wadström developed this multistrain/multifiber synbiotic formula, which in recent years has been extensively used in clinical trials. The choice of LAB for the formulation was done after extensive studies of more than 350 human[85] and more than 180 plant microbial strains[86] and was based especially on the ability of the LAB to produce bioactive proteins, transcribe NF-κB, produce pro- and antiinflammatory cytokines, produce

antioxidants, and most important to functionally complement each other. In recent studies, both the Synbiotic 2000 Forte and a Probiotic 2000 Forte™ (no fiber added), containing 10^{11} of each of the four LABs, that is, 400 billion LAB per dose, have been tried. The effects of Synbiotic 2000 have thus far been investigated in a series of conditions.

8.10.1 Synbiotics in Acute Pancreatitis

In one study, 62 patients with severe acute pancreatitis (SAP) (Apache II scores: Synbiotic 2000-treated 11.7 ± 1.9, controls 10.4 ± 1.5) were given either two sachets/day of Synbiotic 2000 (2 × 40 billion LAB/day and a total 20 g fibers) or the same amount of fibers (20 g) as in Synbiotic 2000 during the first 14 days after arrival to the hospital. [87] Of 33 patients, 9 (27 percent) in the Synbiotic 2000-treated group and 15 of 29 patients (52 percent) in the only fiber-treated group developed subsequent infections. Of 33 Synbiotic 2000-treated patients, 8 (24 percent) and 14 of 29 (48 percent) of the only fiber-treated patients developed SIRS (systemic inflammatory response syndrome), MOF (multiple organ failure), or both ($p < 0.005$).[88] A total of 7 pathogenic microorganisms were cultivated in the synbiotic-treated group compared to 17 in the fiber-only group.

8.10.2 Synbiotics in Polytrauma

In patients with polytrauma, two prospective randomized trials with Synbiotic 2000 and Synbiotic 2000 FORTE have been concluded. The first study compared the following treatments in patients with acute extensive trauma: (1) Synbiotic 2000 (40 billion LAB/day) with (2) a soluble fiber, (3) a peptide diet (Nutricomp, Braun Inc., Germany), and (4) supplementation of glutamine. Treatment with Synbiotic 2000 led to a highly significant decrease in number of chest infections (4/26 patients, 15 percent), compared to peptide diet (11/26 patients, 42 percent, $p < 0.04$), glutamine (11/32 patients, 34 percent, $p < 0.03$), and fiber only (12/29 patients, 41 percent, $p < 0.002$).[89] The total number of infections was also significantly decreased: Synbiotic 2000 5/26 patients (19 percent); fiber only 17/29 patients (59 percent); peptide 13/26 patients (50 percent); and glutamine16/32 patients (50 percent).

In the second study, 65 patients with polytrauma were randomized to receive Synbiotic 2000 Forte (400 billion LAB + 10 g fiber, see above) once daily for 15 days or maltodextrine as placebo. Significant reductions were observed in number of deaths (5/35 vs. 9/30, $p < 0.02$), severe sepsis (6/35 vs. 13/30, $p < 0.02$), chest infections (19/35 vs. 24/30, $p < 0.03$), central line infections (13/32 vs. 20/30, $p < 0.02$), and ventilation days (average 15 vs. 26 days).[90] A total of 54 pathogenic microorganisms were cultivated in the symbiotic-treated group compared to 103 in the fiber-only group.

8.10.3 Synbiotics in Abdominal Surgery

In a randomized controlled study, 45 patients undergoing major surgery for abdominal cancer were divided into three treatment groups: (1) enteral nutrition (EN) + Synbiotic 2000 (LEN), (2) EN + only the fibers in the same amounts (20 g) as in Synbiotic 2000 (FEN), and (3) standard parenteral nutrition (PN). All treatments lasted for 2 preoperative and 7 days postoperative days. The incidence of postoperative bacterial infections was 47 percent with PN, 20 percent with FEN, and 6.7 percent with LEN ($p < 0.05$).[91] A total of 34 pathogenic microorganisms were cultivated in the symbiotic-treated group compared to 54 in the fiber-only group. Significant improvements were also documented in prealbumin (LEN, FEN), C-reactive protein (LEN, FEN), serum cholesterol (LEN, FEN), white cell blood count (LEN) , serum endotoxin (LEN, FEN), and IgA (LEN).

In another prospective randomized, double-blind trial, 80 patients subjected to pylorus-preserving pancreatoduodenectomy (PPPD) received twice daily either Synbiotic 2000 (2 × 40 billion LAB) or only the fibers in composition from the day before surgery and during the first 7 postoperative days.[92] A highly significant difference in infection rate ($p = 0.005$) was observed as only 5 of 40 patients (12.5 percent) in the Synbiotic 2000-treated group suffered infections (4 wound and 1 urinary tract infection) versus 16 of 40 (40 percent) in the fiber-only group (6 wound infections, 5 peritonitis, 4 chest infections, 2 sepsis, and 1 of each of urinary tract infection, cholangitis, and empyema). The infecting microorganisms in the symbiotic-treated group were *Klebsiella pneumoniae* (2 patients), *Enterobacter cloacae* (2 patients), *Proteus mirabilis* (1 patient), and *Enterococcus faecalis/faecium* (1 patient); in the fiber-only group *Enterobacter cloacae* (8 patients), *Enterococcus faecalis/faecium* (7 patients), *Escherichia coli* (7 patients), *K. pneumoniae* (2 patients), *Staphylococcus aureus* (2 patients), and *Proteus mirabilis* (1 patient). Statistically significant differences between the groups were also observed in use of antibiotics (mean: Synbiotic 2000; 2 ± 5 days, fiber-only; 10 ± 14 days).

8.10.4 Synbiotics in Chronic Liver Disease and Liver Transplantation

In a study, 58 patients with liver cirrhosis suffering minimal encephalopathy were randomized into three treatment groups: Group 1 (20 patients) received Synbiotic 2000 (40 billion LAB); group 2 (20 patients) received the same amount of the fibers in Synbiotic 2000; and group 3 (15 patients) received placebo (nonfermentable, nonabsorbable fiber—crystalline cellulose).[93] A significant increase in intestinal LAB flora was observed after 1 month of supplementation in the synbiotic-treated group, but not in the other two groups. Intestinal pH was significantly reduced in both treatment groups, but not in the placebo-treated group. Significant decreases in fecal counts of *Escherichia coli, Staphylococcus,* and *Fusobacterium,* but not in *Pseudomonas* and *Enterococcus,* and significant decreases in ammonias, endotoxins, ALTs (alanine transaminase), and bilirubins (original level 252 ± 182) were observed in the Synbiotic 2000-treated group (84 ± 65, $p < 0.01$) and in the fiber-only

treated group (110 ± 86, $p < 0.05$), while it remained unchanged in the placebo group. The improvements in liver function were accompanied by significant improvements in psychometric tests and in the degree of encephalopathy.

In a follow-up study by the same group of investigators 30 patients with liver cirrhosis were randomized to receive either Synbiotic 2000 or placebo (crystalline cellulose) for 7 days.[94] Viable fecal counts of *Lactobacillus* species, Child-Pugh class, plasma retention rate of indocyanine green (ICG_{R15}), whole blood TNF-α mRNA, IL-6 mRNA, serum TNF-α, soluble TNF receptor (sTNFR)I, sTNFRII and IL-6, and plasma endotoxin levels were measured pre- and posttreatment: Synbiotic treatment was associated with significantly increased fecal lactobacilli counts and significant improvements in plasma retention rate of ICG_{R15} and stage of liver disease (Child-Pugh classification). No significant changes in any study parameter followed placebo treatment, but significant increases in whole blood TNF-α mRNA and IL-6 mRNA, along with serum levels of soluble TNF receptors sTNFRI and sTNFRII, were observed in the Synbiotic 2000-treated patients. TNF-α and IL-6 levels correlated significantly, both at baseline and after synbiotic treatment. Synbiotic-related improvement in ICG_{R15} was significantly associated with changes in IL-6, both at mRNA and protein levels, and unrelated to plasma endotoxin values. It was concluded that even short-term synbiotic treatment can significantly modulate gut flora and improve liver function in patients with cirrhosis. The observed benefits seemed unrelated to reduction in endotoxemia, but could be mediated, at least in part, by treatment-related induction of IL-6 synthesis by TNF-α. These results offer great hope that synbiotic treatment of patients on the waiting list for liver transplantation might prevent septic episodes, improve liver function, and promote successful outcome of surgery.

In another study, 66 patients were randomized to either receive Synbiotic 2000 or only the fibers in Synbiotic 2000 in connection with human orthotopic liver transplantation. The treatment started on the day before surgery and continued for 14 days after surgery. During the first postoperative month only 1 patient in the Synbiotic 2000-treated group (3 percent) showed signs of infection (urinary infection) compared to 17 of 33 (51 percent) in the patients supplemented with only the four fibers.[95] The infecting organisms in the synbiotic-treated group were *Enterococcus faecalis* in 1 patient and in the only fiber-treated group *E. faecalis/faecium* in 11 patients, *E. coli* in 3 patients, *Enterobacter cloacae* in 2 patients, *Pseudomonas aeruginosa* in two patients, and *Staphylococcus aureus* in 1 patient. The use of antibiotics was on average 0.1 ± 0.1 days in the synbiotic-treated patients and 3.8 ± 0.9 days in the fiber-only group.

8.10.5 Synbiotics in Inflammatory Bowel Disease

Daily rectal instillations with Synbiotic 2000 reconstituted in saline were given to 10 patients with distal colitis for 2 weeks. One patient withdrew after 1 week; the remaining patients showed dramatic improvements in various disease scores during the 3 weeks of observation: episodes of diarrhea (decreased from 2.4 to 0.8), visible blood in stool (2.2 to 0.8), nightly diarrhea (0.5 to 0), urgency (1.9 to 1.0), and consistency of stool (1.1 to 0.8).[96] In the study, 2 patients reported significant bloating

and flatulence, but no other adverse or side effects were reported. In another study, 8 patients with active ulcerative colitis (UC) received a synbiotic composed of 4×10^{11} freeze-dried *Bifidobacterium longum* and 6 g of a prebiotic FOS/inulin mix called Synergy daily for 4 weeks. These patients were compared to 8 similar patients receiving placebo.[97] Levels of intestinal bifidobacteria at the end of the study were increased 42-fold compared to 4.6-fold in the placebo group. The sigmoidoscopy score decreased on average by 1.3 compared to an increase of 0.58 in the placebo ($p = 0.06$). The mean histology score was decreased in the synbiotic group and increased in the placebo group. However, due to the small size of the patient group, these changes were not statistically significant. The bowel habit index scores decreased by 20.4 percent in the synbiotic group and the scores increased by 70.4 percent in the placebo group. Human beta-defensin (hBD) (2, 3, and 4), TNF-α, and IL-1 were all decreased after synbiotic treatment, but remained unchanged in the placebo group ($p = 0.05$). These observations are most interesting and promising for future therapies. I fully agree with the statement of the reviewer: "Slowly, the links of diet to the intestinal environment and the association of the intestinal environment to IBD are becoming evident. The prebiotic and probiotic trials reveal the importance of the intestinal environment as a potent regulator of IBD activity."[98]

8.10.6 Synbiotics in Short Bowel Syndrome

Seven malnourished patients aged 2.5 to 24 years with short bowel syndrome and refractory enterocolitis received a synbiotic composition consisting ~1 billion *Bifidobacterium breve* and *Lactobacillus casei* and ~3 g galacto-oligosaccharides three times daily for 15 to 55 months.[99] Improvement of the flora as a whole (general increase in anaerobic bacteria and suppression of pathogenic flora) and an increase in fecal content of SCFAs (from an average of 27.8 to 65.09 ~mol/g wet feces) resulted. Six of seven patients increased their body weight between 1.0 and 4.2 kg/year. Prealbumin was increased in all treated patients ($p = 0.05$). These results in a small study offer hope that other eventually more potent probiotics in combination with other fibers and antioxidants will significantly contribute to the quality of life for patients with short bowel syndrome.

8.10.7 Synbiotics in Irritable Bowel Syndrome

The effects of twice-daily consumption of a probiotic fruit drink ProViva (Skånemejerier, Malmo, Sweden) containing *L. plantarum* 299v (6×10^7 cfu/drink) or placebo for 4 weeks were studied in a controlled study including 40 patients.[100] The vast majority (95 percent of LAB-treated vs. 15 percent of the placebo-treated patients) of individuals in the probiotic consumption group reported general improvement. A total of 20 of 20 patients in the LAB-supplemented group and 11 of 20 patients in the placebo group ($p = 0.0012$) reported resolution of abdominal pain. A similar study, using the same formula, was performed in patients who received the treatment for 4 weeks. A significant enhancement of LAB composition in probiotics-supplemented patients was described. Flatulence was rapidly and significantly reduced in the LAB-

treated group, but no difference in bloating was reported between the groups.[101] The same formula was applied in a cross-over trial of 4 weeks duration in 12 patients. A significant reduction in breath hydrogen was registered after 2 hours of ingestion, without a change in total hydrogen production or any symptomatic improvement.[103] A total of 68 patients with IBS were treated for 12 weeks with a vitamin and plant fiber-enriched diet containing either live or heat-inactivated LAB including 10^9 each of *L. acidophilus*, *L. helveticus,* and *Bifidobacterium* spp.[104] Of the patients, 80 and 40 percent, respectively, reported significant improvements in pain, bloating, constipation, and bowel habits ($p < 0.01$).

8.10.8 Synbiotics in *Helicobacter pylori* Infections

A clinical trial was carried out in a school in a low socioeconomic area of Santiago, Chile. *Helicobacter pylori* (Hp) positive children were randomly distributed into four groups: (1) antibiotic treatment (lanzoprazole, clarythromycin, and amoxicillin) (Ab) daily for 8 days; (2) 250 mg *Saccharomyces boulardii* plus 5 g inulin (SbI) daily for 8 weeks; (3) 1 billion *L. acidophilus* LB (LB) daily; or (4) no treatment.[105] A ^{13}C-urea breath test (^{13}C-UBT) was performed before and after the study and the differences in $^{13}CO_2$ over baseline were calculated (DDOB). Hp was eradicated in 66, 12, and 6.5 percent of the children from the Ab, SbI, and LB groups, respectively, while no spontaneous clearance was observed in the children without treatment. A moderate but significant difference in DDOB was detected in children receiving living SbI (76.31; 95 percent CI: 711.84 to 70.79), but not in those receiving LB (+0.70; 95 percent CI: 75.84 to +7.24). Although more studies are needed to confirm the effects and elucidate the mechanisms, it is clearly an interesting observation that Hp infection was eradicated in 12 percent of synbiotic-treated and 6.5 percent of probiotic-treated Hp-infected children. It is likely that other LAB and larger doses of both LAB and prebiotics might achieve much stronger effects.

8.10.9 Synbiotics in Allergy

A synbiotic combination of *L. casei* subsp. *casei* + dextran prevented cedar pollen-induced nasal and ocular symptoms, increased cedar pollen-specific IgE, and increased the number of eosinophils.[106]

In another randomized study, children > 2 years with atopic dermatitis received either potato starch and *L. rhamnosus*-based synbiotics or the prebiotic alone three times a day for 3 months. The disease score decreased with synbiotic treatment from 39.1 to >20.7 ($P < 0.0001$), and with prebiotic treatment from 39.3 to 24.0 ($P < 0.0001$). No difference was observed after 3 months of treatment ($P = 0.535$).[107]

8.10.10 Synbiotics in Prevention of Cancer

A synbiotic preparation consisting of oligofructose-enriched inulin (12 g) (SYN1), *L. rhamnosus* GG (LGG), and *B. lactis* Bb12 (BB12) (10^{10} cfu), was recently administered in a 12-week randomized, double-blind, placebo-controlled

trial including 37 patients with colon cancer and 43 polypectomized patients.[108] The intervention resulted in significant changes in the fecal microbiota, including elevations of *Bifidobacterium* spp. and *Lactobacillus* spp. and reductions of *Clostridium perfringens*. The intervention reduced colorectal proliferation, the capacity of fecal water to induce necrosis in colonic cells, and improved epithelial barrier function in polypectomized patients. Genotoxicity assays of colonic biopsy samples at the end of the intervention period indicated a decreased exposure to genotoxins in the polypectomized patients. Synbiotic consumption prevented an increased secretion of IL-2 by peripheral blood mononuclear cells in the polypectomized patients and increased the production of interferon-gamma (IFN-γ) in the patients with colon cancer. It was concluded that several colorectal cancer biomarkers may be favorably altered by synbiotic intervention.

8.11 TREATMENT-RESISTANT CONDITIONS

Treatment with synbiotics has failed in two types of patients: those with CD and general intensive care patients.

8.11.1 Crohn's Disease

Attempts in the past to affect CD by probiotic interventions have generally failed. Daily oral administration of 10^{10} of the probiotic LA1, even when instituted early after ileo-cecal resection, failed to exert any protective effect on early endoscopic recurrence in patients with CD. The histological score, the serum inflammatory parameters, and the clinical relapse rate were similar to those of the controls.[109] Two studies with Synbiotic 2000 have also ended with negative outcome. In one study, after an initial treatment with infliximab, 63 patients were randomized to daily receive either Synbiotic 2000 or crystalline cellulose as placebo.[110] Median time to relapse was 9.8 and 10.1 months, respectively. In a second study, patients following surgery were supplemented with either Synbiotic 2000 or crystalline cellulose as placebo. In the synbiotic-treated group, 7 patients completed the scheduled 24-month treatment, as did and 2 patients in the placebo group.[111] No differences were observed between the two groups either in endoscopic findings or rate of clinical relapse. After 3 months of treatment, the Rutgeerts disease scores were 0.6 ± 0.8 in the synbiotic-treated group and 0.8 ± 1 in the placebo group (NS).

8.11.2 General Intensive Care Patients

Two large studies have been performed in a general intensive care population: one with Synbiotic 2000 and one with Synbiotic 2000 Forte. Synbiotic 2000 (40 billion LAB) was given to 162 patients and only the fibers in the synbiotic composition to 168 patients. No difference was observed in mortality or in multiorgan dysfunction.[112] In the other study Synbiotic 2000 Forte was supplemented to 130 patients twice a day throughout the whole intensive care unit stay (2×400 billion LAB) and

compared to 129 patients supplemented with a cellulose-based placebo. No statistical difference was demonstrated between the groups in the incidence of ventilator-associated pneumonia (VAP) (9 and 13 percent, $P = 0.31$), the rate of VAP per 1,000 ventilator days (13 and 14.6, $p = 0.73$), and hospital mortality (27 and 33 percent, $p = 0.32$).[113]

8.12 CHOICE OF LACTIC ACID BACTERIA AS PROBIOTICS

Only a few probiotic strains have thus far shown ability to eliminate or reduce unwanted proinflammatory molecules, such as AGE, ALE, glutenoids, and heterocyclic amines, from food. Furthermore, only a minority of several hundred tested probiotic strains have demonstrated ability to suppress inflammation in the body, when supplemented. Especially desirable strains are those that improve immune function by increasing the number of IgA-producing plasma cells, improve phagocytosis, and the proportion of Th1 cells and NK cells.[114] The genetic differences between different LAB are large, said by some to be larger than those between fish and humans. The choice of probiotics for clinical use is critical, especially as strains that carry the same name have often different and sometimes opposite effects. A recent study selected 46 strains of *Lactococcus lactis* from about 2,600 LAB and compared their ability to induce production of cytokines.[115] Even if the different strains carry the same name, their ability to produce pro- and antiinflammatory cytokines varies widely, which seems to underline the importance of a meticulous choice for clinical studies and use. Some strains, however, are more likely to have strong clinical effects; among them are such strains as *Lactobacillus paracasei* subsp *paracasei, L. plantarum,* and *Pediococcus pentosaceus.* Especially *L. paracasei* has a solid record. It has been shown to induce cellular immunity and stimulate production of suppressive cytokines, such as transforming growth factor beta (TGF-β) and Il-10 and to suppress Th2 activity and CD4 T cells,[116,117] to suppress splenocyte proliferation,[118] and to decrease antigen-specific IgE and IgG_1.[119] *Lactobacillus paracasei* was also shown to be the strongest inducer of Th1 and repressor of Th2 cytokines when more than 100 were compared.[120] A recent study in rats compared the ability of four different strains: *L. paracasei*, *L. johnsonii*, *B. longum*, or *B. lactis* to control *Trichinella spiralis*-induced infection; only *L. paracasei* but not the other LAB was able to reduce the infection-associated Th2 response, muscle levels of TGF-β, COX-2, and PGE2, and attenuate infection-induced muscle hypercontractility.[121] An even more recent study compared three probiotic strains—*B. lactis* NCC362, *L. johnsonii* NCC533, and *L. paracasei* NCC2461—and their effects on stress-induced changes in gut permeability and on sensitivity to colorectal distension. Again, only *L. paracasei* but not the other LAB significantly prevented visceral hyperalgesia, reduced visceral pain, and restored normal gut permeability.[122] However, *L. plantarum* also has an excellent record. When the ability of 50 different LAB to control 23 different *Clostridium difficile* strains was studied, only *L. paracasei* and *L. plantarum* were effective in eliminating all *C. difficile* strains; more than half of the tried LAB strains were totally ineffective, and some against only a few.[123] Some LAB can be

potentiated by simultaneous supply of prebiotic fibers (probiotics + prebiotics = synbiotics), but there are great differences in their ability to utilize semifermentable fibers such as oligofructans. When 712 different LAB strains were tested, only a handful demonstrated ability to ferment inulin and phlein, namely, *L. plantarum* (several), *L. paracasei* subsp. *paracasei, L. brevis,* and *Pediococcus pentosaceus*.[124]

8.13 CONCLUSIONS

Aging and various chronic diseases are all associated with an increasingly deranged function of the neuroendocrine axis resulting in an increased status of systemic inflammation.[125–128] This affects the intestinal microbiota, which become reduced both in diversity and numbers. Continuous supplementation of pro- and synbiotics, as well as plant fibers and antioxidants, provides a promising alternative to suppress systemic inflammation, reduce the risk of developing other chronic diseases or complications to disease, and to considerably improve quality of life. Treatment with lactic specific LAB and plant fibers (Synbiotic 2000) has shown a unique ability to suppress inflammation in animal models—neutrophil accumulation in tissues, release of markers of inflammation: myeloperoxidase, malondialdehyde, nitric oxide—and to prevent destruction of tissues.[129] This offers great hope for the future.

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CHAPTER 9

Probiotics
Recent Human Studies Using Lactobacillus casei *strain Shirota*

Tetsuji Hori

CONTENTS

9.1 INTRODUCTION

9.1.1 Definition of Probiotics

As defined by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) in 2002, probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host." Probiotics (literally "for life") are friendly bacteria or yeasts and are a concept in contrast to antibiotics. Lactobacilli and bifidobacteria are the most common probiotic bacteria, but the yeast *Saccharomyces cerevisiae* and some *Escherichia coli* strains are also used as probiotics. Probiotics can be found in the form of food or dietary supplements in the United States. Potential health benefits from probiotics may vary depending on the type of probiotics consumed.

9.1.2 *Lactobacillus*

Lactobacillus is a genus of Gram-positive, nonspore-forming, catalase-negative, facultative anaerobic or microaerophilic rods, which commonly produce lactic acid as their major metabolite. Lactobacilli are widespread in nature, found in human and other animal digestive systems. At present, more than 125 *Lactobacillus* species have been identified. Some *Lactobacillus* species aid in production of "fermented foods," such as pickles, kimchi (kimchee), cheese, yogurt, and fermented milk. Lactobacilli have been used to enhance the storage stability of foods and improve taste, but recently attention has been paid to their beneficial effects on human health.

9.1.3 *Lactobacillus casei*

Lactobacillus casei is broadly distributed in nature and isolated from dairy products, silage, and the intestinal tracts of various animals. This particular species, *L. casei,* is suggested to have a wide range of pH and temperature. The most common application of *L. casei* is industrial, specifically for dairy production. *Lactobacillus casei* is typically the dominant species of nonstarter lactic acid bacteria used in the manufacture of fermented dairy beverages.

Several stains of *L. casei* have been found, and many aspects of their biological activities have been intensely studied.

9.1.4 *Lactobacillus casei* strain Shirota

In 1930, Dr. Minoru Shirota was at the Microbiological Laboratory of Kyoto Imperial University's School of Medicine, where he successfully cultured a bacterial strain that was able to survive throughout the intestines. This strain, selected from a large collection of lactic acid bacteria, was later named *Lactobacillus casei* strain Shirota and was found to act as a probiotic agent (Figure 9.1).

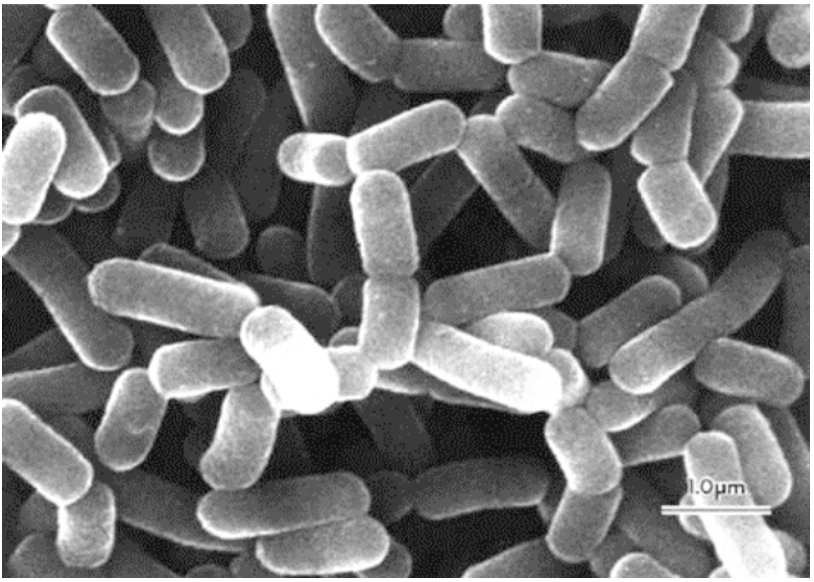

Figure 9.1 Scanning electron microscopy of *L. casei* Shirota. The cells of *L. casei* Shirota are about 0.5 μm in diameter and about 1.5 μm in length (original magnification ×17,000). (Photo courtesy of Yakult Central Institute for Microbiological Research.)

9.1.5 Focus Point in This Chapter

There are more than 70 years of research studies that indicate the various health benefits of regular consumption of *L. casei* Shirota, including regulation of the digestive tract and strengthening of the immune system. Nowadays the research field is expanding more and more to include prevention of infection, allergies, autoimmune diseases, and several cancers.

In this chapter, recent human studies conducted not only in Japan, but also in other countries, are presented. (Please see Reference 1 for more information on the reviews of *L. casei* Shirota.)

9.2 MODIFICATION OF INTESTINAL FUNCTION

9.2.1 *Lactobacillus casei* Shirota Reaches the Intestines Alive and Modifies the Composition of Intestinal Flora in Humans

Matsumoto et al.[2] investigated the effect of consumption of a probiotic milk product containing 4.0×10^{10} cells of *L. casei* Shirota for 2 weeks on the gastrointestinal tract of 40 healthy Japanese subjects. Over 1.0×10^{7} colony-forming units (CFU)/g feces of *L. casei* Shirota was recovered, and the number of bifidobacteria and their

percentage in the total number of fecal bacteria increased significantly compared with the levels before intake.

Tuohy et al.[3] conducted a double-blind, placebo-controlled study with 20 healthy European volunteers to investigate the effect of consumption of two 65 mL bottles of fermented milk (6.5×10^9 CFU of *L. casei* Shirota/bottle) for 3 weeks on the survival of the probiotic in the gastrointestinal tract. After 7 days of fermented drink intake, *L. casei* Shirota was recovered from the test group's fecal samples at $10^{7.1 \pm 0.4}$ CFU/g feces (mean ± SD) and numbers were maintained at this level for 3 weeks (Figure 9.2).

Spanhaak et al.[4] performed a similar study to assess the effect of consumption of a fermented drink containing *L. casei* Shirota in healthy Europeans. The treatment group ($n = 10$) received 100 mL of a fermented milk containing 1.0×10^9 CFU/mL three times a day, while the control group ($n = 10$) was given the same amount of unfermented milk (placebo) in the same manner. As a result, more than 1.0×10^7 CFU/g feces of *L. casei* Shirota were recovered, and the significant increase of *Bifidobacterium* was found in comparison to the placebo group.

Shioiri et al.[5] investigated the effect of consumption of *L. casei* Shirota and transgalactosylated oligosaccharides on the microflora of elderly Japanese subjects. The volunteers were administered a fermented milk beverage containing *L. casei* Shirota at 3.0×10^{10} CFU/bottle and 2.5 g of transgalactosylated oligosaccharides once a day for 2 weeks. By weeks 1 and 2 of ingestion of the fermented milk beverage, the numbers of fecal *Bifidobacterium* and *Lactobacillus* were significantly higher than those of the placebo group. On the other hand, the numbers of fecal lecithinase-positive *Clostridium* and Enterobacteriaceae in the

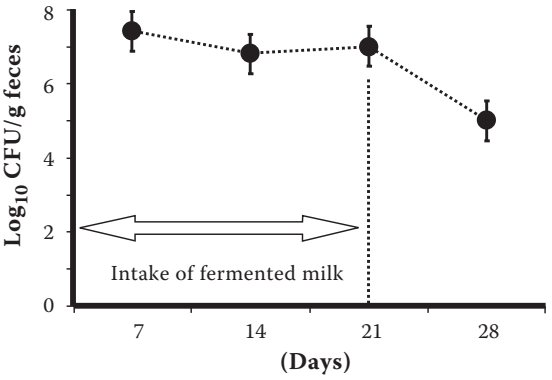

Figure 9.2 Survivability of *L. casei* Shirota in feces by drinking of a fermented milk beverage containing *L. casei* Shirota. Healthy subjects drank two 65-mL bottles of fermented milk for 3 weeks. On days 7, 14, 21, and 28 after subjects stopped drinking sample for 7 days, the fecal numbers of *L. casei* Shirota were measured. Each black circle represents the mean value of Log_{10} CFU/g feces, and each bar expresses standard deviations (error bar). (From Tuohy, K.M. et al., *J. Appl. Microbiol.*, 102, 1026–1032, 2007. With permission.[3])

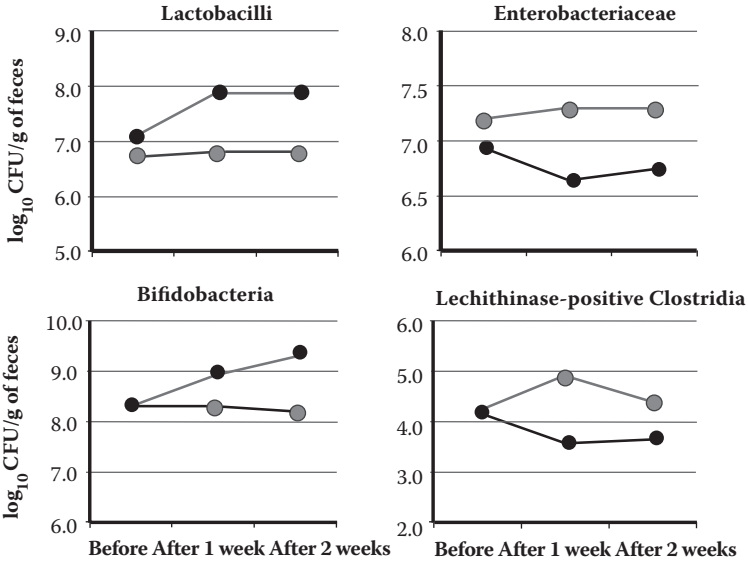

Figure 9.3 Change of the intestinal flora in healthy elderly people by drinking a fermented beverage containing *L. casei* Shirota and transgalactosylated oligosaccharides. Healthy elderly subjects were administered a fermented milk drink beverage (gray circles) or placebo (black circles) once a day for 2 weeks. Before and after intake of a fermented drink or placebo, feces were collected and the number of each bacterium was measured. (From Shioiri, T. et al., *Biosci. Microflora,* 25, 137–146, 2006. With permission.[5])

L. casei Shirota group were significantly lower than those of the placebo group (Figure 9.3).

These results suggest that *L. casei* Shirota reached the intestines alive in both the Japanese and European subjects, and modified the composition of the intestinal flora.

9.2.2 Suppression of the Intestinal Production and Accumulation of Putrefactive Substances

Proteins we ingest are degraded by intestinal bacteria into potentially toxic metabolites, such as ammonia, and phenolic compounds, such as *p*-cresol. These metabolites cause intestinal putrefaction and are related to the pathogenesis of certain diseases. It has also been shown that these metabolites undergo further hepatic transformation, and their metabolites are then excreted in the urine.

To evaluate the effect of ingestion of *L. casei* Shirota on intestinal putrefaction, a randomized, placebo-controlled, cross-over study was conducted on 19 healthy European subjects. Healthy volunteers were administered a probiotic beverage containing 6.5×10^9 cells of *L. casei* Shirota or placebo drink for 2 weeks twice daily.[6] By ingesting *L. casei* Shirota, the urinary excretion of ^{15}N, which is a biomarker of NH_3, and *p*-[$^2H_4$] cresol, were significantly lower compared with ingestion of placebo (Figure 9.4).

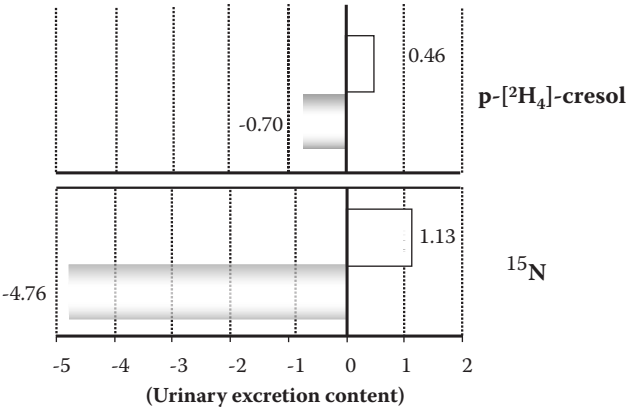

Figure 9.4 Suppression of putrefaction products by intake of *L. casei* Shirota. Healthy subjects ingested a probiotic drink or placebo for 2 weeks twice daily. Before and after intake of probiotic beverage (black bar) or placebo (gray bar), urines were collected and ^{15}N and *p*-[$^{2}H_4$]-cresol were measured. (From De Preter, V. et al., *Br. J. Nutr.*, 92, 439–446, 2004. With permission.[6])

These results suggest that oral administration of *L. casei* Shirota suppressed the intestinal production and accumulation of putrefactive substances, such as NH_3 and *p*-cresol.

9.2.3 Improvement of Bowel Movement

Koebnick et al.[7] investigated the effect of the daily intake of a fermented milk beverage containing *L. casei* Shirota (6.5×10^9 CFU/bottle) on the gastrointestinal symptoms in patients with chronic constipation by conducting a double-blind, placebo-controlled, randomized study in Europe. The consumption of a fermented milk drink containing *L. casei* Shirota for 2 weeks resulted in a significant improvement in the self-reported severity of constipation and stool consistency. At the end of the 4 weeks, although the occurrence and degree of flatulence or bloating sensation did not change, the occurrence of moderate and severe constipation was significantly improved by ingesting a fermented milk drink containing *L. casei* Shirota (Figure 9.5).

It has also been reported that ingestion of 4.0×10^{10} cells of *L. casei* Shirota for 2 weeks was effective for the Japanese subjects to improve defecation frequency, the stool smell, and the feeling of completion of voiding.[2] These results suggest that *L. casei* Shirota improved the state of bowel movements, and may contribute to people's quality of life.

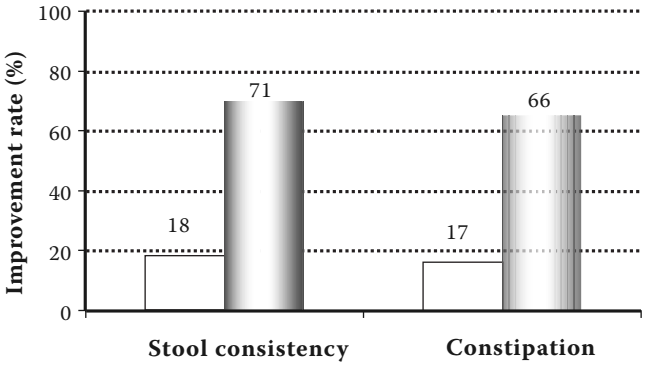

Figure 9.5 Improvement of constipation by drinking of fermented milk containing *L. casei* Shirota. Patients with chronic constipation were administered a 65-mL fermented beverage (black bar) or placebo (gray bar) for 4 weeks. After that, all patients were questioned on gastrointestinal symptoms and stool habits. (From Koebnick, C. et al., *Can. J. Gastroenterol.*, 17, 655–659, 2003. With permission.[7])

9.3 IMMUNOMODULATORY EFFECTS

9.3.1 Normalization of Natural Killer Cell Activity

Natural killer (NK) cells are a type of cytotoxic lymphocyte that constitutes a major component of the innate immune system. NK cells play an important role in the rejection of tumor cells and cells infected by viruses. As for the relationship between the level of NK cell activity and the occurrence rate of cancer, it has been reported that men and women with low NK cell activity were more likely to develop cancer.[8]

Takeda et al.[9] studied whether or not the habitual intake of fermented milk containing 4.0×10^{10} cells of *L. casei* Shirota for 3 weeks would increase NK cell activity in Japanese subjects. This study was conducted on volunteers who had relatively low NK cell activity. The result was that NK cell activity significantly increased, and the elevated NK cell activity was maintained 3 weeks after cessation of intake.

Morimoto et al.[10] investigated the effect of NK cell activity by supplementation of fermented milk containing 4.0×10^{10} cells of *L. casei* Shirota for 3 weeks in Japanese habitual smokers. It has been reported that habitual smoking significantly reduces NK cell activity.[11] By ingesting fermented milk containing *L. casei* Shirota, average NK cell activity in the test group was significantly higher than that of placebo.

On the other hand, Spanhaak et al.[4] has reported that oral intake of fermented milk drink containing *L. casei* Shirota for 4 weeks did not affect the immune system, including NK cell activity in healthy volunteers.

These results suggest that *L. casei* Shirota augmented NK cell activity only in subjects with low NK cell activity, and did not affect healthy subjects with normal NK cell activity. So, it may be important to take *L. casei* Shirota continuously to maintain innate immunity.

9.3.2 Possibilities That *L. casei* Shirota Protects against Allergic Rhinitis

Ivory et al.[12] investigated the effect of the daily ingestion of a fermented milk beverage containing *L. casei* Shirota (6.5×10^9 CFU/bottle) over a period of 5 months on seasonal allergic rhinitis in 20 people by conducting a double-blind, placebo-controlled study. First, the antibody levels of the plasma were measured; next, the peripheral blood mononuclear cells were cultured; and finally, their cytokine levels were measured. By intake of a fermented milk drink containing *L. casei* Shirota, the level of specific immunoglobulin G (IgG) increased while the level of IgE decreased. Furthermore, ingestion of the fermented drink decreased the production of antigen-induced interleukin 5 (IL-5), IL-6, and interferon gamma (IFN-γ). These results suggest that *L. casei* Shirota modulated the immune response in allergic rhinitis, but further studies are needed to investigate the effect of *L. casei* Shirota on allergic rhinitis symptoms.

Tamura et al.[13] conducted a randomized, double-blind, placebo-controlled trial in subjects with allergic rhinitis triggered by Japanese cedar pollen. Subjects were given a fermented beverage containing 4.0×10^{10} CFU of *L. casei* Shirota or a placebo drink for 8 weeks. Consequently, oral administration of *L. casei* Shirota delayed the deterioration of nasal symptoms by 1 week, compared to the placebo group. In comparing the subgroups of mild and moderate-to-severe nasal symptoms, the nasal symptom scores in moderate-to-severe cases in the *L. casei* Shirota group were lower than that of placebo group at 4 and 5 weeks. These results suggest that *L. casei* Shirota may delay the onset of the allergic symptoms in patients with moderate-to-severe scores.

9.4 ANTITUMOR EFFECTS

9.4.1 Preventive Effect on the Recurrence of Bladder Cancer

Aso et al. have reported that *L. casei* Shirota preparation (Biolactis® Powder, BLP, which contains 1.0×10^{10} cells of viable *L. casei* Shirota per gram, Yakult Honsha, Tokyo, Japan) was effective for reducing the recurrence of bladder cancer.[14,15]

Ohashi et al.[16] conducted an epidemiological study on the effect that lifestyle habits (such as smoking or habitual intake of a fermented drink containing *L. casei* Shirota) has on the risk of developing bladder cancer. Smoking was concluded to be a 1.6 times higher risk factor than not smoking, and a frequent intake of this probiotic beverage (once to twice a week) was related to about 50 percent reduction risk of bladder cancer compared to occasional intake of *L. casei* Shirota (once to twice a month).

9.4.2 Preventive Effect on Colorectal Cancer

Ishikawa et al.[17] investigated whether the administration of dietary fiber and *L. casei* Shirota prevented the occurrence of colorectal tumors. The subjects in this

study were 398 Japanese, at the time free from tumor and who previously had at least two colorectal tumors removed. They were randomly assigned to four groups and were administrated wheat bran (7.5 g/day), *L. casei* Shirota preparation (3.0×10^{10} cells/day), both, or neither. The primary end point was the presence or absence of new colorectal tumor(s) diagnosed by colonoscopy after 2 and 4 years. There were no significant differences in the development of new colorectal tumors with administration of either wheat bran or *L. casei* Shirota preparation after 2 years (20 percent risk reduction), but the occurrence rate of tumors with a grade of moderate atypia or higher was significantly decreased by ingestion of *L. casei* Shirota preparation after 4 years (35 percent risk reduction) (Figure 9.6). These results suggested that *L. casei* Shirota may prevent development of colorectal tumors.

9.5 CLINICAL APPLICATIONS

Barrett et al.[18] studied the effect of daily intake of a fermented milk drink containing *L. casei* Shirota (6.5×10^9 CFU/bottle) for 6 weeks on small intestinal bacterial overgrowth (SIBO) of 18 patients with irritable bowel syndrome (IBS). SIBO occurs in up to 78 percent of patients with IBS, and may be directly related to the genesis of IBS symptoms.[19] To evaluate SIBO, a lactulose breath test was conducted. By ingesting a fermented milk beverage containing *L. casei* Shirota, the median time of the first rise in breath hydrogen increased significantly from 45 to 75 min (Figure 9.7). While there was no significant result for bloating, a significant improvement was seen in the passage of wind.

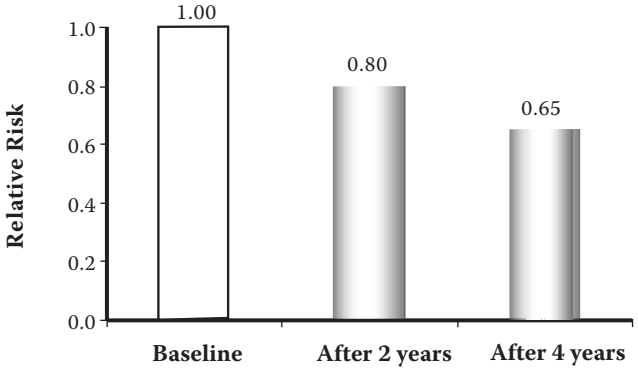

Figure 9.6 Reduction in the risk of colorectal tumors by intake of *L. casei* Shirota. Subjects who had at least two colorectal tumors surgically removed previously were given *L. casei* preparation (Biolactis®), wheat bran, both, or neither. Before the experiment (white bar) and after 2 years (gray bar) and 4 years (black bar), endoscopic investigation was undergone. As a baseline, the risk of not taking *L. casei* Shirota was taken as "1.00." After 4 years, the relative risk was significantly ($p < 0.05$) lower than that of baseline. (From Ishikawa, H. et al., *Int. J. Cancer,* 116, 762–767, 2005. With permission.[17])

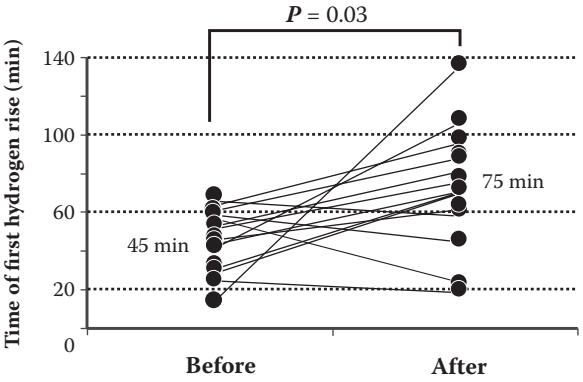

Figure 9.7 Increase of the mean time of first rise of hydrogen in breath by intake of *L. casei* Shirota. Patients with irritable bowel syndrome were administered a fermented milk drink for 6 weeks. Before and after ingestion of a fermented beverage, a lactulose breath test was undergone. The median time of first rise in breath hydrogen before and after intake of probiotic drink was 45 and 75 min, respectively. (From Barrett, J.S. et al., *World J. Gastroenterol.,* 14, 5020–5024, 2008. With permission.[18])

Candy et al.[20] reported a case study that *L. casei* Shirota is effective for the patient with short bowel at 12 months of age. Short bowel syndrome is characterized by impaired digestion and absorption mainly due to extensive bowel resection. The subject ingested 15 mL of a fermented milk beverage containing more than 1.5×10^9 cells of *L. casei* Shirota three times a day. As a result, abundant *L. casei* Shirota was detected from patient's stool after 3 days, stool frequency decreased from 12 to 4 per day, and the concentration of sodium in the urine increased. After 2 years of taking *L. casei* Shirota, the patient's development became normal.

Matsuzaki et al.[21] conducted a study to determine whether or not consumption of fermented milk containing *L. casei* Shirota is effective for patients with human T-cell lymphotropic virus type-1-associated myelopathy (HAM) or tropical spastic paraparesis (TSP). It has been reported that HAM/TSP is a chronic progressive myelopathy.[22] The precise mechanism that causes HAM/TSP is not clear, but it is thought that virus–host immunological interactions are most important in causing this disease. In the study, 10 patients with HAM/TSP were administered 4.0×10^{10} cells of *L. casei* Shirota twice a day for 4 weeks. Significant improvement of urinary symptoms and spasticity were seen after *L. casei* Shirota administration.

Naito et al.[23] evaluated whether or not *L. casei* Shirota could enhance the effect of epirubicin (an anticancer drug). After transurethral resection for superficial bladder cancer, patients were randomly administered either epirubicin intravesically or epirubicin intravesically plus oral administration of *L. casei* Shirota preparation (3 g/day) for 1 year. As a result, there were no serious adverse drug reactions in either group, and the 3-year recurrence-free rates in the epirubicin plus *L. casei* Shirota group were significantly higher than that of the epirubicin group (74.6 percent vs. 59.9 percent).

9.6 CONCLUSIONS

Lactobacillus casei Shirota was found to have various biological activities through its use in human trials conducted both in Japan and in other countries. Now, the beneficial effects of *L. casei* Shirota have been acknowledged not only for healthy subjects, but also for patients suffering from various diseases. In some studies, a randomized, double-blind, placebo-controlled clinical trial is needed to definitively prove the effectiveness of *L. casei* Shirota.

ACKNOWLEDGMENTS

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PART III

Physiological Functions of Prebiotics and Probiotics

CHAPTER 10

Prebiotics and Lipid Metabolism

Jonathan E. Teitelbaum

CONTENTS

10.1 INTRODUCTION

Coronary heart disease (CHD) is a major cause of morbidity and mortality in industrialized countries. Several epidemiologic and clinical studies reveal a positive correlation between elevated total serum cholesterol levels, mainly reflecting the low-density lipoprotein (LDL) cholesterol fraction, and risk of CHD.[1] Specifically, large-scale epidemiologic surveys suggest that elevated fasting triglyceride levels are associated with a greater risk of CHD, and that this effect is independent of any association with high-density lipoprotein (HDL) cholesterol.[2] Elevated postprandial triglyceride concentrations may also predict CHD risk.[3,4] Intervention studies have

then gone on to prove that reduction in total plasma cholesterol levels in patients with primary hypercholesterolemia can lower the incidence of coronary thrombosis.[5]

Various drugs have been developed to regulate cholesterol metabolism based on our current understanding of the key enzymes, receptors, and transporters in cholesterol biosynthesis and transfer.[6] In addition, current dietary strategies for prevention of CHD include low fat/low saturated fat diets.[7] Although these diets seem effective, they are difficult to maintain on a long-term basis and their efficacy diminishes over time. Alternative dietary interventions include the use of soluble fibers, soy protein, plant sterols, probiotic bacteria, and prebiotic compounds.[8] The effect of prebiotics on lipid metabolism in animal and human studies has been the subject of various reviews.[9–12] Indeed, a study in which inulin was added to a moderately high carbohydrate/low fat diet was shown to decrease hepatic lipogenesis and plasma triacylglyceride concentrations.[13] More to the point, a study of the effects of inulin on atherosclerotic plaque formation in male apo E deficient mice revealed the prebiotic group to have 32 to 25 percent less atherosclerotic lesion area than controls.[14]

10.2 CHOLESTEROL METABOLISM

Cholesterol is important in cell membranes, as well as acting as a precursor molecule for the synthesis of steroid hormones, vitamin D, and bile salts. It is derived from the diet or synthesized within the body. The typical human diet contains 200 to 500 mg of cholesterol. Cholesterol also enters the intestine via bile (800 to 1,200 mg/day) and desquamated intestinal epithelial cells (300 mg/day). Between 30 and 60 percent of intestinal cholesterol is absorbed, with losses occurring through unabsorbed bile salts or dietary cholesterol, as well as through sebum. Approximately 900 mg of cholesterol needs to be synthesized daily to balance out losses.[15] The principal sites of cholesterol synthesis are in the liver and central nervous system.

The principal plasma lipoproteins are the chylomicrons, very low density lipoproteins (VLDL), LDL, and HDL. Chylomicrons are rich in triglycerides and are secreted by enterocytes into the lacteals of the intestine and enter the blood from lymph. Triglyceride is the principal fat in the diet and is absorbed from mixed micelles formed in the intestinal lumen as fatty acids and monoglycerides after hydrolysis by intestinal and pancreatic lipases. In the enterocyte, triglyceride is resynthesized and complexed with Apo B_{48} to form chylomicrons. Short-chain fatty acids escape this process and enter the portal vein directly. Free cholesterol is largely reesterified and packaged with the triglyceride to form the core of the chylomicron.

Once chylomicrons enter the circulation they come in contact with lipoprotein lipase on the luminal surface of the vascular epithelium of skeletal muscle, adipose tissue, and lactating breast. This enzyme hydrolyzes the triglyceride in the chylomicron which then becomes smaller, cholesterol-rich chylomicron remnants. The fatty acids and monoglycerides released are then taken up by local adiposites, myosites, or hepatocytes. The remnants are also taken up by the liver.

The liver also exports cholesterol to the tissue via secreted VLDL and to a lesser degree as HDL. Triglycerides that cannot be accommodated in VLDL accumulate in

the liver giving rise to fatty liver disease. Once in the circulation, VLDL accepts cholesterol ester from HDL and LDL. This transfer occurs because of CETP (cholesteryl ester transfer protein) in human plasma. Other species, such as the rat, which has lower levels of circulating LDL, do not contain the CETP. Of note, another interspecies difference in cholesterol metabolism is that in humans the liver secretes largely unesterified cholesterol, whereas in the rat it is esterified before secretion. During its circulation, VLDL undergoes progressive removal of triglyceride from its core by lipoprotein lipase leaving smaller cholesterol-rich LDL. The LDL is small enough to cross the vascular epithelium to supply tissues with cholesterol. In the adult human, HDL can transfer excess cholesterol from the tissue back to the liver.

10.3 BILE ACID METABOLISM

Cholic and chenodeoxycholic acids are the two primary bile acids of humans and are synthesized from cholesterol. The first reaction in bile acid synthesis is catalyzed by a liver-specific microsomal cholesterol 7α-hydroxylase. This enzyme is regulated in part by negative feedback of bile acids returning by way of the portal vein during their enterohepatic recycling. However, different bile acids vary in the strength of this negative feedback, so that whereas primary bile acids successfully downregulate synthesis, those with a 7β-hydroxy group, such as ursodeoxycholic acid, do not. Factors that influence cholesterol 7α-hydroxylase activity cause concomitant changes in 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme for cholesterol synthesis. This allows for maintenance of a constant cholesterol pool size. After the synthesis of 7α-hydroxycholesterol, modifications to the steroid nucleus result in oxidoreduction and hydroxylation. The final step is the conjugation of cholic and chenodeoxycholic acids to the amino acids glycine and taurine within peroxisomes.

The final products, referred to as primary bile acids, are secreted in canalicular bile and stored in gallbladder bile. The gallbladder concentrates the bile and releases it into the duodenum during meals. This raises the intraluminal concentration of bile salts above the critical micellar concentration, allowing formation of micelles (macromolecular aggregates with phospholipids and cholesterol). Micelles promote solubilization of nonpolar dietary constituents and assist in the delivery of lipids to the intestinal absorptive surface.

Bile acids are efficiently absorbed in the distal ileum by a carrier-mediated transport mechanism, returning to the liver by the portal vein. The total bile acid pool circulates approximately twice with each meal. Bacterial enzymes metabolize primary bile acids to secondary bile acids with different physicochemical characteristics. 7α-Dehydroxylation of cholic and chenodeoxycholic acids results in the formation of the secondary bile acids deoxycholic and lithocholic acids, which are relatively insoluble and thus poorly absorbed. They make up the largest proportion of fecal bile acids. The large portion (95 percent) of bile acids that are reabsorbed results in feedback inhibition of new bile acid synthesis.

10.4 PROBIOTICS AND LIPID METABOLISM

The Maasai people of Africa consume large amounts of meat, blood, and milk. Despite this atherogenic diet, the incidence of cardiovascular disease is low. It has been hypothesized that it is their consumption of milk fermented with a wild *Lactobacillus* strain that offers protection against disease. A study of these people found that when one group consumed higher amounts of the fermented milk (up to an average of 8.3 L/day) there was a decrease in cholesterol concentration despite an increase in body weight.[16]

Animal studies of the effect of probiotics on lipid metabolism have demonstrated positive results. A study of rats randomized to receive yogurt with or without bifidobacteria found that the total cholesterol of all the rats fed the yogurt decreased. The probiotic group had a notable increase in HDL, and a 21 to 27 percent lower LDL compared to the rats fed whole milk.[17] Gilliland studied pigs on high cholesterol diets and found that supplementation with *L. acidophilus* resulted in a smaller increase in total cholesterol compared to the unsupplemented group. The authors speculated that the bacteria modified the cholesterol within the lumen of the intestine, making it unavailable for absorption.[18] Akalin et al.[19] in a study of rats fed water, yogurt, or *L. acidophilus* yogurt found that the probiotic group had lower total cholesterol concentration after 28 days of feeding, with levels 22 percent lower than controls. By day 56, the difference was 31 percent, with HDL and triacylglyceride being unaffected.[19] Finally, a group compared the cholesterol-lowering effects of a group of bacteria including bacilli, lactobacilli, streptococci, *Clostridium butyrium, Saccharomyces cervisiae,* and *Candida utilis* with those of *L. acidophilus* or *Streptococcus faecalis.* The group of rats receiving the mixture had a greater reduction in cholesterol concentration than did those receiving a single supplement.[20]

Investigations into the cholesterol-lowering effects of probiotics on human subjects reveal conflicting results. While some studies of patients with normal or borderline high cholesterol levels failed to reveal any effect,[21,22] a study in which subjects were randomized to either placebo or *Enterococcus faecium* supplementation did show an effect. The supplemented group had a 6 percent decrease in total cholesterol and a 10 percent decrease in LDL at 6 weeks.[23] A similar study using the same probiotic in 87 normolipidemic men and women found a significant decrease in LDL in the supplemented group after 1 month compared to placebo. However, by 6 months there were no differences in cholesterol reduction between to the two groups.[24] Studies of probiotics in individuals with elevated cholesterol levels also reveal varied results with a study by Bertolami demonstrating a small positive effect on cholesterol and LDL lowering after 2 months,[25] whereas Sessions was unable to prove any effect in a hypercholesterolemic population after 3 months of a probiotic.[26]

The mechanism by which probiotics might lower serum cholesterol levels is unclear. Observations that HMG-CoA reductase in the liver decreased significantly with probiotic consumption points toward a decrease in cholesterol synthesis. Further, increases in the amount of fecal bile acids suggest there is a compensatory

increased conversion of cholesterol to bile acids.[20] The cholesterol-lowering effect seen in culture media is thought to be secondary to precipitation of cholesterol with free bile acids formed by bacterial bile salt hydrolases.[27] Hydrolation of bile salts *in vivo* may also decrease cholesterol. Those bacteria that hydrolyze efficiently lead to faster cholesterol conversion to bile acids, and thus lower serum cholesterol. Indeed, studies demonstrate that bile acids are eliminated faster in normally nourished rats than in germ-free rats.[28]

10.5 PREBIOTICS AND LIPID METABOLISM

As evidence exists that alteration in gut flora via probiotics may reduce serum cholesterol levels, it allows for the study of prebiotics, which encourage the growth of various prebiotic strains, to determine if they too can alter lipid metabolism. This approach holds promise as prebiotic substances are not subject to viability problems and have greater possibilities for incorporation into a wide range of common foods.

10.5.1 Experimental (Animal) Studies

The use of animal models often forms the basis to test theory and allows for the development of future interventional studies in humans. Convincing evidence indicates that the intake of inulin-type fructans and oligofructose has beneficial effects on blood lipid changes in animals. However, lipid metabolism in animals is not identical to that in humans, and the conditions that exist within the laboratory are often more homogeneous as compared to complexity of human studies, which inherently contain more variable factors that cannot be controlled including genetics, diet, bacterial colonization, and compliance.

The addition of inulin-type fructans,[29] fermented resistant rice starch,[30] raw potato, or high amylase cornstarch[31] to the diet of nonobese rats or hamsters fed a high carbohydrate diet resulted in a decrease in hepatic and serum triacylglycerol. Delzenne[32] studied the influence of dietary fructo-oligosaccharides (FOS) on lipid metabolism in rats. Animals were fed oligofructose for 30 days, at a dose of 20 g/100 g food. He reported a large decrease in the concentration of liver and serum triglycerides in the study animals versus controls. The total cholesterol did not change, but there was an increase in HDL/LDL ratio. Similar observations were made by Leverat[33] who fed rats 10 percent inulin by weight. Trautwein et al.[34] fed Syrian hamsters cholesterol-enriched diets containing differing amounts of inulin (8, 12, and 16 percent) for 5 weeks. Significant hypocholesterolemic and hypotriglyceridemic effects were seen, especially at inulin levels of 12 and 16 percent. Alterations in bile acids profiles were seen at all three concentrations. A study in obese Zucker rats fed oligofructose revealed an increase in body weight without a change in serum triglycerides at 7 weeks. However, at 10 weeks there was a 57 percent decrease in hepatic triglycerides versus controls.[35]

10.5.2 Human Studies

Various human studies have been done based on the promising results of those in animals. However, the results are conflicting and differences may be based on study design or patient population studied. A meta-analysis of 15 human studies from 1995 through 2005 on the effects of inulin was associated with a significant decrease in serum triacylglycerides by 7.5 percent. Effects on total cholesterol were not as evident.[36] In addition, human studies typically use lower doses than animal studies as human subjects often report adverse events when given doses greater than 15 g/day. The type of prebiotic used or the study duration does not seem to influence the results. Human effects of prebiotics may also be affected by the fact that inhibition of hepatic fatty acid synthesis, a major site of action for the cholesterol-lowering effects of inulin and oligofructose, is relatively inactive in humans unless a high carbohydrate diet is fed, the subject is obese, or has hypertriglyceridemia. Indeed, individuals with serum cholesterol over 250 mg/dL tend to have the greatest reduction in cholesterol after inulin supplementation.

A study among 12 healthy young men fed 9 g/day of inulin within a ready-to-eat breakfast cereal demonstrated a 27 percent reduction in fasting triglycerides and 5 percent decrease in total cholesterol.[37] There was no effect on the number of bile acid dehydroxylating bacteria in the test subjects, thus arguing against an affect mediated by such bacteria.

However, other studies among healthy individuals failed to show any significant cholesterol-lowering effects. One study by Pedersen et al.[38] on 64 young women involved a randomized, double-blind, cross-over design over weeks using 14 g of inulin as the intervention. The authors reported no differences in serum cholesterol, HDL, LDL, or triglyceride concentration. Two similar studies by Luo et al.[39,40] with 12 young healthy men, or 10 adults with noninsulin-dependent diabetes ingesting 20 g FOS/day for 4 weeks also failed to reveal any significant cholesterol-lowering effects. Similarly, a large study of 215 infants during the first 6 months of life compared cholesterol levels in breastfed, formula-fed, and prebiotic-supplemented groups. There was no difference in serum cholesterol levels of the formula-fed groups with or without prebiotic supplementation.[41] Finally, the long-term, 6 months, administration of 10 g/day of inulin and oligofructose versus placebo to 17 healthy subjects failed to produce a significant cholesterol-lowering effect, and cholesterol synthesis was not altered as there was no change in circulating mRNA concentrations of key regulatory genes of cholesterol metabolism.[42]

The use of prebiotics in humans with elevated cholesterol appears more promising. A study of a synthetic oligofructose in people with noninsulin-dependent diabetes reported an 8 percent reduction in total cholesterol and a 10 percent reduction in LDL after 14 days compared to a control group. Within the group, greater decreases were observed among those who were hypercholesteroloemic.[43] The lack of a cross-over design where subjects serve as their own control brings the results into some question. In a supporting randomized, double-blind, cross-over study in 21 adults

with mild to moderate hypercholesterolemia in which subjects consumed 18 g/day of inulin-containing foods for 6 weeks, there was a significant reduction of 14.4 percent in LDL, and 8.7 percent in total cholesterol comparing the control period and the inulin period. The significance was due to a rise in these levels during the control period rather than a reduction in cholesterol during the inulin period. Thus, the authors suggest that the inulin prevented the increase in cholesterol during the control period.[44]

Furthermore, a study of 54 subjects with moderate hypercholesterolemia consuming 10 g/day of inulin or placebo over 8 weeks revealed no difference in serum cholesterol; however, there was a 19 percent decline in fasting serum triglycerides. This effect was lost 4 weeks after discontinuation of treatment.[45] Another study by Causey on men with hypercholesterolemia also showed a decline in triglycerides with 20 g/day of inulin after 3 weeks.[46]

10.6 MECHANISM BY WHICH PREBIOTICS EXERT THEIR EFFECT ON LIPID METABOLISM

10.6.1 Effects on Hepatic Cholesterol Synthesis

It is commonly accepted that the principal mechanism by which oligofructose and inulin produce a cholesterol-lowering effect is linked to a decrease in *de novo* hepatic lipogenesis.[29] A decrease in the expression of hepatic lipogenic enzymes, reflected by a decrease in fatty acid synthase messenger RNA, has been demonstrated after fructan or resistant starch supplementation. Kok et al.[47] showed that oligofructose supplementation to rats can protect them against the rise in free cholesterol concentrations induced by high-fat diets, without preventing the accumulation of cholesterol in liver tissue. This hypothesis is further supported by the Trautwein et al.[34] study in which there was a reduction in plasma VLDL particles indicating a decreased production and secretion of VLDL. Others have observed a significant postprandial triglyceride lowering effect after administration of oligofructose to male rats fed a standard, fiber-free, or high-fat diet.[48] It has also been shown that FOS reduces serum insulin and glucose,[47] as well as increases intestinal peptides (i.e., GIP and GLP-1)[49] all of which are regulators of hepatic lipogenesis.

10.6.2 Fermentation Products as Mediators of the Systemic Effects

Intestinal breakdown of prebiotics leads to the production of substantial amounts of short-chain fatty acids, mostly acetate, propionate, and butyrate. Butyrate is widely metabolized by erythrocytes, while Wolever[50] found that rectal infusion of short-chain fatty acid fermentation products, acetate or propionate, are absorbed into the blood. When acetate enters the hepatocyte, it is activated by the cytosolic acetyl-coenzyme A synthetase 2, and then enters the cholesterolgenesis and lipogenesis pathways. Conversely, propionate is a competitive inhibitor of the protein that is devoted to the entry of acetate into the hepatocyte, thus decreasing lipogenesis and

cholesterolgenesis. Levrat et al.[33] showed that high levels of propionic acid fermentations were present in the cecum of rats fed moderate amounts of inulin. Similarly, Eberhard et al.[51] showed inulin supplementation in piglets decreased cecal acetate. This suggests that one role of prebiotics or probiotics is to alter the proportion of these breakdown products produced during fermentation. While intriguing, this fact is controversial and does not seem to play a major role in the cholesterol-lowering effects of prebiotics.[9]

10.6.3 Increase in Cholesterol Excretion

Studies suggest that an interruption of the enterohepatic circulation of bile acids and enhanced fecal excretion may have a major impact on the hypocholesterolemic effect of prebiotics. In a study by Vanhoof and Schrijver,[52] normocholesterolemic rats were fed a bread diet with cornstarch or 6 percent inulin in either cholesterol-free diets or diets with 1 percent cholesterol and 0.1 percent cholic acid. There was significant reduction in plasma cholesterol in those rats fed inulin and a cholesterol-free diet. Also seen was a tendency toward greater fecal excretion of neutral steroids. The authors speculated that the greater cholesterol excretion could be due to a decrease in cholesterol absorption as a result of a higher viscosity in the upper intestinal tract. Fecal loss results in higher hepatic cholesterol catabolism. This is supported by an inverse relationship between liver cholesterol concentrations and daily fecal bile acid excretion. Greater excretion is facilitated by a lower cecal pH as seen in those rats consuming inulin. At a lower pH, the amount of soluble bile acids decreases, resulting in less lipid absorption. A similar experiment with hypercholesterolemic rats, however, showed a tendency toward greater bile acid excretion, but no effect on serum or hepatic cholesterol.[52]

In humans, a study of 12 healthy volunteers fed short-chain fructo-oligosaccharides for 4 weeks revealed an increase in fecal cholesterol concentration during ingestion, and a return to baseline 4 weeks after completion of the study. This was correlated with a rise in the number of fecal bifidobacteria and a decrease in fecal pH during the study period, with a subsequent return to baseline.[53]

10.6.4 Effect on Bacterial Flora

Most prebiotics promote lactic acid–producing bacteria. As previously discussed, in animals the use of fermented dairy products to lower cholesterol has been demonstrated. The combination of different types of bacteria, such as *Lactobacillus acidophilus, L. casei,* and *Bifidobacterium bifidum,* may be responsible for the cholesterol-lowering action of dairy products.[54] Although animal studies appeared promising regarding the ability of probiotics to lower cholesterol, their effect in humans is unclear. The mechanisms by which probiotics exert an effect were previously discussed.

10.7 CONCLUSION

The data available at present are still inconsistent regarding whether prebiotics have a significant cholesterol-lowering effect in humans although overall they may lower triacylglycerides. However, animal models do seem to indicate that intake of moderate amounts of inulin or oligofructose affect lipid metabolism. The difficulty in demonstrating an equivalent effect in humans may be species or dose related. There does seem to be a greater effect of prebiotics in those individuals with elevated baseline cholesterol levels as opposed to those with normal levels. Clearly, more research will be needed to further define the role of prebiotics in manipulating lipid metabolism in humans. Studies need to investigate the mechanism by which these products exert their action, as well as build on preliminary data suggesting the efficacy of synbiotics in lowering serum lipds.[55]

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CHAPTER 11

Fermentation of Prebiotics and Short-Chain Fatty Acid Production

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CONTENTS

11.1 INTRODUCTION

The concept of prebiotics has gained much attention in recent years as evident in the scientific literature and the emergence of functional foods marketed with health benefits associated with its prebiotic properties. Prebiotics and other nondigestible carbohydrates (including dietary fiber) are fermentable substrates that have been associated with favorable effects on both colonic and systemic health.[1] Furthermore, specific end products of bacterial fermentation, such as the short-chain fatty acids (SCFAs), have also been associated with reducing the risk of gastrointestinal disorders, cancer, and cardiovascular disease (CVD).[2] Therefore, the purpose of this chapter is to discuss the benefits of prebiotic fermentation and SCFA production.

11.2 PREBIOTICS AND FERMENTATION

Gibson and Roberfroid have refined their original definition of a prebiotic whereby "a prebiotic is 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."[3] The original definition of a prebiotic took into account only the associated microbial changes in the colon;[4] however, the current proposed definition considers the additional health benefits associated with the targeted stimulation of particular microorganisms. Any substrates or food components that are not digested may enter the colon intact and be a potential prebiotic. However, to be classified as a prebiotic, three criteria must be met. These include (1) resistance to gastric acidity, digestion, and absorption; (2) fermentation by intestinal microflora; and (3) selective stimulation of the growth and/or the activity of those intestinal microflora that contribute to the health and well-being of the host.[5]

To date, much of the interest in prebiotics has been focused on nondigestible oligosaccharides, specifically inulin-type fructans, such as inulin and oligofructose, which meet all three criteria for classification as prebiotics. Inulin-type fructans are oligo- or polymers of D-fructose joined by β(2-1) bonds with an α(1-2) linked D-glucose at the terminal end. Oligofructose is referred to those with degrees of poly-merization (DP) between 3 to 10, and inulin to those with a DP between 10 and 65.[6] Other possible candidates, such as gluco-oligosaccharides, isomalto-oligosaccharides, lactosucrose, polydextrose, soybean oligosaccharides, and xylo-oligosaccharides, are being investigated for their prebiotic activity.[5]

The nondigestible and fermentable nature of inulin-type fructans has been shown to selectively stimulate the growth of specific bacteria that are beneficial to health, especially bifidobacteria and lactobacilli, which have defined metabolic functions.[7] In studies involving patients with ileostomies, inulin and oligofructose have been shown to be resistant to hydrolysis and 88 and 89 percent, respectively, are recovered in the effluent in the intact unhydrolyzed form.[8,9] Furthermore, inulin and oligofructose are not recovered in the feces suggesting they are completely fermented in the colon.[10,11] This is supported by studies using various *in vitro* fermentation systems, with mixed or pure cultures of human fecal microflora, demonstrating that the fermentation of both inulin and oligofructose result in the selective stimulation of bacterial growth, specifically bifidobacteria.[12–14] In a study by Gibson et al.,[15] intake of 15 g/day of oligofructose or inulin for 15 days resulted in a significant increase in bifidobacteria from 8.8 to 9.5 $\log_{10}$/g stool and 9.2 to 10.1 $\log_{10}$/g stool, respectively. The total bacterial counts remained unchanged indicating that the increase in bifidobacteria resulted in a shift in the balance of microflora in the large intestine, where decreases in bacteroides, clostridia, and fusobacteria were observed.[15] Numerous human studies with varying dose, substrate, duration, and subject population have also resulted in similar outcomes of increased fermentation and bifidobacteria.[15–22] Furthermore, increases in breath hydrogen excretion, as an indirect marker of colonic fermentation, have also been observed with intake of oligofructose and inulin.[11,15,23] It has

been suggested that prebiotic intake of about 5 to 20 g/day is sufficient to induce a significant increase in colonic microflora.[1,3,24]

11.3 SCFA PRODUCTION AND HEALTH

The major end products of colonic fermentation of nondigestible carbohydrates are production of SCFAs (acetate, proprionate, and butyrate), gases (CO_2, CH_4, and H_2), heat, and bacterial cell mass.[25,26] Increased SCFA production has been associated with various health benefits including decreased pH, which may reduce the potential pathogenic bacteria, decreased bile acid solubility, increased mineral absorption (indirectly), and reduced absorption of ammonia by protonic dissociation of ammonia and other amines (i.e., conversion of the diffusible NH_3 to less diffusible NH_4^+).[1,26–29] In general, fecal SCFA production is in the order of acetate > propionate > butyrate in a molar ratio of ~60:20:20, respectively.[30] However, the relative ratio between the three primary SCFAs is dependent on a number of factors, including the number and types of microflora present in the colon,[1] type of substrate,[2] and gut transit time.[2,31,32] SCFAs produced in the colon are efficiently absorbed, where as little 5 to 10 percent are excreted in the feces.[1,33–35]

11.4 ACETATE

Acetate is readily absorbed in the colon where 50 to 70 percent of the absorbed acetate is taken up by the liver and the remainder enters the systemic circulation.[1] As a result, acetate is often used to monitor colonic events in human studies because it is the main SCFA in blood. The presence of acetyl-coenzyme A (CoA) synthetase in the cytosol of adipose tissue and mammary glands allows the use of acetate for lipogenesis once it enters the systemic circulation.

Acetate is the primary substrate for cholesterol synthesis and has been associated with hyperlipidemia. Subjects given rectal infusions of acetate and propionate in equivalent ratios showed a dose-dependent increase in serum total cholesterol and triglyceride, providing indirect evidence that SCFA is utilized for lipid synthesis.[36] However, the methodology used in this study may have resulted in greater than physiological levels of acetyl-CoA from the rapid uptake of acetate. As a result, SCFA may have been diverted from oxidation to lipid synthesis.[37] It is possible that substrate-dependent SCFA produced by fermentation inhibits cholesterol synthesis.[38,39] However, uniform agreement has not been reached on the effect of increased colonic fermentation on lipid metabolism, because the possibility exists that different substrates of varying chemical composition and properties may produce different effects.[36, 40]

The intake of resistant starch has been shown consistently to raise fecal butyrate levels.[41–44] Fermentation of starch primarily yields acetate and butyrate, whereas fermentation of pectin and xylan yields acetate alone as the main product.[45] Human studies found that acute ingestion of nondigestible monosaccharide,

L-rhamnose (25 g), yields more propionate relate to acetate,[46] but longer-term studies have not shown reductions in serum lipids.[47] Lactulose, a rapidly fermented dietary fiber, has been shown to increase colonic fermentation and serum cholesterol compared to a control group that did not receive the intervention.[48] The increase in cholesterol may be a result of increased production and absorption of colonic acetate, which is a substrate for increased hepatic lipogenesis.[48] Other substrates such as psyllium, which are viscous fiber sources, are less fermentable and have been shown to be very effective in reducing serum lipids.[38,49] This effect may be related to the increase in fecal losses of bile acids. These fermentable substrates may also generate propionate,[37,38] which have been suggested to reduce serum cholesterol levels by offsetting the hyperlipidemic effect of acetate generation. However, results from human studies have been inconsistent. Intakes of 2.7 g of sodium propionate given in bread[50] and 7.5 g of sodium taken as capsule[51] did not affect serum lipids. Only one study showed that 5.4 g of propionate given daily for 2 weeks decreased total cholesterol and low-density lipoprotein cholesterol (LDL-C) in subjects with total cholesterol > 5.5 mmol/L.[52] In healthy young men and women, rectal infusions of propionate (180 mmol) did not affect serum lipids or triglycerides.[53] However, when propionate (60 mmol) was infused with acetate (180 mmol), free fatty acids decreased by an additional 10 percent and negated the increase in total and LDL-C seen when acetate was given alone.[53] Therefore, it appears that the ratio of propionate to acetate may be one of the mechanisms of action by which propionate reduces serum lipids.[53–56]

11.5 PROPIONATE

Propionate is produced through two main pathways: (1) fixation of CO_2 to form succinate, which is subsequently decarboxylated (the "dicarboxylic acid pathway"); and (2) from lactate and acrylate (the "acrylate pathway").[26] Propionate is a substrate for hepatic gluconeogenesis and has been associated with the inhibition of cholesterol synthesis in hepatic tissue.[51] However, propionate appears to have two competing and opposing effects on gluconeogenesis. It is both a substrate for gluconeogenesis and an inhibitor of gluconeogenesis. Propionate enters the Krebs cycle at the level of succinyl CoA. The inhibition of gluconeogenesis by propionate may be related to its metabolic intermediaries, methymalonyl CoA and succinyl CoA, which are specific inhibitors of pyruvate carboxylase.[57] Propionate enhances glycolysis, possibly by depleting hepatic citrate,[58] which is an important metabolic inhibitor of phosphofructokinase. Propionate may also have an indirect effect on hepatic glucose metabolism by lower concentrations of plasma free fatty acids, which, in itself, is known to be closely related to the actual rate of gluconeogenesis.[59] Much of the knowledge about the nutritional fate of propionate comes from studies of ruminants. Due to the presence of microbiota in the rumen of ruminants, which digest and ferment carbohydrates, intestinal glucose update is minimal. Therefore, the production of SCFA constitutes the major source of ruminant energy,[60] where propionate is

the primary precursor for gluconeogenesis. However, in humans, the metabolism of propionate is less well understood.

Propionate may also have systemic effects in humans, including a potential hypolipidemic action. Results from animal studies suggest that propionate inhibits cholesterol synthesis by inhibiting both 3-hydroxy-3-methylglutaryl-CoA synthase and 3-hydroxy-3-methylglutaryl-CoA reductase.[61,62] As mentioned earlier, polyfructans are bifidogenic and may improve the acetate:propionate ratio, which is associated with a reduction in serum lipids. The use of polyfructans (e.g., Neosugar, inulin) in individuals with type 2 diabetes mellitus (8 g/day)[63] and hyperlipidemia (18 g/day)[64] resulted in decreases in serum cholesterol. However, no hypolipidemic effect (20 g/day) was observed in healthy subjects.[65] Other studies have also investigated the effect of polyfructans on blood lipids in the dose range of 8 to 20 g/day, but have yielded inconsistent results.[66] This inconsistency in human intervention studies, in contrast to animal experiments, may be related to species differences. Numerous mechanisms have been proposed to be responsible for the observed lipid-lowering effect, with increased production of propionate being one of the possible mechanisms of action. Increased production of propionate, through fermentation, may inhibit hepatic cholesterol synthesis.[39,54,61,67–69] This effect has been supported in studies with hyperlipidemic experimental animals,[38,39] but not supported in other animal studies.[70–72] To date, there are limited experimental studies in humans that have quantified the production of acetate and propionate specifically related with the use of prebiotics. Absorption of propionate from the human colon is more efficient than acetate,[73,74] and studies in ruminant mucosa show that propionate is activated to its CoA derivative (a step required for its oxidation) to a greater extent than acetate.[75] During a single pass, the liver extracts 90 percent of propionate, as opposed to 75 percent of acetate[76,77] and colon infusions of equal amounts of acetate and propionate suggest that the amount of colonic propionate reaching peripheral blood is only 25 percent of the amount of colonic acetate doing so.[53]

11.6 BUTYRATE

Butyrate is an important SCFA not only as the preferred fuel of the colonic epithelial cells, but it also plays a major role in the regulation of cell proliferation and differentiation and may be beneficial for inflammatory bowel disease.[1,25,78,79] It is estimated that 70 to 90 percent of butyrate is metabolized by the colonocyte, thus making it the most important SCFA in colonocyte metabolism.[2] Butyrate is used preferentially over propionate and acetate in a ratio of 90:30:50,[2] and is preferred over glucose or glutamine supplied by blood.[80] More than 70 percent of the oxygen consumed by human colonic tissue is due to butyrate oxidation. Sodium butyrate exerts an antiproliferative effect on many cell types, and evidence from animal and cell line studies suggests that it also has preventive effects on colon cancer and adenoma development.[81] Similar effects have been shown with acetate and propionate where apoptosis was induced in colorectal tumor cell lines, but to a much lesser extent than butyrate.[82,83] Butyrate also stimulates immunogenicity of cancer cells.[84]

The mechanisms of action of butyrate on colon cancer are not clearly defined. It has been suggested that butyrate induces p21WAFI/Cip1 protein and mRNA levels.[85–87] As a result, cell cycle is blocked at G1 leading to the inhibition of cell proliferation. The blockage of cell cycle at G1 may allow DNA checkpoint-mediated repair of genomic instability or mutations.[88] Through the inhibition of histone deacetylase, butyrate has been shown to induce apoptosis through hyperacetylation of histones (H3 and H4),[89] resulting in the DNA being in a more open form.[90] Ideally, the open form of the DNA would be necessary if DNA damage had occurred and repair enzymes were needed to approach the damaged DNA. However, the open form of the DNA may be more susceptible to mutation in the presence of a carcinogen.[91] The inhibition of histone deacetylase by butyrate may have a role in reversing epigenetic events.[92] Butyrate can also induce differentiation of neoplastic colonocytes *in vitro*, producing a phenotype typically associated with normal mature cells.[92]

Accumulation of SCFAs decreased the colonic pH, which results in reduced solubility of free bile acids. This drop in pH decreases the production of secondary bile acids, which have potential tumor-promoting activity.[93] Furthermore, increased colonic acidification (pH below 6 to 6.5) may inhibit colonic bacterial enzyme 7α-dehydroxylase, which degrades primary bile acids to secondary bile acids.[94] The decreased colonic pH also increases the availability of calcium for binding to free bile acids and fatty acids, rendering them insoluble.[95]

In vitro and *in vivo* studies have shown that butyrate is the preferred energy substrate and stimulates cell proliferation in normal colonocytes,[78,79] yet it suppresses proliferation of colon adenocarcinoma cells. This observed inconsistency has been termed the "butyrate paradox."[88,91] Possible reasons for this discrepancy may be differences between *in vitro* and *in vivo* environments, the timing of butyrate administration in relation to the stage of cancer development, the amount of butyrate administered, the source of butyrate (i.e., different dietary fibers), and interaction with dietary fat.[91]

SCFA enemas, especially with butyrate, have also been used as a possible treatment for bowel inflammation, including diversion and ulcerative colitis. It has been demonstrated that colonocytes of individuals with active and quiescent ulcerative colitis have reduced butyrate oxidation compared with controls.[96] Harig et al.[97] administered a SCFA enema solution of sodium acetate (60 m*M*), sodium propionate (30 m*M*), and sodium *n*-butyrate (40 m*M*) to five patients with diversion colitis for a period of 2 to 6 weeks. This study was the first to provide evidence that an absence or near absence of SCFAs resulted in rectosigmoid colitis, suggesting that a local nutrient deficiency resulted in a state of inflammation. The use of either surgical reanastomosis or SCFA irrigation to resupply nutrients led to marked improvements by endoscopic appearance and histologic findings. However, another study using the same SCFA enema solution in 13 patients with diversion colitis resulted in no endoscopic or histologic changes after 2 weeks.[98] SCFA irrigation for the treatment of distal ulcerative colitis has also produced inconsistent results,[99] some showing it to be an effective treatment,[100–102] whereas other have not.[102,103] Possible explanations for the inconsistencies include type of SCFA used (mixture or butyrate alone), SCFA concentrations, frequency of administration, and duration of treatment.

Many mechanisms of action have been proposed to explain the use of SCFA irrigation as a possible treatment of bowel inflammation. These include a lack of luminal SCFAs (i.e., a nutritional deficiency of colonic epithelium) and a block in the uptake or oxidation of SCFA by colonocytes,[104,105] possibly related to a reduction in CoA which is required for fatty acid (SCFA) oxidation.[96] It has been suggested that the latter may result from the production of sulfur-containing compounds by colonic microflora.[106] However, this block in uptake and oxidation may be overcome by "mass action," in other words, by raising SCFAs to higher than normal concentrations in the colonic lumen.[105] Overall, the use of SCFA irrigation as a treatment for bowel inflammation still remains inconclusive and further research needs to be pursued.

11.7 CONCLUSION

The fermentable nature of nondigestible carbohydrates, specifically the inulin-type fructans (i.e., inulin and oligofructose), may have significant implications for systemic health. In particular, the end products of fermentation, specifically the SCFA end products, have been associated with reducing the risk of developing gastrointestinal disorders, certain cancers, and cardiovascular disease. However, currently there are limited human studies quantifying the alterations in SCFA production from intake of prebiotics and its link to outcomes that reduce the risk of chronic disease. Further studies in this area will contribute to the growing body of evidence supporting the health-promoting aspects of prebiotics as a functional food.

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CHAPTER 12

Probiotics and Prebiotics in Inflammatory Bowel Disease

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CONTENTS

12.1 INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) refers primarily to two major disorders, ulcerative colitis (UC) and Crohn's disease (CD), but is a collective term for a group of intestinal conditions characterized by uncontrolled inflammation in the

gastrointestinal (GI) tract. IBD is most prevalent in North America and Europe (1.4 and 2.2 million sufferers, respectively)[1] while other, previously low-incidence areas have reported an increased occurrence in recent years.[2] Environmental factors, such as diet and degree of sanitation, are believed to play a role in the development of IBD.[3] There is also a large body of evidence suggesting a genetic predisposition to IBD, with genes such as CARD15/NOD2, OCTN1 and 2, and DLG5 all linked to the development of IBD.[4] The role of genetics has been comprehensively reviewed by Henckaerts and colleagues.[3] Although the exact etiology of IBD remains unknown, it is believed to be the result of a dysfunctional interaction between the gut microbiota and the mucosal immune system.[6] While many speculate both CD and UC may be instigated by similar mechanisms, there are a number of differences between the two conditions. UC occurs primarily in the colon, extending proximally from the rectum.[7,8] It is characterized by continuous inflammation of the colon, superficial mucosal inflammation, increased neutrophil presence in the lamina propria and crypts, and the production of proinflammatory mediators such as interleukin (IL)-12 and tumor necrosis factor (TNF)-α.[9,10] CD is characterized by the aggregation of macrophages which promotes the formation of noncaseating granulomas.[11] In contrast to UC, CD can occur in any region of the GI tract, but is most common in the terminal ileum.[7] CD lesions often present as patchy, typically transmural, inflammation.[12] In addition to UC and CD, other conditions including collagenous colitis, lymphocytic colitis, and Behçet's syndrome are classified as IBD. Symptoms of IBD include abdominal pain, GI bleeding, malnutrition, and bloody diarrhea[13]; extraintestinal manifestations have also been reported, and can include disorders of the liver, lungs, eyes, and joints.[14] The mortality rates are 1.4 percent and 1.0 percent for CD and UC, respectively.[7] Common therapies for IBD, including 5-aminosalicylates, antibiotics, steroids, and growth factors, have been comprehensively reviewed by Kozuch and Hanauer.[11]

Although the exact pathogenesis of IBD remains unknown, four mechanisms have been proposed to initiate the disorder. The first theory suggests that microbial pathogens (e.g., mitogen-activated proteins) are detected by the host immune system, which initiates an inflammatory immune response.[12] The second theory proposes that an imbalance between commensal and pathogenic bacteria in the microbiota leads to a reduced ratio of protective: aggressive bacterial species, as well as reducing the availability of short-chain fatty acids (SCFAs), the primary energy source for colonic epithelial cells.[12] Defective host-immunoregulation is the third possible mechanism in which the host immune system is unable to distinguish between harmful and commensal bacteria.[12] It is unclear what event would act as a trigger in this scenario, as a defective immune system could be present from birth, although the disease may not manifest until later in life. Environmental factors could trigger these events in a genetically susceptible host. This would elicit an immune response against commensal bacteria and disrupt gut homeostasis. Finally, host genetic defects leading to defective bacterial killing and mucosal barrier function have been proposed.[12] Increased permeability of the epithelial barrier facilitates the transfer of harmful luminal antigens into the surrounding intestinal tissue, while

defective bacterial killing would reduce the ability of the host to control pathogen levels in the gut.

The above hypotheses describe mechanisms by which the intestinal bacteria and epithelium initiate the pathogenesis of IBD. As the intestinal microbiota appears to play a significant role in IBD, its manipulation has been identified as a potential therapeutic option. Previous studies have demonstrated the ability of probiotics to modify and improve the intestinal environment and subsequently reduce the severity of intestinal inflammation associated with IBD.[12,14,15]

12.2 PROBIOTICS

Probiotics are defined as living, nonpathogenic microorganisms that exert a positive influence on host health and/or physiology when ingested.[16] Probiotics have demonstrated efficacy for a number of inflammatory conditions, including arthritis, vernal keratoconjunctavitis,[17] necrotizing enterocolitis,[18] intestinal mucositis,[19] UC,[20,21] CD,[22] and atopic eczema.[23]

The mechanisms underlying the beneficial effects of probiotics are not completely understood. Numerous bacterial strains have been identified as probiotics, many of which differ markedly in their mode of action. The mechanisms of probiotic action are numerous and the activities of these strains can also be dependent on a number of other factors including the presence of other bacteria in the intestinal environment, or even the disease setting in when the strain is being used.[24]

There are, however, some common mechanisms of action that have been reported for a majority of probiotic strains (Table 12.1). One general mechanism is the adherence of the probiotic to the intestinal epithelium, which not only stimulates the immune system but also reduces pathogen colonization and subsequent infection.[25] Evidence for this mechanism has been demonstrated in various *in vitro* systems, for example, *Lactobacillus rhamnosus* GG, *L. rhamnosus* LC705, *Bifidobacterium breve* 99, and *Propionibacterium freudenreichii* ssp. *shermanii* JS have all been demonstrated to reduce the adhesion of a number of pathogenic species to human intestinal mucus.[25] The ability of probiotics to modulate cell proliferation and apoptosis is also common among different species. Intragastric administration of 10^8 or 10^9 colony

Table 12.1 Common Mechanisms Involved in the Beneficial Effects of Probiotics

Stimulation of the host immune system[25,28–31]
Reduction of pathogen colonization[25,28–32]
Modulation of cell apoptosis-to-proliferation ratio[26]
Downregulation of proinflammatory cytokines[14,33–38]
Stimulation of antiinflammatory cytokines[39]
Elimination of microbial pathogens[40–42]
Maintenance of intestinal barrier function[43,44]
Provide energy source for colonic enterocytes through SCFA production[45]

forming units (cfu)/mL of *L. rhamnosus* was shown to significantly decrease the cell apoptosis-to-proliferation ratio in ulcerated rat gastric epithelium.[26] The reduction of this ratio was hypothesized to occur due to upregulation of ornithine decarboxylase and B-cell lymphoma 2 (growth factors critical to ulcer healing).[26] *Lactobacillus rhamnosus* GG has been found to increase epithelial cell proliferation in the small intestine and distal colon of rats,[26] facilitating repair of epithelial damage. This was most likely the result of polysaccharide fermentation by the probiotic strain, thus increasing SCFA availability for the epithelial cells.[27] Stimulation of the mucosal immune system is a further mechanism, with evidence suggesting that some probiotics have potential antiinflammatory properties.[14] Lorea-Baroja et al.[14] describe a number of potential mechanisms for the antiinflammatory effect of probiotics, such as modulation of the balance between T-helper 1 (Th1), Th2, and regulatory T (T_{reg}) cells; downregulation of proinflammatory cytokine production (e.g., IL-12, TNF-α) and/or stimulation of antiinflammatory cytokines (e.g., IL-10); enhanced elimination and permeation of proinflammatory antigens; and as a response to antagonism against potentially pathogenic or proinflammatory endogenous bacteria.[14] It is likely, however, that there are further mechanisms of action that have not yet been elucidated, as such, a wide range of candidate strains continue to be screened *in vitro*, *in vivo,* and in clinical trials.

12.2.1 Probiotics in IBD

12.2.1.1 In Vitro Models

There has been a recent increase in the number of comprehensive cell culture experiments investigating the effects of probiotics using *in vitro* model systems of IBD. Miyoshi and colleagues investigated the relationship between mucus adhesion-promoting protein (MapA) and *L. reuteri* in Caco-2 cells.[46] *Lactobacillus reuteri* has been shown to attenuate visceral pain[47] and moderate diarrhea,[48] but the mechanism behind the adhesion of the bacteria to the GI tract was previously unknown. This study demonstrated that MapA plays a key role in the adhesion of *L. reuteri* as it binds to receptor-like molecules on the Caco-2 cells, as well as revealing the existence of multiple receptor-like molecules in Caco-2 cells, which may also be involved.[46] Further studies could involve competitive binding assays between *L. reuteri* and pathogenic bacteria to determine whether this is a mechanism by which *L. reuteri* exerts its beneficial effect. In addition to competitive binding, a recent study has identified production of the potent, broad-spectrum antimicrobial compound reuterin as another mechanism by which *L. reuteri* could exert a beneficial effect in the GI tract.[41] Four *L. reuteri* strains were investigated, and each produced different amounts of reuterin. The reuterin derived from each strain was then shown to inhibit the growth of pathogenic bacteria (enterohemorrhagic and enterotoxigenic *Escherichia coli*, *Salmonella enterica*, *Shigella sonnei,* and *Vibrio cholera*) to a similar extent, indicating no strain specificity. Live *L. reuteri* displayed greater pathogen-inhibitory activities than reuterin alone, indicating that other microbial factors

were likely to be important for the inhibition of bacterial pathogens; and that future studies should focus on isolating and testing these compounds.

Schlee and colleagues[40] investigated the mechanism via which the antimicrobial human beta defensin-2 (hBD-2) gene (which is important for the maintenance of intestinal barrier function) was induced by the probiotic strains: *L. fermentum* PZ-1138, *L. acidophilus* PZ1138, *E. coli* Nissle 1917, and VSL#3 (a combination of eight bacterial strains).[40] It was determined that hBD-2 induction by probiotic bacteria was both time and dose dependent, and that deletion of the NF-κB and activator protein-1 binding sites on the hBD-2 promoter completely inhibited the probiotic effect. Furthermore, inhibition of mitogen-activated protein kinase (MAPK) also impeded hBD-2 induction. Schlee and colleagues demonstrated that selected lactobacilli and VSL#3 were able to strengthen intestinal barrier function via the upregulation of hBD-2 through the induction of MAPKs and the proinflammatory NF-κB and AP-1 pathways.[40] In addition to improving barrier function, further studies using *L. fermentum* highlight other potentially beneficial effects. *Lactobacillus fermentum* ACA-DC 179 displayed antimicrobial immunomodulatory activity as it reduced *Salmonella enterica* viability and increased IL-10 levels *in vitro*.[42]

In support of the findings of Schlee and colleagues, *E. coli* Nissle 1917 was also demonstrated to improve intestinal barrier function, although this effect was detected in an *in vitro* model of intestinal inflammation induced by an *E. coli* challenge.[43] Following DNA micro-array analysis, Nissle 1917 has been shown to alter both the distribution and expression of zonula occludin (ZO)-2 proteins and a number of protein kinase C isotypes; both of which are involved in the maintenance of tight junctions within the epithelial barrier. Although it is possible these changes occurred in conjunction with the effect on hBD-2 observed by Schlee and colleagues, the findings of this study are potentially of greater relevance to IBD treatment (assuming that microbial pathogens are involved in the disorder) as they occur following pathogen-induced damage to the cell monolayer. In addition to the maintenance of barrier function, Nissle 1917 has also been shown to have an antiinflammatory effect on human epithelial cells *in vitro*.[33] Following the addition of TNF-α, treatment with Nissle 1917 reduced the production of proinflammatory IL-8 without altering transactivation pathways, such as NF-κB activation, nuclear translocation, or nuclear binding. The ability of *E. coli* Nissle 1917 to increase both intestinal barrier function and antiinflammatory cytokine production makes it a promising therapeutic option for IBD. Indeed, clinical trials have been performed and are discussed here.

In addition to reducing pathogen adhesion, Candela and colleagues reported that *Bifidobacterium longum* Bar33 and *L. acidophilus* Bar13 were able to reduce the production of proinflammatory IL-8.[34] Interestingly, the experiments were performed on two different cell lines, with pathogen competition observed in Caco-2 cells, and immunomodulation reported in the HT-29 cell line. Probiotic activity can be influenced by the environment; therefore, further studies should investigate whether these effects are repeatable in multiple cell lines, and whether they are observed *in vivo.* Similarly, Jankowska and colleagues reported that *L. paracasei* IBB2588 reduced adhesion of harmful *S. enterica* to Caco-2 cells,[32] finding that displacement

of pathogens was dependent on the time of bacteria–epithelial cell contact, as preincubation with the probiotic reduced *S. enterica* adhesion sevenfold compared to the fourfold reduction observed following coincubation. Studies involving preincubation are less common than those investigating coincubation; however, these findings suggest a greater need for the former. Future studies should compare the effects of probiotics in these two treatment regimens, as it could identify a method of improving their efficacy. Unfortunately, it is impossible to predict the onset of IBD; accordingly, pretreatment with probiotics may be more beneficial in intestinal disorders, such as chemotherapy-induced mucositis. Somewhat surprisingly, *S. enterica* displayed far greater adherence properties compared to *L. paracasei*, indicating that the reduced adhesion observed following coincubation and preincubation was likely to be due to both competition for epithelial cell receptors and secreted antimicrobial compounds. This was further supported by the inability of the culture supernatant to exert a similar effect.[32]

12.2.1.2 Animal Models of IBD

Numerous published reports describe the beneficial effects of probiotic consumption in both genetically and chemically induced murine models of IBD.[49,50] Ukena and colleagues demonstrated that treatment with the probiotic *E. coli* Nissle 1917 resulted in an upregulation of the tight junction molecule ZO-1 at both mRNA and protein levels, and reduced intestinal barrier permeability in BALB/c mice with dextran sulfate sodium (DSS)-induced experimental colitis.[44] In addition to the upregulation of ZO-1, *E. coli* Nissle 1917 has been shown to reduce proinflammatory cytokine expression, myeloperoxidase (MPO), activity and disease activity in DSS-treated mice.[35] By comparing the efficacy of *E. coli* Nissle 1917 in wild-type and Toll-like receptor (TLR)-2 and TLR-4 knockout mice, this study also determined that the bacteria exerted their beneficial effect via TLR-2 and TLR-4 dependent pathways. TLRs are expressed on numerous cell types in the GI tract and serve to defend against microbial pathogens through four mechanisms: recognition of pathogen-specific molecular patterns, expression at the interface with the environment of the GI lumen, initiation of secretion of either pro- or antiinflammatory cytokines and chemokines, and induction of antimicrobial effector pathways.[51] The inability of *E. coli* Nissle 1917 to exert its beneficial effect in the absence of TLR-2 and TLR-4 signaling indicates that it may improve the ability of TLRs to recognize microbial pathogens, improving the host immune response. *Escherichia coli* Nissle 1917 has also demonstrated efficacy in the trinitrobenzenesulphonic acid (TNBS) model of colitis, where it has been used to significantly reduce visceral hyperalgesia, believed to be involved in the manifestation of a number of GI disorders.[52] This effect was not unique to this probiotic strain; however, as attenuation of visceral pain has been reported in a number of *in vivo* studies using *L. paracasei*,[53] *L. reuteri,*[47] and *L. farciminis*.[54]

Oral administration of *L. plantarum* HY115 to mice with DSS colitis has recently been shown to reduce colon shortening and to inhibit MPO activity and NF-κB activation.[36] Probiotic treatment also inhibited mRNA expression of the proinflammatory

cytokines IL-1β, TNF-α, and interferon (IFN)-γ, reduced protein levels of colonic IL-1β and IL-6, and reduced the bacterial degradation activities of chondroitin sulfate and hyaluronic acid. Similarly, Osman and colleagues described a reduction in disease activity, MPO activity, and bacterial translocation following *L. plantarum* administration in the DSS model of colitis,[55] while Schultz and colleagues reported efficacy of *L. plantarum* in the IL-10-deficient (IL-$10^{-/-}$) model of colitis as indicated by decreased IL-12 and IFN-γ production.[37] Furthermore, Bujalance and colleagues demonstrated the ability of *L. plantarum* to improve immune function in immunocompromised hosts.[56] The various beneficial mechanisms of *L. plantarum* highlight its therapeutic potential in GI disorders, such as IBD.

Peran and colleagues demonstrated the preventative effects of *L. reuteri* and *L. fermentum* in the rat TNBS colitis model.[45] Oral administration of these probiotics reduced colonic inflammation scores, MPO activity, colonic TNF-α expression, and inducible NO synthase expression when compared to untreated rats. Interestingly, only *L. fermentum* treatment lowered colonic cyclo-oxygenase-2 expression and increased SCFA production in the colonic contents, indicating a greater efficacy of *L. fermentum* in the treatment of experimental colitis. These findings are supported by Zoumpopoulou and colleagues who also reported efficacy of *L. fermentum* in a mouse model of TNBS-induced colitis.[42] In a separate study, Peran and colleagues demonstrated the ability of *L. acidophilus*, *L. casei,* and *B. lactis* to reduce intestinal inflammation in the TNBS model.[38] Interestingly, each probiotic displayed a unique antiinflammatory profile: *L. acidophilus* reduced MPO activity and leukotriene B_4 production; *B. lactis* reduced colonic TNF-α production edema; and *L. casei* decreased cyclooxygenase-α expression in the colon. These findings further highlight the different mechanisms by which probiotics can exert their beneficial effects.

IL-$10^{-/-}$ mice spontaneously develop colitis following colonization with conventional flora, and have been frequently used to screen probiotics for therapeutic potential. Neonatal IL-$10^{-/-}$ mice typically possess low levels of colonic lactobacilli, and Madsen and colleagues reported normalization of lactobacilli levels following rectal administration of *L. reuteri*.[57] Furthermore, *L. reuteri* treatment reduced levels of colonic mucosal adherent and translocated bacteria and prevented the development of colitis. Administration of *L. gasseri* (10^9 cfu/mL), a strain that produces high levels of manganese superoxide dismutase (MnSOD, an antioxidant), reduced intestinal inflammation compared to untreated animals in the IL-$10^{-/-}$ model.[58] When compared to wild-type *L. gasseri*, treatment with the MnSOD-producing strain led to significantly lower histological inflammation scores and provides an example of how probiotics can be used as vehicles to deliver therapeutic compounds as well as exerting their own beneficial effects. Both *L. salivarius* UCC118 and *B. infantis* were shown to attenuate the development of colitis in IL-$10^{-/-}$ mice.[28] The authors concluded that this was a result of reduced Th1-type cytokine production, as well as maintenance of transforming growth factor (TGF)-β levels.

Gnotobiotic mice have been used to elucidate the effects of probiotics on the host immune response. Menard and colleagues tested a number of *Bifidobacterium*

strains in gnotobiotic mice, reporting a host of immunomodulatory responses, including induction of Th1 and Th2 cytokines, increased IL-10, IL-4, IFN-γ, and TNF-α secretion and increased TGF-β gene expression.[39] These results further highlight the difficulties involved in isolating the "ideal" probiotic as the influence of probiotics on the immune system may be highly strain specific. The ability to modulate the immune response is characteristic of a number of probiotic strains, with Park and colleagues reporting immunoenhancing effects, including increased numbers of immunoglobulin A^+ cells and $CD4^+$ T cells, in gnotobiotic mice treated with *L. fermentum* PL9005,[29] while Shima and colleagues observed an upregulation of genes involved in immune function following administration of *L. casei* Shirota.[30] The increase in gene expression following *L. casei* treatment was more pronounced in the ileum than in the colon, indicating site specificity for probiotic effects of *L. casei*. Interestingly, *L. casei* was present at greater levels in the colon than in the ileum, suggesting the difference in gene expression may be due to the function of the probiotic changing as a result of being in a different environment. Menard et al.[39] and Park et al.[29] also reported immunomodulatory effects in the small intestine, but did not investigate potential probiotic effects in the colon. With UC typically extending proximally from the rectum, probiotics that could exert their beneficial effects in the colon would likely be most successful as a therapeutic strategy.

Bioengineered probiotics have demonstrated therapeutic capacity in a number of *in vivo* models. Steidler and colleagues reported that *Lactococcus lactis* mIL10, which had been developed to secrete biologically active murine IL-10, was able to reduce histological damage in both the DSS and $IL\text{-}10^{-/-}$ models of colitis.[31] Further studies using this strain showed it to also be successful in preventing food-induced anaphylaxis.[59] *Lactobacillus lactis* has also been engineered to secrete ovalbumin (OVA), a protein used to stimulate allergic reactions.[60] Oral administration of the probiotic in OVA T-cell receptor transgenic mice led to antigen-specific tolerance, indicated by reduced IFN-γ and increased IL-10 levels. Despite their therapeutic potential, there has been limited research into bioengineered probiotics. Their effectiveness will depend greatly on further research into the pathogenesis of IBD. Once this is known, probiotics could be designed to specifically target the trigger, whether it is a specific antigen or a pathogen. If the cause of IBD itself could not be targeted, strains similar to that developed by Steidler et al.[31] could be designed to produce antiinflammatory compounds and, hence, reduce intestinal damage.

12.2.1.3 Human Studies/Clinical Trials

The efficacy of probiotics in the setting of IBD has been investigated in a number of clinical studies; however, there remain an insufficient number of large, randomized, double-blind, placebo-controlled trials that investigate the efficacy of candidate probiotics.[7] Key findings from clinical trials have recently been reviewed comprehensively by Hedin and colleagues.[61]

Promising results involving the use of probiotics in IBD treatment have been reported in the setting of pouchitis. Pouchitis is a nonspecific, idiopathic inflammation

of the ileal reservoir and is characterized by symptoms, such as rectal bleeding, increased stool frequency, abdominal cramping, and fever.[62] Gionchetti and colleagues[62] investigated the use of VSL#3 as a treatment for active mild pouchitis, as defined by a pouchitis disease activity index (PDAI) between 7 and 12. In the study, 23 consecutive patients were treated with two sachets twice a day. According to the PDAI, 3,600 billion bacteria/day for 4 weeks, and symptomatic, endoscopic, and histologic evaluations were taken before and after probiotic treatment. Patient quality of life was also assessed using the Inflammatory Bowel Disease Questionnaire. Of the 23 patients, 16 (69 percent) were in remission following probiotic treatment, and median total PDAI scores, before and after treatment, were 10 and 4, respectively. The median questionnaire score was also improved, from 110 to 200. Patients determined to be in remission were placed on a maintenance treatment regimen consisting of one sachet twice a day (1,800 billion bacteria). None of the 16 patients receiving the maintenance treatment reported relapse of pouchitis within the experimental period.[62] VSL#3 has also been investigated by Bibiloni and colleagues, in the setting of active UC.[13] In this study, 34 patients with active UC were treated with two sachets twice a day (3,600 billion bacteria/day) for a period of 6 weeks. Using the ulcerative colitis disease activity index (UCDAI) as a guide, patients were determined to be in either remission (UCDAI ≤ 2); response (decrease in UCDAI ≤ 3 points, but final score ≥ 3); no response or worsening (increase in UCDAI). Of the 32 patients who completed the trial, 18 (53 percent) were determined to be in remission, while 8 (24 percent) reported a positive response to treatment. No response was reported in 3 (9 percent) patients, and another three (9 percent) reported a worsening of UC.

Positive results have also been observed for UC treatment using the BIO-THREE tablet formulation.[20] Tsuda and colleagues investigated this probiotic combination, which comprised *Streptococcus faecalis* T-110, *Clostridium butyricum* TO-A, and *Bacillus mesentericus* TO-A in 20 patients with mild to moderate distal UC. Patients ingested 9 tablets daily for a period of 4 weeks, with UCDAI scores obtained prior to and following treatment. By using a system similar to that described by Bibiloni et al.,[13] treatment was determined to elicit remission, response, no response, or worsening. Remission was observed in 9 (45 percent) patients, response in 2 (10 percent), no response in 8 (40 percent), and worsening in only 1 (5 percent). Fecal samples were also obtained from patients, with the microbiota analyzed via the terminal-restriction fragment length polymorphism (T-RFLP) method. An increase in bifidobacteria was the principal alteration to the intestinal microflora following probiotic treatment. This was particularly interesting, as no bifidobacteria were administered within the probiotic supplement. The reason for this increase remains unknown, but could represent a consequence of the treatment altering the environment to facilitate the growth of bifidobacteria, perhaps by removal of competing pathogens.

Administration of *E. coli* Nissle 1917 has been reported to both induce and maintain remission of UC in numerous studies.[63–65] In a randomized, double-blind clinical trial of patients in remission from UC, treatment with Nissle 1917 led to relapse rates statistically similar to patients receiving the antibiotic mesalazine.[63] These findings were confirmed in a larger, double-blind, double-dummy trial, during which relapse rates of UC patients receiving Nissle 1917 or melasalazine were

not significantly different.[64] In addition to maintaining remission, Rembacken and colleagues reported that Nissle 1917 administrated to patients with active UC led to similar remission rates to those treated with melasalazine, with mean time to remission, and duration of remission also similar between the two treatment groups.[65]

Mechanistic studies have also been performed in humans to elucidate the mode of action of specific probiotic strains. Lorea-Baroja and colleagues examined the effect of yogurt supplemented with *L. rhamnosus* GR-1 and *L. reuteri* RC-14 on T_{reg} cells, cytokines in T cells, monocytes, dendritic cells (DC), and fecal and serum cytokine concentrations.[14] The proportion of T_{reg} cells increased significantly in patients with IBD both before and after treatment, but no significant difference was observed in controls. Basal proportion of TNF-α^+/IL-12^+ monocytes and myeloid DC decreased in both groups, but only in stimulated cells of patients with IBD. Probiotic treatment significantly decreased serum IL-12 concentration in both controls and patients with IBD, and also decreased serum TNF-α concentration in healthy patients. No significant changes in serum or fecal TNF-α or IL-10 were observed as a result of probiotic treatment.

12.2.1.4 Summary of Probiotics in IBD

Probiotics have demonstrated efficacy *in vitro*, *in vivo*, and in a clinical setting of IBD. However, not all probiotics have decreased disease severity and, indeed, some strains, in fact, have worsened the condition.[15] To gain the maximum benefits from probiotics, a greater understanding of the role of the intestinal microbiota in the pathogenesis of IBD is required. This will facilitate the development of effective microbial therapies as specific targets for manipulation will be identified. Furthermore, detailed studies investigating interactions between probiotics and commensal bacteria are required, as it is unlikely that the effects of a single probiotic would be uniform throughout a population. This knowledge will aid in the identification of the optimal treatment regimen for each patient, and may help to reduce the incidence of disease worsening. Finally, the long-term effects of probiotic treatment and the regimens required for long-term colonization of the GI tract need to be investigated further.

12.3 PREBIOTICS

The use of prebiotics to manipulate the intestinal microbiota offers another potential therapeutic option for IBD sufferers. Prebiotics are defined as "nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one, or a limited number of bacteria in the colon, thus improving host health."[68] A healthy microbiota is predominantly saccharolytic, and contains a high concentration of bifidobacteria and lactobacilli.[69] Treatment with prebiotics can alter the saccharolytic activity of the gut,[70] as well as elevate the number of beneficial bacterial strains present in the microbiota.[71] Improving the health

Table 12.2 Common Mechanisms Involved in the Beneficial Effects of Prebiotics

Reduction of neutrophil aggregation (determined by MPO activity)[73–76]
Stimulation of beneficial bacteria[77,78]
Provide energy source for colonic enterocytes through SCFA production[79]
Downregulation of proinflammatory cytokines[73]
Increased expression of TLRs[80]

of the microbiota has many benefits, including improved barrier function, prevention of mucosal colonization by aerobic enterobacteria, reduced luminal pH, and an increase in SCFA production.[70,72] A number of prebiotics have been demonstrated to be effective in the manipulation of the microbiota. These include inulin, germinated barley foodstuff (GBF), and oligosaccharides, such as oligofructose.[70] Other suggested mechanisms of prebiotic action are listed in Table 12.2. Similarly to probiotics, there is a lack of conclusive clinical studies supporting the use of prebiotics as a treatment for IBD.

12.3.1 Prebiotics in IBD

12.3.1.1 Prebiotics in Animal Models of IBD

Kanauchi and colleagues demonstrated that GBF, a mixture of glutamine-rich protein and hemicellulose-rich dietary fiber, had prebiotic characteristics when tested in the rat model of DSS colitis, where it decreased the incidence of bloody diarrhea and mucosal injury.[81] Furthermore, GBF has been shown to be more efficacious than a probiotic mixture of lactobacilli and *Clostridium butyricum*.[82] Active hexose correlated compound (AHCC) demonstrated prebiotic activity as evidenced by an antiinflammatory effect in the TNBS model of colitis in female Wistar rats.[73] Administration of AHCC to rats 2 days prior to TNBS challenge led to increases in body weight and food intake; reduced colonic inflammation, expression of proinflammatory cytokines, MPO activity; and improved the colonic weight-to-length ratio and intestinal damage score. While TNBS treatment increased colonic clostridia levels, AHCC-treated rats had increased aerobic and lactic acid bacteria counts.

Goats milk oligosaccharide recently demonstrated efficacy in the DSS model of colitis, with treated rats showing reduced MPO activity and higher body weights than DSS-treated controls.[74] Fructo-oligosaccharide (FOS) administration has also been reported to decrease the severity of DSS colitis, reducing disease activity and damage in the distal colon, while producing more rapid recovery from damage.[83] In contrast, Moreau and colleagues observed a reduction of inflammation in the cecum, but not the colon of FOS-treated rats with DSS colitis.[84] Winkler and colleagues[83] administered FOS via intragastric gavage to C57BL/6 mice, while Moreau and colleagues[84] added FOS to the solid diet of Sprague-Dawley rats; therefore, the contrasting results could have been due to species differences and/or route of administration. Indeed, delivery of FOS in liquid form may have increased the rate of

passage through the stomach of mice, and subsequently altered the interaction of the prebiotic with the intestinal epithelium.

Lactulose is another prebiotic that has recently demonstrated a capacity to reduce the severity of DSS colitis in rats.[75] Twice daily prebiotic administration for 6 days was shown to significantly reduce colonic lesions and MPO activity; however, the effects on the microbiota were not determined. Furthermore, inulin administration has also proved efficacious in the setting of DSS colitis, reducing mucosal inflammation, MPO activity, and release of inflammatory mediators, such a prostaglandin E2.[76] Interestingly, this effect was observed only following oral administration, as rectal administration of the prebiotic showed no beneficial effects. This is surprising as the method of administration should not have affected prebiotic availability and hence its ability to exert it beneficial effects.

As is the case with probiotics, not all prebiotics have demonstrated antiinflammatory effects in the setting of IBD, with some prebiotics actually increasing the severity of damage. FOS is one prebiotic that has demonstrated antagonistic effects in the intestine. FOS, administered as a dietary supplement (6 percent w/w of total diet), has been shown to stimulate lactobacilli and bifidobacteria[77,78] and increase SCFAs in the large bowel (a result that has been replicated in humans with ulcerative colitis[85]).[86] Therefore, FOS has been proposed to have the capacity to be beneficial in the IBD setting; however, it has also been demonstrated that, while FOS could decrease the colonization of pathogenic bacteria, it actually increased translocation of bacteria, increased mucosal irritation, and increased cecal and colonic MPO activity.[78] The proposed mechanism of injury involves elevated FOS levels in the cecum promoting rapid bacterial fermentation, thus increasing organic acid concentrations. These organic acids then damage the mucosa of the cecum and colon.[87] Interestingly, however, when FOS was administered by oral gavage in the TNBS rat model of colitis, it was shown to decrease the severity of damage, indicated by increased lactic acid bacteria, lactate, and butyrate and decreased inflammation scores and MPO activity.[79] The effect of the route of administration on the efficacy of the prebiotic is similar to those reported by Moreau et al.[84] and Winkler et al.[83] described above, with oral administration leading to an increased efficacy of treatment. These inconsistent findings may be due to differences in the model of colitis, differences between the remainder of the diet between trials (i.e., levels of fiber, indigestible carbohydrates), or a result of alterations in the delivery or dosage of FOS, leading to different rates of fermentation, and in turn SCFA production. Optimizing SCFA production by the microbiota is an important determinant of probiotic efficacy, as SCFAs are a vital energy source for intestinal epithelial cells.

12.3.1.2 Prebiotics in Human Trials

Lactulose was recently shown to have no beneficial effects in human IBD patients, despite promising findings from murine models.[88] Patients were treated with either 10 g of lactulose combined with standard medication or standard medication alone for 4 months. The study group comprised both UC and CD sufferers, but the results

did not differ greatly between conditions. No significant improvement in clinical activity index, endoscopic score, or immunohistochemical parameters was observed, although UC sufferers did report a significant increase in quality of life. The absence of a sole-lactulose treatment group prevented the determination of the prebiotic effect in human IBD, and the failure to replicate the positive results observed *in vivo* could have occurred as a result of a nonideal combination with medication. Despite the effect of lactulose not being supported by the investigated parameters, the increase in quality of life following administration reported by UC sufferers indicates further investigation is warranted. UC appears to be suited to prebiotic treatment, with GBF reducing disease severity both clinically and endoscopically as well as increasing the concentration of fecal butyrate.[89] Another dietary fiber, derived from the *Plantago ovate* seed, has also been demonstrated to have therapeutic effects. It has been shown to increase fecal butyrate levels, and was as effective as conventional mesalamine treatment to maintain remission in patients with UC in an open-label study of 102 patients.[90]

FOS administration has yielded promising results in studies involving patients with CD. Lindsay and colleagues reported increased fecal bifidobacteria concentrations and a decrease in disease severity.[80] Interestingly, FOS also increased levels of DCs expressing TLR-2 and TLR-4, as well as IL-10$^+$ DCs. Immunomodulatory effects of prebiotics have not been studied extensively, but indicate another mechanism via which they could be associated with efficacy in IBD treatment. Furthermore, combination with probiotics that exert similar beneficial effects could increase potency of the treatment. Hussey and colleagues reported efficacy of FOS administration, although it was delivered in combination with inulin and whey protein.[91] Once again, disease severity scores were reduced following treatment, as were erythrocyte sedimentation rates, a biochemical marker of inflammation. The effects of FOS administration alone were not determined, but may have been useful in identifying the most active component of the combination and, hence, potential methods of increasing its potency.

Prebiotics have also demonstrated efficacy in the setting of pouchitis. Welters and colleagues reported a decrease in both histological and endoscopy scores of patients with pouchitis following inulin administration.[92] Inulin administration was shown to reduce the concentration of *Bacteroides fragilis*, a bacteroide hypothesized to initiate inflammation in pouchitis and associated with villous atrophy, but had no effect on commensal lactobacilli or bifidobacteria concentrations. Fecal butyrate levels were also increased by inulin administration, leading to increased energy availability for colonic epithelial cells, which may have aided to tissue repair and regeneration.

Prebiotics have demonstrated efficacy both in animal models *in vivo* and in clinical trials. Similar to probiotics, a greater understanding of the role of the intestinal microbiota in IBD is required in order to optimize their efficacy. This would facilitate the development of more effective microbial therapies as specific targets for manipulation will be identified. In addition to exerting their own beneficial effects, prebiotics could also be utilized to manipulate the microbiota to facilitate the survival of probiotic species or increase the efficacy of other therapeutics.

12.4 SYNBIOTICS

Administration of probiotics and prebiotics in conjunction is referred to as a synbiotic,[93] and is a further potential treatment for IBD. The rationale behind synbiotic treatment is that the desired probiotic and prebiotic (presumably with independently demonstrated efficacy) would exert a beneficial effect greater than would be observed when each was administered individually. Indeed, a prebiotic that was not efficacious when administered singularly may stimulate probiotic species, significantly enhancing its beneficial effects on intestinal health. There are currently few well-conducted studies that examine the effects of synbiotic therapy in IBD; however, it remains a logical and viable treatment option.

Bomba and colleagues demonstrated that a synbiotic combination of *L. paracasei* and maltodextrin decreased *E. coli* colonization, while a combination of *L. paracasei* and FOS led to an increase in *Lactobacillus* and *Bifidobacterium* and decreased *Clostridium* and *Enterobacterium* in the jejunum of piglets.[94] Furthermore, Su and colleagues determined that treatment with the prebiotics soybean oligosaccharide, FOS, and inulin were able to increase both survival time and retention period of the probiotics *B. lactis* LAFTI B94, *L. casei* L26 LAFTI, and *L. acidophilus* LAFTI L10.[95] Beneficial effects on the human intestinal ecosystem by synbiotic administration have been reported by Casiraghi and colleagues, who observed an increase in fecal bifidobacteria and lactobacilli counts.[96] In addition, Kanamori and colleagues demonstrated that synbiotic treatment with *B. breve*, *L. casei Shirota,* and galacto-oligosaccharide for over 12 months increased fecal SCFA levels, increased fecal bifidobacteria, and lactobacilli concentrations and improved the rate of body weight gain in patients with short bowel syndrome.[97]

Studies into the effectiveness of synbiotics as a therapy for IBD have delivered contrasting findings. Geier and colleagues reported that treatment with FOS and the probiotic *L. fermentum* BR11 failed to reduce the severity of DSS colitis in rats.[98] However, investigations into the efficacy of FOS delivered alone determined that the prebiotic actually increased some indicators of colonic injury, indicating that FOS may not be a suitable prebiotic for use in this synbiotic combination. Shultz and colleagues reported an improvement in colonic inflammation of colitic rats treated with a combination of *L. acidophilus* 5, *B. lactis* Bb-12, and inulin.[99] Synbiotic administration increased the diversity of the gut microbiota, although the two probiotics were not detected. This led to the suggestion that the antiinflammatory effects of the treatment may have been due to the prebiotic. Chermesh and colleagues reported the failure of "Synbiotic 2000" (a combination four prebiotics and four probiotics) to reduce the postoperative recurrence of CD.[100] These findings were not unexpected, however, as probiotics and prebiotics have typically demonstrated a greater efficacy in the treatment of UC rather than CD. Indeed, the prebiotic mixture Synergy 1®, combined with *B. longum* in a double-blind, randomized controlled pilot study was able to improve sigmoidoscopy scores, decrease β-defensin mRNA, TNF-α, and IL-1α, and reduce inflammation seen in biopsies of active UC.[101]

The potential benefits of synbiotic therapy are clear; however, the great challenge is to determine the best combination for each disease setting and for each individual patient. Logically, the first investigations should focus on combining probiotics and prebiotics that have demonstrated individual benefits, and to determine the specific properties that a prebiotic requires to be beneficial to a probiotic, and to select the prebiotic accordingly.

12.5 FUTURE DIRECTIONS

12.5.1 Inactivated Bacteria

Traditionally, it has been thought that probiotics need to be living to exert their beneficial effects. However, recent evidence suggests that inactivated bacteria may also possess therapeutic properties. It is postulated that the protective effect of probiotics may be mediated, to some degree, by their own DNA; hence, the bacteria do not need to be "live" to exert their therapeutic effect.[102] This challenges the previous dogma suggesting that probiotic bacteria must survive passage through the GI tract to exert their beneficial effects. The use of inactivated bacteria for therapeutic benefit has a number of advantages as it reduces the risk of sepsis potentially associated with administration of live bacteria. This could provide a safer means to deliver probiotics to immunocompromised patients as well as providing greater quality control and longer storage life. The efficacy of dead and inactivated bacteria has been tested in a number of *in vitro* and *in vivo* models of diseases of the GI tract, but has yet to be examined in clinical trials.

12.5.1.1 In Vitro *Studies of Inactivated Bacteria*

Zhang and colleagues compared the ability of live and inactivated *L. rhamnosus* GG (LGG) to decrease TNF-α-induced IL-8 production, a proinflammatory cytokine observed at increased levels in IBD, using Caco-2 cells.[103] Cells were treated with LGG at a range of doses (10^4 to 10^{10} cfu/mL), in the presence or absence of TNF-α or antibiotic (penicillin or streptomycin). Both live and heat-inactivated LGG were reported to reduce the TNF-α-induced IL-8 production. However, when IL-8 levels were examined in cells treated with 10^{10} cfu/mL LGG in the absence of TNF-α, cells produced more IL-8 than untreated cells and cells treated with TNF-α alone. In contrast, an identical dose of heat-inactivated LGG only slightly increased IL-8 levels compared to untreated controls. The addition of antibiotics did not alter these results, indicating no detrimental effect on probiotic efficacy. Although the effect was not as apparent using dead bacteria, the increase in IL-8 levels following high-dose treatment indicates a degree of risk of inflammation associated with both live and inactivated LGG administration. Interestingly, Roselli and colleagues reported that heat-killed LGG did not have the same beneficial effect as live bacteria in Caco-2 cells.[104] Live and heat-killed LGG and *B. animalis* MB5 were tested for their ability to reduce *E. coli*-induced neutrophil transmigration, but only live bacteria were able to induce a significant

decrease. These two studies provide further insight into the complexity of the mechanisms of probiotic action. Although both studies used live and dead LGG in Caco-2 cells, different parameters were measured. While dead bacteria were able to reduce inflammation,[103] they had no impact on neutrophil transmigration,[104] indicating different mechanisms of action for each probiotic. This indicates that probiotic-induced effects may be mediated by different bacterial-derived pathways, some which are dependent on viable bacteria, while others, mediated by probiotic structures or secreted products, do not require live bacterial cells. Differences in the efficacy of dead LGG also may have been due to the challenge applied to the cells. Although LGG was effective against TNF-α-induced damage, it was unable to counteract damage caused by enterotoxigenic *E. coli*. Depending on the disease setting, heat-killed LGG still provides a therapeutic option.

12.5.1.2 In Vivo *Studies of Inactivated Bacteria*

Laudanno and colleagues tested live and heat-killed forms of the commercially available *Bioflora* probiotic, which contains four species of bacteria: *L. casei*, *L. plantarum*, *Streptococci faecalis,* and *B. brevis*.[105] Female Wistar rats were challenged orally with 50 mg/kg of indomethacin to induce gastric necrotic lesions and erosions of the small intestine, and treated (either subcutaneously or orally) with 1 mL of either live or dead *Bioflora* probiotic. Regardless of administration route, both the live and heat-killed bacteria prevented indomethacin-induced lesions and reduced MPO activity. Live and heat-inactivated *Bioflora* was also able to reduce inflammation in the carrageenin-induced model of plantar edema. Rachmilewitz and colleagues provided further evidence supporting the theory that bacterial DNA could be responsible for the beneficial effects of certain probiotics.[106] Live, irradiated (nonviable), and heat-killed forms of VSL#3 were administered to rats with DSS colitis. However, in contrast to the findings of Laudanno and colleagues, only the live and irradiated probiotics attenuated the severity of colitis. The contrast between heat-killed and irradiated VSL#3 was surprising, suggesting that during the heating process the bacterial DNA may have been damaged. It is unclear why this effect did not occur for the *Bioflora* probiotic, but it may have been due to differences between the properties of the probiotic strains. Future studies should investigate whether the probiotic DNA structure was damaged during the inactivation process. Irradiated VSL#3 did not ameliorate DSS colitis in TLR-$9^{-/-}$ mice, indicating a key role for the TLR-9 pathway in the attenuation of colonic inflammation. Furthermore, irradiated bacteria treated with DNase also failed to replicate the beneficial effects of untreated irradiated bacteria, suggesting that DNA was the component of the probiotic that exerted this beneficial effect, most likely through stimulation of the host immune system. Immunostimulatory DNA has further been shown to inhibit colonic proinflammatory cytokines and chemokines[107] as well as to promote regulatory T-cell production.[108]

12.5.2 Probiotic Supernatants

Recently, there has been increasing interest in the use of probiotic supernatants in the treatment of intestinal disorders. Probiotic supernatants are devoid of bacterial cells, but contain a mixture of secreted products. If deemed to have therapeutic potential, the use of bacterial supernatants would reduce the minor risk of sepsis associated with administration of live bacteria. The use of supernatants will also facilitate the delivery of these secreted products in a more controlled manner, which does not require the colonization and survival of the bacterium.[109] Bacterial supernatants could also be more effective therapeutics as they would have a longer shelf life than live bacteria, facilitating greater quality control during production. The exact composition of the secreted products is not known, but would vary dependent on species, strain, and culture conditions. Studies have reported probiotic supernatants to contain SCFAs,[110] phospholipids,[111] bacteriocins,[112] and proteins.[109]

Frick and colleagues investigated the ability of *L. fermentum* supernatant to inhibit the proinflammatory responses of HeLa 229 cells on *Yersinia enterocolitica* infection.[111] *Yersinia enterocolitica* treatment was shown to induce two proinflammatory responses: NF-κB activation and increased IL-8 production. Treatment with *L. fermentum* supernatant inhibited IL-8 secretion and decreased NF-κB activation following infection. The antiinflammatory effect of *L. fermentum* supernatant was diminished upon treatment with phospholipase C, indicating a key role for a secreted phospholipid in the antiinflammatory effect. Similarly, Roselli and colleagues demonstrated the efficacy of both *B. animalis* MB5 and LGG in the treatment of E. *coli* K88-infected Caco-2 cells.[104] Supernatant administration decreased *E. coli* K88 adhesion, counteracted IL-8 upregulation, and inhibited neutrophil translocation. This supernatant exerted identical beneficial effects following protease digestion, suggesting that proteins were not the active constituent. *Escherichia coli* viability was unaffected by treatment, eliminating bactericidal activity of the probiotic or its supernatant. The mechanism for these beneficial effects needs to be further elucidated. Interestingly, only treatment with live bacteria prevented the pathogen-induced increase in expression of IL-1β and TNF-α and the decrease of TGF-α. This study provided an example of the differing impact of live bacteria and supernatants, and highlights that not all therapeutic benefits of probiotic bacteria are mediated by their secreted products.

Yan and colleagues performed the first study in which proteins were characterized and purified from a probiotic supernatant, and shown to exert beneficial effects on colonic epithelial cells.[109] In this experiment, two proteins (p75 and p40) were isolated from LGG and tested in four settings: young adult mouse colon epithelial cells, kinase suppressor of Ras^{-1} knockout mouse colon epithelial cells, human HT-29 colon cells, and cultured C57BL/6 mouse colon explants. Cells and colon explants treated with p75 and p40 displayed increased Akt activation, inhibition of cytokine-induced epithelial cell apoptosis, and growth promotion. TNF-induced epithelial cell apoptosis was also significantly reduced by both p75 and p40. These findings elucidate key mechanisms behind the therapeutic effects of LGG, and indicate potential

for its use as a therapeutic for cytokine-mediated GI diseases. All of the mechanisms responsible were not identified, however; an earlier study also reported that soluble products of LGG were able to activate MAP-kinases and induce cryoprotective heat shock proteins in intestinal epithelial cells, further mechanisms that could contribute to the beneficial clinical effects of LGG.[113]

The production of multiple bioactive compounds by probiotic bacteria has previously been reported in *L. johnsonii* NCC 533.[114] The supernatant was shown to contain products capable of catalyzing the synthesis of the antimicrobial compound, hydrogen peroxide, in addition to the previously identified lactic acid and other bacteriocins. Production of hydrogen peroxide was also observed in eight other *L. johnsonii* strains, suggesting a degree of species, rather than strain, specificity.

12.5.3 Efficacy of Probiotics, Prebiotics, and Synbiotics

Probiotic, prebiotic, and synbiotic treatments have the potential to decrease the severity of IBD. A number of potential mechanisms have been identified, including increased SCFA production, reduction of proinflammatory cytokine secretion and gene expression, strengthening of the intestinal epithelial wall and improvement of barrier function, improvement of the Th1/Th2 balance, and the elimination of pathogenic bacteria, among others. As a result of their variable successes, concerns remain related to the use of probiotics as therapeutics for IBD. Although some exert beneficial effects, many strains have been reported to be ineffective while some have been shown to exacerbate disease severity. A critical step to improve the effectiveness of these therapies is to gain a better understanding of the intestinal microbiota and its relationship with disease development. This information would facilitate the identification of specific targets for manipulation and allow for strategic selection of the most beneficial probiotics for a given disease. Furthermore, a greater mechanistic understanding of probiotics, prebiotics, and synbiotics would facilitate the selection of the strains and combinations most suited to each gut disorder. Finally, it is essential that the manner in which the probiotic treatments interact with the commensal bacteria be determined. The microenvironment differs between individuals and it is feasible to predict that treatments may be selected to suit the individual based on their own unique bacterial profile.

The risk of sepsis associated with the administration of live probiotic bacteria is low, but nevertheless worthy of consideration. Probiotic-related cases of sepsis are rare and usually observed in immunocompromised patients with impaired barrier function. In addition, the difficulties associated with maintaining a high degree of quality control is another problem hindering the development of probiotic-based therapeutics.[115] Both of these issues can be addressed through the use of either inactivated probiotic bacteria or the supernatant products of probiotics. Inactivated bacteria and supernatants have been tested *in vivo* and *in vitro* and have demonstrated efficacy in the setting of intestinal inflammation. They could potentially allow the same beneficial effects of probiotics to be exerted, without the risk of sepsis or harmful interactions with the host microbiota. Furthermore, once the sources of beneficial effects associated with probiotics have been identified, whether it be the microbial

DNA, a secreted product, or an array of factors, these could be isolated and harnessed to produce a more potent therapeutic. Inactivated probiotic bacteria and probiotic supernatants also have the benefit of facilitating greater quality control and longer shelf life as therapeutics.

IBD is a complex disorder for which the exact pathogenesis has not yet been determined, nor has a definitive treatment been developed. Probiotics and prebiotics have demonstrated therapeutic promise in this disorder, and have the potential to be employed as either alternative or cotherapeutics. Nevertheless, further studies are required to gain a more detailed understanding of the mechanisms behind the beneficial effects of probiotics and prebiotics in order to optimize their applicability for prevention or treatment of IBD.

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CHAPTER 13

Prebiotics and Probiotics in Pediatric Diarrheal Disorders

Rosemary J. Young

CONTENTS

13.1 INTRODUCTION

Prebiotics and probiotics are components of foods that produce positive physiological effects through their interrelationships with the gastrointestinal tract. Whereas the benefits of prebiotics have come to light in more recent years, recognition of probiotic effects dates back to the seventeenth century when Louis Pasteur postulated the importance of microorganisms in human life. More formalization to the study of probiotic organisms came about in 1908 when Eli Metchnikoff *made observations that human health and longevity are associated with the ingestion of lactic acid-producing bacteria*. His observation stemmed from the fact that Bulgarian peasants, who lived longer, consumed large quantities of sour milk containing what is now known as *Lactobacillus bulgaricus*.[1] Prior to refrigeration, live bacteria and other microorganisms were commonly ingested in food as organisms were extensively

utilized for food preservation. Currently, there is a greater consumption of processed foods in addition to a sterile food supply, and the ingestion of food-based pre- and probiotics has become more limited.

13.2 DEFINITIONS

Prebiotics are defined as "a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or modifying the metabolic activity of one or a limited number of bacterial species in the colon that have the potential to improve host health."[2] Prebiotics are found naturally in many foods, are present in breast milk, and can also be isolated from plants (e.g., inulin from chicory root) or synthesized (e.g., enzymatically from sucrose). The major prebiotics for bacterial growth in humans are dietary carbohydrates that have not been digested in the upper gastrointestinal tract. These most often include resistant starch, nonstarch polysaccharides, and nondigestible oligosaccharides.[3] It is primarily the nondigestible oligosaccharides, such as human milk oligosaccharides, fructo-oligosaccharides (FOS), and galacto-oligosaccharides (GOS) that have been found to selectively stimulate beneficial bacteria to the point of providing a quantifiable benefit. Although some proteins and lipids are partially nondigestible, their prebiotic benefits are not as well characterized.

Several definitions of probiotics exist. For example, a probiotic has been defined as "a live microbial food ingredient that, when ingested in sufficient quantities, exerts health benefits."[4] Similarly, the Joint FAO/WHO (Food and Agriculture Organization / World Health Organization) Working Group on drafting "Guidelines for the Evaluation of Probiotics in Food" has recommended more specifically that probiotics be defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host."[5] Therefore, the focus becomes microorganisms that are not just safe, but also must have a demonstrable benefit to the host. Probiotic microorganisms can be found both in supplement form and as components of foods. Examples of probiotics include certain strains of *Lactobacillus, Bifidobacterium, Streptococcus, Enterococcus,* and *Saccharomyces.* Many are consumed in foods such as yogurts and other cultured dairy products.

13.3 COLONIZATION

Normally individuals receive their first exposure to bacteria during the birthing process. Prior to that time, the gastrointestinal tract is sterile. During childbirth, an infant swallows bacteria present in the birth canal; these bacteria rapidly colonize the small and large intestine, and the intestinal immune system learns to recognize these bacteria as desired residents of the gastrointestinal tract. Besides extrinsic factors, such as mother's dietary intake or use of probiotics, type of birth (vaginal or surgical), gestational age, and primary source of nutrition (bottle or breastfed); intrinsic factors including underlying neonatal health, immunologic status, gastrointestinal

transit time, pH, and stress all affect the process of colonization and the types of organisms established.[6] Because they are not exposed during birth to maternal flora, infants born via cesarean section may have delayed colonization as well as a greater acquisition of environmental flora than the vaginally born infant. Infants delivered via cesarean section have been demonstrated to have delayed acquisition of anaerobes, particularly with *Bacteroides,* which require very close contact for transmission.[7]

Dietary factors also play a major role in gut colonization of the newborn. Human milk is thought to create an environment favorable for the growth of bifidobacteria; however, studies are conflicting and it may be that the higher counts in this group are due to the overall lower proportional levels of other bacterial groups. Once established, the intestinal flora is relatively stable throughout life and difficult to change permanently. It is recognized as native and typically beneficial to that individual. *Escherichia coli* are the predominant enterobacteria to colonize the infant initially, but later bifidobacteria increase in numbers. Formula-fed infants typically have a more complex microflora including bacteroides, clostridia, and streptococci in equal proportions to the bifidobacteria probably due to greater exposures.[8] The bifidobacteria strains that predominate in infants, *B. bifidum* type B, B. *infantis* ssp. *Infantis,* and *B. longum* ssp. *longum* type B rarely occur in adults, implying that dietary/environmental exposure plays a significant role in initial colonization.[9]

By the end of the first month of life, bifidobacteria levels are equal in both groups of infants.[10] Once solid foods are introduced to formula-fed infants, their flora adjusts with increase in anaerobic flora. By the second year of life, assuming similar diet and environmental exposure, bacterial populations on both formula-fed and breastfed infants resemble that of adults in both number and composition.[11]

Premature infants and term infants requiring intensive care are slower to acquire bifidobacteria flora. Premature infants are also more susceptible to pathogenic colonization, which predisposes them to infection. Animal studies have shown that bacteria considered to be nonpathogenic to adults may be harmful in the early human neonatal stages[12] and may in part explain the occurrence of necrotizing enterocolitis.[13] In general, colonization of beneficial intestinal bacteria has been shown to stimulate normal mucosal defense systems and inhibit pathogenic organisms.

In adults, bacteroides species represent the most prevalent groups in the large intestine, but others are also present, including bifidobacteria, lactobaccilli, staphylococci, enterobacteria, streptococci, and clostridia species. While these resident commensual bacteria are important, they should not be automatically considered probiotic species unless these native microorganisms can be specifically characterized and studied.[14] *Lactobacillus rhamnosus* GG is an example of a human-derived bacterium that has been specifically studied in this regard.

Temporary alterations in intestinal flora are related to the health of the individual and can be altered by diet, environment, antibiotic therapy, radiation or chemotherapy, or modifications in the individual's immune system. Ingesting specific prebiotics as well as probiotic bacteria that are not currently a part of the individual's daily intake can result only in the transient changes in the flora. Sterilization of our

food supply has limited our exposure to the more beneficial organisms previously consumed on a daily basis. The science of prebiotics and probiotics is now focused on attempting to identify those specific beneficial nutrients and species.

13.4 MECHANISMS OF ACTION

Prebiotics and probiotics are not as similar as their names suggest. Prebiotics basically provide the food for all sorts of microorganisms. A beneficial prebiotic increases the number of less aggressive or beneficial organisms in the bowel, produces short-chain fatty acids, which protect the bowel lining and prevent invasion of harmful organisms, lowers the intestinal pH, which alters the growth of some organisms as well as increases calcium absorption and possibly has some immunomodulation effects.[2] Prebiotics, therefore, act only on natural flora already present in the bowel.

To be a successful probiotic, microorganisms must be ingested in live or dormant form, be able to maintain sufficient viable microorganisms that survive the host's digestive process, as well have demonstrable health effects without significant adverse effects.[15] Probiotics act by numerous different mechanisms; however, adherence to the intestinal epithelium is often felt to be important for the interaction with the gastrointestinal immune system by inducing the immunomodulating benefits, such as enhancing immunoglobulin A (IgA) production and stimulating cytokines.[16] Other functions of probiotic bacteria include their ability to produce antimicrobial substances, such as bacteriocins, hydrogen peroxide, and biosurfacants. They may also act to lower intestinal pH by stimulating lactic acid-producing organisms, which favors growth of more beneficial organisms. Some probiotics enhance colonization resistance by competing with pathogens for binding and receptor sites and for available nutrients required by pathogenic organisms.[17] A probiotic is most beneficial when it can adapt to healthy intestinal flora, not displace the native bacteria already present.

13.5 CLINICAL STUDIES

13.5.1 Prebiotics

As a result of the relatively recent recognition of the potential benefits of prebiotics, the number of randomized controlled studies is limited. It has been recognized that the human milk oligosaccharides, the third most abundant component of breast milk, are bifidogenic and one mechanism for the protective effect of the breastfed infant against many diarrheal conditions.[18,19] The composition of human milk oligosaccharides is very complex and more than 100 different oligosaccharide-like structures are known. The concentration of these compounds in breast milk changes according to different lactation phases; it is higher in colostrum than in transitional and mature milk. These findings have led to the study of supplementing

infant formulas with various prebiotics to obtain the protective benefits conferred with the breast milk prebiotics.

The addition of GOS and FOS to formula has been shown to positively affect the bifidobacteria content of the infant's feces,[20] as well as to induce a reduction of clinically relevant pathogen germs in the feces of formula-fed preterm infants.[21] Stahl et al.[22] found that GOS/FOS can be detected in stools of prebiotic-supplemented formula-fed infants in amounts similar to those displayed in infants given human milk oligosaccharides via breast milk. Furthermore, the pattern of fecal short-chain fatty acids in infants fed an oligosaccharide mixture was found to be similar to that of breastfed infants and significantly different from that of a group of infants fed with a formula without added prebiotics.[23] A study by Euler et al.,[24] however, identified that not only the amount but also the type and origin of prebiotic used in the formula are key in obtaining demonstrable clinical benefits, as they were unable to demonstrate any change in fecal flora with two different doses of FOS.

In a group of preterm infants, the addition of a combination of GOS/FOS to the formula was shown in a double-blind, placebo-controlled study to reduce stool viscosity and gastrointestinal transit time without any adverse events.[25] Boehm et al.[26] tested in preterm infants a mixture of 90 percent GOS and 10 percent FOS, with a distribution of molecules and a concentration of total oligosaccharides close to human milk, added to a standard preterm formula. The supplementation resulted in a clear bifidogenic effect, accompanied by more frequent softer stools. It was also observed that the Ca/P ratio in the urine was similar to that observed in breastfed infants, suggesting also an influence of prebiotics on calcium absorption.

Ziegler et al.[27] recently reported the use of a prebiotic supplemented formula in a group of healthy term infants and found that the supplemented group had comparable growth to the placebo group with no adverse events. The prebiotic-supplemented group also had a stool pattern that more closely resembled breastfed infants than the group fed the standard infant formula. A study with term infants has evaluated the nutritional efficacy and bifidogenic characteristics of an infant formula containing partially hydrolyzed whey proteins, modified fats, and prebiotics with starch and reported satisfactory growth and higher counts of bifidobacteria in the feces with no adverse side effects.[28] Another prospective study suggested that infants with "minor" gastrointestinal symptoms (such as colic, regurgitation, and constipation) improved within 2 weeks of feeding the same type of supplemented formula.[29]

The use of oligofructose-supplemented infant cereal was found in a randomized, blinded trial to give fewer loose stools, fewer physician visits for diarrhea, and fewer days missed from daycare because of diarrhea in the group receiving the supplemented cereal.[30] However, there was no difference in the incidence of diarrhea or other infections. More recently Duggan et al.[31] demonstrated that oligofructose-supplemented cereal given to community-based infants in Peru also had no effect on diarrhea incidence, use of healthcare resources, and response to *Haemophilus influenzae* immunization. It was speculated that the high rate of breastfeeding in both the control and treatment group may have negated the effect.

A study on oligofructose supplementation was performed in a group of healthy 7- to 19-month-olds attending daycare and found that compared to a placebo group

they tended to have greater bifidobacteria counts and fewer pathogenic clostridia, but not salmonella.[32] The oligofructose-supplemented group had less flatulence and fewer episodes of vomiting, diarrhea, and febrile episodes than the control group, but the effects did not persist beyond the supplementation period. General immune system enhancement has been demonstrated by Arslanoglu et al.[33] in a study, using a mixture of neutral short-chain GOS and long-chain FOS. In this study, the incidence of recurring infections, particularly respiratory infections, was decreased during the first 6 months of life in the prebiotic group as compared to the placebo group.

Antibiotic use is frequent in children and at times leads to antibiotic-associated diarrhea. Brunser et al.[34] conducted a randomized, double-blind study of the effects of a prebiotic-supplemented formula given to a group of infants 1 to 2 years of age receiving amoxicillin for acute bronchitis. They found that the antibiotic usage decreased total fecal bacteria and increased clostridia; however, with prebiotic supplementation there was increased fecal bifidobacteria and lactobacilli without a change in gastrointestinal symptoms. Another common problem in infants is the rising incidence of atopic dermatitis due to formula or breast milk intolerance. Many children concurrently have gastrointestinal symptoms, such as vomiting, diarrhea, and failure to thrive. Moro et al.[35] found that a mixture of GOS/FOS-supplemented hydrolyzed formula given to infants at high risk for atopy reduced the incidence of atopic dermatitis including regurgitation and crying during the first 6 months of life as compared to the unsupplemented group.

13.5.2 Probiotics

The use of probiotics in the treatment of acute diarrheas, particularly viral diarrhea, has been extensively studied by several groups in placebo-controlled studies in both Europe and the United States. In these studies, *Lactobacillus* GG*, L. reuteri, L. acidophilus* Lb, *Saccharomyces boulardii,* and a combination product of *Streptococcus thermophilus, L. acidophilus,* and *L. bulgaricus* led to decreased severity and duration of diarrhea in both developed and in developing countries when administered alone or as part of oral rehydration therapy. Four meta-analyses have concluded that probiotic therapy reduced the duration of acute diarrheal illness by approximately 1 day.[36–39] The probiotic with the most consistent results was *Lactobacillus* GG. Two studies have, however, demonstrated no benefit demonstrated of *Lactobacillus* GG in the treatment of acute diarrhea children with severe diarrhea.[40,41] A study of *L. paracaseii* ST11 also noted no benefit in severe cases of pediatric diarrhea; however, some benefit in less severe, nonrotavirus diarrhea was noted.[42]

The prevention of nosocomial infectious diarrhea may be affected by the use of probiotics. A double-blind, randomized control trial using *Lactobacillus* GG in children ages 1 to 36 months showed a significant reduction in the risk of rotavirus gastroenteritis 2.2 percent versus 6.7 percent.[43] However, in a larger double-blind, randomized study there was no statistically significant protective effect of the same probiotic for nosocomial rotavirus infection.[44] Another randomized trial looking at 55 infants admitted to a chronic care pediatric hospital showed a lower risk of

developing nosocomial diarrhea when infants were fed formula containing bifidobacteria and streptococci 7 percent versus 31 percent.[45]

Randomized controlled studies suggest a modest protective effect of probiotics in decreasing community-acquired diarrheal episodes. A Peruvian study of 204 malnourished children showed a reduction of the number of episodes of diarrhea per child per year from 6.02 to 5.21 in those receiving *Lactobacillus* GG (46). A second study from Finland involving 571 children attending daycare centers did not show a significant difference in the number of days with diarrhea when *Lactobacillus* GG was used. However, there was a 16 percent reduction in the number of days of absence due to gastrointestinal and respiratory illnesses.[47] Another study involving 210 healthy children in child healthcare centers using *L. reuteri* and *B. lactis* showed a lower frequency and duration of diarrhea as compared to a control group.[48]

The most common alteration of intestinal flora in children occurs with antimicrobial therapy, especially with broad-spectrum antibiotics. Positive effects in pediatric antibiotic-associated diarrhea have been identified with *Lactobacillus* GG. Arvola et al.[49] performed a double-blind trial in 119 children (mean age 4.5 years) receiving antibiotics for respiratory infections in Finland. They administered *Lactobacillus* GG twice a day during antibiotic therapy and demonstrated significantly fewer incidences of diarrhea in the probiotic group (5 percent vs. 16 percent). In this study, actual changes in gut microflora were also identified in patients who had diarrhea as defined by three or more loose stools per day. Vanderhoof et al.[50] also reported a placebo-controlled study of 188 children receiving antibiotics for common upper respiratory infections that demonstrated fewer episodes of diarrhea, as defined by increased stool looseness and frequency, in the group receiving the probiotic *Lactobacillus* GG (48 percent vs. 17 percent).

A meta-analysis of data from five randomized, controlled trials showed *Saccharomyces boulardii* to be moderately effective in preventing antibiotic-associated diarrhea in children and adults treated with antibiotics.[51] Not all probiotics are equally effective in this condition as a combination of *L. acidophilus* and *L. bulgaricus* was ineffective in preventing diarrhea in children receiving amoxicillin therapy during a double-blind, placebo-controlled trial.[52] Hospitalized children receiving limited enteral intake and broad-spectrum antibiotics may significantly benefit from concurrent probiotic therapy. Biller[53] reported a positive effect in an open-label case series of four pediatric patients using *Lactobacillus* GG for recurrent *Clostridium difficile* infection.

Necrotizing enterocolitis (NEC), a condition seen predominantly in premature infants, often results in small bowel resection in severe cases. In three studies, the use of a combination probiotic therapy administered to premature infants reduced the incidence of NEC.[54–56] Other investigators, however, were unable to demonstrate any benefit of *Lactobacillus* GG in NEC prevention.[57]

A new area of research has demonstrated that probiotics may be particularly effective not only in intestinal inflammation, but may also affect the systemic immune response that occurs with food-related allergies in infants and children. Probiotics appear to redirect the immune system toward producing chemical mediators that are more useful in controlling infections, rather than mediators that induce the allergic response. Studies in infants with eczema receiving formulas supplemented with

Lactobacillus GG have shown benefit in decreasing both gastrointestinal symptoms and eczema.[58,59] When *Lactobacillus* GG or placebo was given to pregnant mothers with a strong family history of eczema, allergic rhinitis, or asthma and to their infants for the first 6 months after delivery, the frequency of developing atopic dermatitis in the offspring was significantly reduced at 2 years[59] and 4 years.[60] Another placebo-controlled study showed significant improvement in children with atopic dermatitis after a 6-week administration of *L. rhamnosus* 19070-2 and *L. reuteri* DSM 122460.[61] Children with high IgE levels and one or more positive skin tests were more responsive to probiotic therapy. In a large controlled study, infants with atopic eczema and cow's milk allergy responded more effectively to hydrolyzed whey formula when *Lactobacillus* GG was added to the formula.[62] When *L. paracasei*-33 was given for 30 days to 80 children with perennial rhinoconjunctivitis, the quality of life questionnaire scores significantly improved relative to placebo.[63] However, *L. rhamnosus* supplementation failed to show any benefit in birch pollen allergic children in a placebo-controlled trial.[64] These positive effects in the gastrointestinal tract may be due to a probiotics ability to alter intestinal permeability as well as to a direct effect on the gut-associated lymphoid tissue.

The systemic effect of probiotics on the immune system has been demonstrated in two placebo-controlled studies examining an antibody response to typhoid vaccine in adults and to rotavirus vaccine in children when given the probiotic *Lactobacillus* GG.[65,66] In two similar, but separate, controlled studies done in pediatric patients with cystic fibrosis and in healthy children in a Finnish daycare, it has been demonstrated that *Lactobacillus* GG therapy decreased the number of respiratory infections requiring antibiotic therapy over an extended period of time.[67,68] Recently it has also been shown that the episodes of pulmonary exacerbations and hospital admissions were significantly decreased in patients with cystic fibrosis receiving LGG compared to a placebo group.[69]

13.6 SAFETY

Short-term safety, adequate growth, and effects on the total number of bifidobacteria in stools have been demonstrated with prebiotics; however, no long-term studies on the effects have been conducted. Although the induction of softer stools may be beneficial in infants with constipation, a hypothetical concern regarding fluid balance should be considered. Animal data suggest that there may be an increased risk of *Salmonella* translocation and possible adenoma formation may occur with use of FOS,[70,71] but this has not been observed in human studies to date.

Probiotics available as food ingredients or dietary supplements containing microorganisms have been used extensively in food processing for years, with a long history of safety and no adverse effects on metabolism.[72,73] However, when considering the safety of probiotics, potential adverse effects include systemic infections, altered metabolism, and gene transfer. Children with abnormal immune function should use these products with caution as they could become potential opportunistic pathogens.[74] Despite the theoretical risk of immunomodulation, especially

in immunocompromised hosts or those with autoimmune disorders, few reports of probiotic-related disease have been reported.[75–77]

13.7 CONCLUSION

Well-designed research studies suggest that supplementary consumption of certain prebiotic and probiotic strains may temporarily alter the intestinal microflora of infants and children to produce a beneficial effect. However, clinical benefit is dependent on numerous factors, such as the type of prebiotic ingredient or specific bacteria, dosing regimen, delivery method, and other underlying host factors. Many claims are made by manufacturers of these products; however, their use needs to be directed through careful review of double blind, placebo-controlled studies in humans. Recommendation of a specific product for any condition requires thoughtful analysis of these issues and the avoidance of overgeneralization of results.

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CHAPTER 14

Anticarcinogenic Effects of Probiotics, Prebiotics, and Synbiotics

Shalini Jain, Mukesh Yadav, Saji Menon, Hariom Yadav, and Francesco Marotta

CONTENTS

14.1 INTRODUCTION

Cancer is a complex disorder, characterized by the uncontrolled growth and spread of abnormal cells. The prevalence of cancer is increasing rapidly and it has been predicted that the prevalence will increase further in the coming years. At present, around the world, more than 10 million cancer cases occur annually. Cancer is a leading cause of death around the world, causing more than 6 million deaths a year. The exact causes of most types of cancer are still not known, and there is not yet a cure for cancer. It is known that the risk of developing many types of cancer can be reduced by adopting certain lifestyle changes, such as quitting smoking and eating a nutritional balanced diet.

The prevalence of cancer is more common in industrialized nations, but its prevalence in developing countries is also increasing, particularly as these nations adopt the diet and lifestyle habits of industrialized countries. The risk of cancer exists for every person in this universe, and it is believed that anyone can get cancer at any age; however, about 80 percent of all cancers occur in people over the age of 55. Cancer appears to occur when the growth of cells in the body is out of control and cells divide too rapidly. It can also occur when cells "forget" how to die. Cancer is a disorder that can affect any site in the body. About 100 human cancers are recognized. Four most common cancers have been reported in most of the population: lung, colon/rectum, breast, and prostate. A report from National Cancer Institute (NCI) states that the incidence rates for these four types of cancer have continued to decline since 1990; however, even with a decrease, NCI indicates that colon cancer is the second most frequently diagnosed cancer in the United States. Colorectal cancer is one of the most common causes of death in populations of developed countries who consume "Western-style diets" (World Cancer Research Fund, American Institute for Cancer Research, 1997). Studies report that dietary patterns, lifestyle exposure, physical inactivity, and obesity increase colorectal cancer risks, especially in genetically predisposed populations (Potter, 1999). Colorectal cancer is thus causally related to both genes and environment. The environment delivers risk factors that cause mutations and initiate cancer or enhance growth by genetic and epigenetic mechanisms (Ferguson, 1999). Nutrition may supply products that may counteract the causative factors (Johnson et al., 1994) and that can be recommended on the basis of a wholesome and complete diet (Pool-Zabel, 2005).

14.2 CARCINOGENESIS PROCESS IN COLORECTAL CANCER

Cancer is a combination of various metabolic and physiologic disturbances in the cell, which are directly or indirectly related to the involvement of genetic makeup (Giovannucci, 2007). Generally, all cancers involve the malfunction of genes that control cell growth and division. The process by which cancers develop is called carcinogenesis. Figure 14.1 shows how colorectal cancer progresses in various stages. Generally, the carcinogenesis process usually starts when chemicals or radiation (carcinogen) damages DNA, the genetic structure inside cells (Toft and Arends, 1999). Viruses are also potent inducers of cancer, and they normally induce carcinogenesis by introducing new DNA sequences (Khalili et al., 2001). Normal cells have DNA repair machinery, so that most of the time when DNA becomes damaged, the cell is able to repair it. In cancer cells, however, the damaged DNA is not repaired. Normal cells with damaged DNA die by the process of apoptosis, whereas cancer cells with damaged DNA continue to multiply and make multiple copies of cells. The exact mechanisms for the development of cancer mediated through mutations are obscure; it is not exactly known how mutations in DNA develop cancer and how many mutations are required for the development of the complete carcinogenesis process, as carcinogenesis is a multistep process, in which as many as 10 distinct mutations may have to accumulate in a cell before the cell becomes cancerous. Normal cell

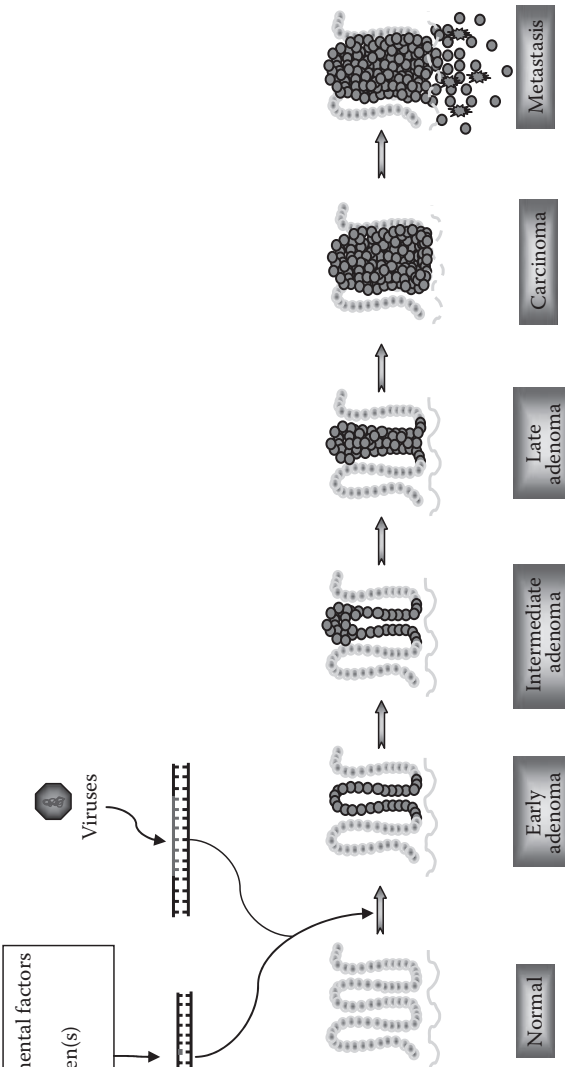

Figure 14.1 Causative agents of cancer induction and various progressive stages of colon carcinogenesis.

growth is controlled by various cell cycle checkpoints and shows a normal growth pattern. These cell cycle checkpoints are regulated by various genes and/or protein machinery. During development of cancer, various genes related to cell growth become mutated, which leads to progression of the cancer phenotype. When cells become cancerous, they start to divide in an uncontrollable manner and accumulate in a particular area of the body. Uncontrolled dividing cells make lumps, which are abnormal accumulations of cells and are called tumors/neoplasms. A tumor, or neoplasm, is an abnormal lump or mass of tissue that may compress, invade, and destroy normal tissue. Tumors may be benign or malignant.

Based on the area affected, the names of different cancers vary. In colorectal cancer, surface or epithelial cells become cancerous; thereby it is called adenoma. Colorectal cancer progresses through following stages: (1) early adenoma, (2) intermediate, (3) late adenoma, (4) carcinoma, and (5) malignant or metastasis (Figure 14.1).

1. ***Early adenoma***: When normal gut epithelial cells are exposed with various alterations in the genetic makeup and lose normal growth control, they start to multiply uncontrollably. This stage of colorectal cancer is called early adenoma.
2. ***Intermediate adenoma***: In this stage cancerous cells start to accumulate on the surface area of the epithelial membrane and make abnormal aberrant crypt foci (ACF), characterized by overconvolution in the gut surface.
3. ***Late adenoma***: This is also a progressive step for overaccumulation of cancerous cells, which makes other cells too sensitive and they also lose contact inhibition. Up to this stage adenoma may be benign and may have a noncancerous phenotype, if growth is suppressed at some point.
4. ***Carcinoma***: In this phase, cancerous cells become overreactive and start to grow very fast and produce an overgrown tumorlike structure. Cancerous cells start to break the border between tissues and the circulatory system.
5. ***Metastasis***: Circulatory system barriers are broken down in this stage, and cancerous cells start to spread in the whole body via the circulatory system, that is, blood and/or lymphatic system. These circulatory cancerous cells accumulate in other tissues and make new tumors far away from the origin, and also invade other tissues.

14.3 ANTICARCINOGENIC POTENTIAL OF PROBIOTICS AND PREBIOTICS

The increasing prevalence of human colorectal cancer is receiving the attention of health professionals and researchers who seek better therapeutic and prevention strategies. Although early detection and surgery have significantly reduced both mortality or morbidity in patients affected by colorectal cancer, survival after surgical treatment for advanced colorectal cancer, even if is followed by a number of adjuvant therapies, has not seen significant improvement in recent years. Hence, prevention of the development of colorectal cancer appears to be the more rational and effective strategy. The multistep nature of colorectal cancer together with the concept of carcinogenesis, that is, the phenomenon by which independent premalignant

foci may progress concurrently and at a different rate to give rise to multiple primary tumors, makes the colon a peculiarly suitable target organ for any given chemoprevention study. Indeed, chemoprevention of colorectal cancer in humans has been the focus of a number of studies where fibers, vitamins, calcium, low fat, and nonsteroidal antiinflammatory drugs have all been shown to affect the incidence of this disease (Duris et al., 1996; Langman and Boyle, 1999; Reddy, 1999). Approximately 70 percent of colorectal cancer is associated with environmental factors, probably mainly the diet (Saikali et al., 2004). Thus, much attention has focused on decreasing cancer risk through diet alterations, particularly consumption of probiotics and increasing intake of dietary fiber (prebiotics). The term *probiotics* is defined as "a viable microbial dietary supplement which beneficially affects the host through its effects on the intestinal tract" (Gibson and Roberfroid, 1995). A prebiotic is defined as a "indigestible food ingredient which beneficially affects the host by selectively stimulating the growth and/or activating the metabolism of one or a limited number of health promoting bacteria in the intestinal tract, thus improving the host's intestinal balance" (Gibson and Roberfroid, 1995). It has been reported that ingestion of probiotics, prebiotics, or combinations of both (synbiotics) plays an important role in the prevention of colorectal cancer, and represents a novel new therapeutic option. Probiotics and prebiotics act to alter the intestinal microflora by increasing concentrations of beneficial bacteria, such as lactobacilli and bifidobacteria, and reducing the levels of pathogenic microorganisms. Probiotics and prebiotics may regulate colorectal cancer by the following possible mechanisms (Figure 14.2):

1. Changes in the colon pH
2. Alteration of gut xenobiotic metabolism
3. Modulation of immune system
4. Antioxidant property
5. Demutagenic effect

Lactobacilli and bifidobacteria are the two well-known probiotics that could lower the risks of colon cancer and may act as most potent chemopreventive organisms. Goldin and Gorbach have demonstrated that dietary administration of some specific lactobacilli strains significantly decreased the incidence of 1,2-dimethylhydrazine-induced experimental colon cancer (Goldin and Gorbacj, 1980; Goldin et al., 1996). Although the first set of strategies for cancer control is ideally the removal of causative agents, such an approach remains very elusive for colorectal malignancies, as yet. Several studies have suggested that the effect of diet on cancer development is indirect, primarily by affecting the ability of the host to metabolize procarcinogens to proximate carcinogens whose activation, in the case of colon cancer, may be mediated by the bacterial flora in the large bowel. A number of bacterial enzymes have been implicated in producing or enhancing mutagens, carcinogens, and various tumor promoters, such as β-glucuronidase, azoreducatse, 7-α-hydroxy-steroid dehydrogenase, glycocholic acid hydrolase, and cholesterol dehydrogenase (Goldin and Gorbach, 1976). Indeed, a number of studies have provided strong evidence in favor of a key role played by certain resident gut bacteria

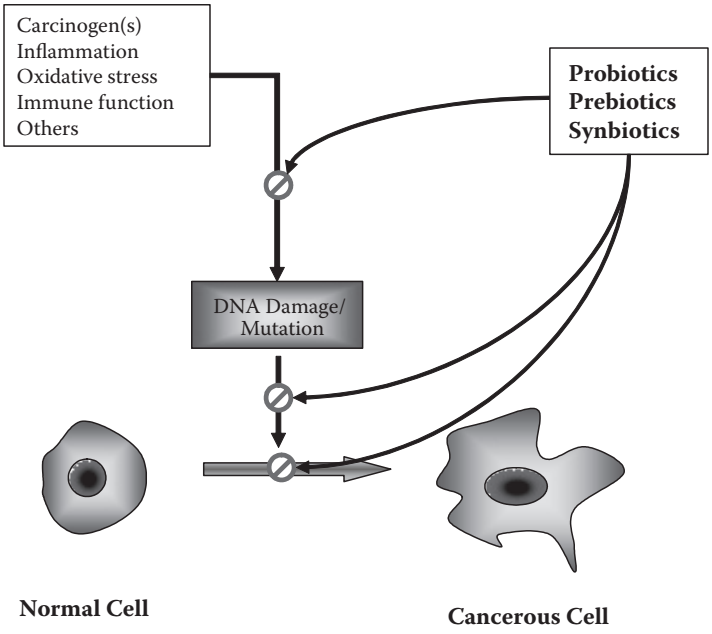

Figure 14.2 Purported mechanisms of action of probiotics, prebiotics, and synbiotics on the transition of normal cells toward cancerous cells.

in the development of large bowel cancer (Gorbach and Goldin, 1990; Kanazawa et al., 1996; Kulkarni and Reddy, 1990; Moor and Holdeman, 1975). These latter findings have given rise to a number of chemopreventive studies with probiotics in colon cancer models in the last decade (McIntosh et al., 1999; Pool-Zobel et al., 1996; Rao et al., 1999; Rowland et al., 1998; Wollowski et al., 1999, 2001; Yamazaki et al., 2000). Many of these studies have aimed at affecting the occurrence of ACF, such as demonstrated by Marotta et al. (2003), because such cellular abnormalities possess several biological aberrations including cell mutation and amplification (Bird, 1995) and are generally regarded as relevant end point lesions of colonic cancers both in the rat and in other species. ACF are regarded as preneoplastic lesions inducible in rat colon by exposure to azoxymethane, a colon-specific carcinogen (McLellan and Bird, 1988) and the risk of malignancy is correlated with the number of foci and the degree of aberrancy as measured by the number of crypts per focus. A new promising research on a novel strain, still to be clearly classified from a taxonomic viewpoint, is named bacillus oligonitrophilus (KU-1); a Russian and an Italian group have demonstrated its potential antitumor effect (Malkov, 2006a) both in implanted mammary tumors in dogs and in some ongoing clinical trials (Malkov et al., 2006b). Some anecdotal reports (Figure 14.3 and Figure 14.4) have shown striking results in case of metastatic localizations, which have been either halted in their progression or even reverted to fibrosis. More detailed studies are in progress aimed to identify the mechanisms of action and its applicability in larger clinical settings. In addition to

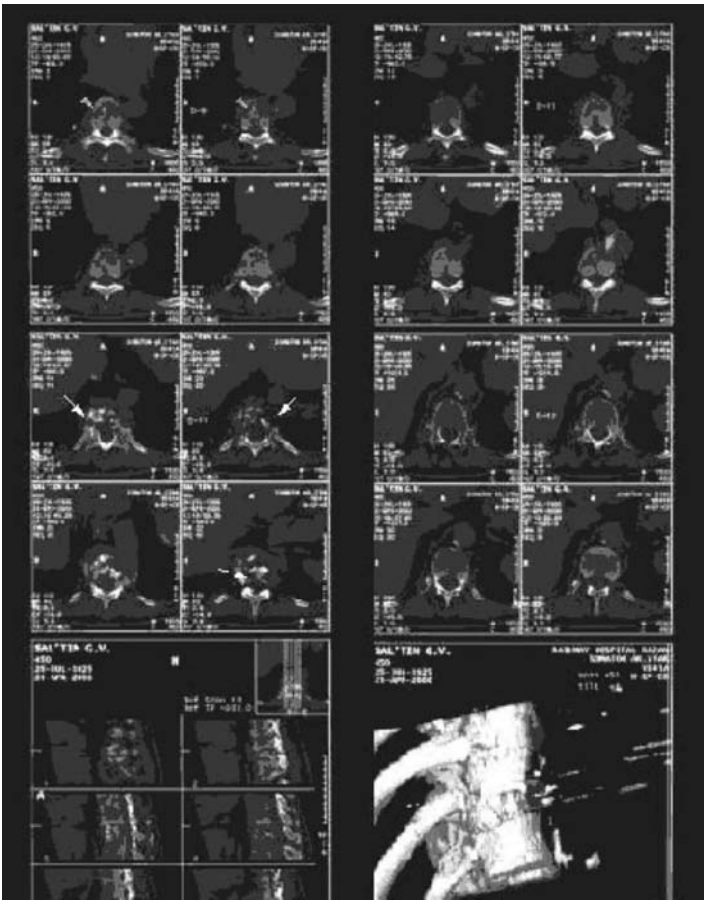

Figure 14.3 Magnetic resonance images (MRIs) of patient with backbone metastases (before treatment with bacteria). Compression fracture of the Th.XI vertebral body with wedge-shaped deformity and slight consequent kyphosis are detected. There are sclerotic bony fragments, but the presence of lytic process is also evident; this is most conspicuous in the vertebral arches. Surrounding soft tissues are somewhat widened. There is another lytic area (approximately 1.5 × 2.3 cm) on the left anterior aspect of the Th.X vertebral body adjacent to Th.X intervertebral space, affecting the cortical bone as well. A third lytic area is demonstrated in Th.IX vertebral body on the right side with a size of approximately 1 × 2.5 cm. It has lobulated contours with sclerotic margins. There are moderate sclerotic degenerative appositions at the Th.XI facet joints. There is no significant spinal canal stenosis at this point. Lytic areas in Th.XI vertebra involving the arches: white arrows; lytic lesion in Th.IX vertebral body with sclerotic margin: short arrow; lesion in Th.X vertebral body at its lower rim. (Adapted from Maklov et al., 2006a. With permission).

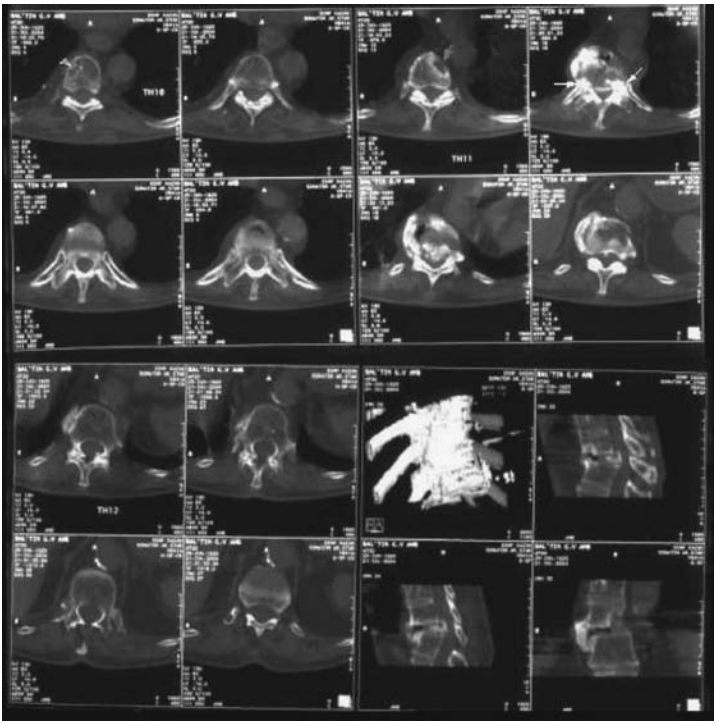

Figure 14.4 MRIs of patient with backbone metastases (after treatment with bacteria). There is progressive spinal deformity; collapse of the anterior part of Th.XI vertebral body is complete with more prominent wedge-shaped deformity. Posterior sclerotic bony elements shifting toward the canal cause significant stenosis (at least 50 percent in AP direction). Remarkable osteophytic appositions have developed on the right lateral aspect of Th.X–XI intervertebral spaces. There is marked progression of sclerotic degenerative changes in the facet joints as well. There is sclerosis in the vertebral arches instead of the formerly observed lytic areas. No evidence of soft tissue mass. The lytic area affecting the left anterior aspect of Th.X vertebral body became demarcated by irregular sclerotic margin. The lesion in the Th.IX vertebral body is unchanged. Compression fracture of Th.XI vertebral body was probably caused by lytic bone pathology, which seemed to affect Th.X vertebral body as well. From the available data, the nature and integrity of this process cannot be determined with confidence; both malignant neoplasia (metastasis) and benign tumors/tumor-like lesions may be taken into consideration. The follow-up, however, reveals progression only in the secondary deformity and the accompanying degenerative changes with consolidation and demarcation of the lytic components. This could be explained by an effect of successful antineoplastic, bacterial-based treatment of a malignant tumor. The probability of malignancy would set lower. The lesion in the Th.IX vertebral body had slightly different imaging characteristics that have not changed in the follow-up period; hence, it may represent benign pathology different from the one affecting Th.X–XI segments. The most striking finding of the follow-up scan is the evolution of significant bony spinal canal stenosis. Sclerosis in place of former lytic areas in Th.XI vertebra: white arrows; unchanged lytic lesion in Th.IX vertebral body with sclerotic margin: short arrow; lesion in Th.X vertebral body at its lower rim with sclerotic margins. (Adapted from Maklov et al, 2006a. With permission.)

probiotics, prebiotics as they are indigestible have been associated with reduced risk of colon cancer mainly by production of short-chain fatty acids, such as butyrate. The possible mechanism by which both probiotics and prebiotics mediate their effect in preventing colon cancer is discussed in the following sections.

14.3.1 Changes in Colon pH

pH in gut plays a very important role as an innate immune barrier. Lactic acid bacteria have the potential to produce various free fatty acids, organic acids, and other metabolites, which lead to decreased pH in the gut. Decrease in colon pH is considered as one of the potent properties of probiotic bacteria in reducing the incidence of colon cancer. Reddy et al. (1997) observed that a stimulated growth of bifidobacteria in the colon could lead to the inhibition of azoxymethane-induced colon carcinogenesis. This inhibition in ACF and its multiplicity was attributed to the pH-lowering effect of bifidobacteria in the colon, which subsequently inhibited the growth of *Escherichia coli* and clostridia. The decrease in growth of pathogenic microorganisms may also produce modulation of such bacterial enzymes as β-glucuronidase that can convert procarcinogens to carcinogens (Kulkarni and Reddy, 1994). Moreover, a prebiotic-induced decrease in luminal colonic pH may function to improve mineral solubility and uptake, namely, calcium, magnesium, and iron. In particular, enhanced bacterial fermentation has also been shown to have this effect on calcium ions, through the fermentation of such substances as phytate (myo-inositol hexaphosphate), which binds to divalent cations, such as calcium. Improved calcium absorption would provide adequate calcium for various physiological processes (Roberfroid et al., 1995; Younes et al., 2001). Additionally, calcium is suggested to be beneficial toward colorectal cancer, with increasing evidence that it inhibits proliferation and enhances differentiation and apoptosis of mucosal cells (Lamprecht and Lipkins, 2003). Further, an acidic luminal environment may reduce procarcinogenic enzyme activity, such as that of 7a-hydroxylase and nitroreductase (Ballongue et al., 1997).

14.3.2 Altering Xenobiotic Metabolism in Gut System

Various chemical substances are responsible for the induction of colon cancer. These substances appear either to come with food or to be produced by gut commensal flora. A xenobiotic is "a chemical found in organisms, but not expected to be produced or present in them," and many, if not most, human carcinogens are xenobiotics. A range of enzymes (xenobiotic metabolizing enzymes, or XME) are classed as either phase 1 or phase 2, which function to convert these exogenous compounds into reactive metabolites or carry out conjugation reactions in order to detoxify reactive compounds for excretion, respectively (Lhoste et al., 2001). Phase 1 enzymes include the cytochrome P450s (CYP) and phase 2 enzymes include glutathione-*S*-transferase (GST) and NAD(P), quinine reductase (quinone reductase), UDP-glucuronosyltransferase (UGT), sulfotransferases, and *N*-acetyl transferase (NATs) (Hashimoto and Degawa, 1995; Joseph and Jaiswal,1994; Lin et al., 1994).

Although the liver is predominantly responsible for biotransformation of ingested compounds, as it contains the majority of the XME, the colon and other tissues also show activity (Helsby et al., 2000).

There are 57 CYPs encoded in the human genome, mainly catalyzing the metabolism of steroids, bile acids, eicosanoids, drugs, and xenobiotic chemicals (Guengerich, 2003). However, some of the P450s are also active carcinogens. Some epidemiological research has shown increased risk of colon cancer in individuals with high P4501A2 activity. The metabolic activation of food-borne heterocyclic amines to colon carcinogens in humans is hypothesized to occur via N-oxidation followed by O-acetylation to form the *N*-acetoxy arylamine that binds to DNA to yield carcinogen–DNA adducts. These steps are catalyzed by hepatic cytochrome P4501A2 and acetyltransferase-2 (NAT-2), respectively (Lang et al., 1994). It has been postulated that probiotics, such as *Bifidobacterium,* could lower the risks of colon cancer, by producing metabolites that could affect the mixed-function of P450s and subsequently affect the conversion of azoxymethane from proximate to ultimate carcinogen (Campbell and Hayes., 1976). These properties of probiotics to alter the xenobiotic metabolizing enzyme suggest that probiotics could suppress colon cancer.

Similarly, Helsby et al. (2000) showed that wheat bran fed at 10 or 20 percent dietary levels to Wistar rats led to changes in the levels of activity and expression of several XMEs, both in hepatic and colonic tissues. Other authors have shown differential effects of wheat bran, carrot fiber, and oat bran, to suggest that the nature or source of the dietary fiber influences which, if any, enzyme activities are modified (Nugon-Baudon et al., 1996). However, the extent to which bacterial modification is associated with these changes in expression of XMEs is not always clear. There are at least two possible mechanisms by which prebiotics may affect hepatic or colonic XMEs through actions on the microbiota (Ferguson et al., 2005; Kirlin et al., 1999). Digestion and fermentation of dietary fiber carbohydrates leads to the production of short-chain fatty acids, of which butyrate in particular has been shown to induce phase 2 enzymes. Other authors (Ferguson et al., 2005; Helsby et al., 2000) have also pointed out that the action of colonic esterases may lead to the release of hydroxycinnamic acids from certain dietary fibers in the human colon, and these acids also have modulatory effects on XMEs in mammalian cells.

Binding of carcinogens to bacterial cell walls has been suggested to protect against colorectal cancer. El-Nezami and colleagues (El-Nezami et al., 1998; Eaton and Gallagher, 1994; Henry et al., 1999) demonstrated such binding with aflatoxin B1 (AFB1), a fungal dietary contaminant causing mutagenic and carcinogenic effects in both animals and humans. Binding of AFB1 was strain-specific, with *Lactobacillus rhamnosus* strain GG (LBGG) and *L. rhamnosus* strain LC-705 (LC-705) the most effective. *In vivo*, health benefits would work through preventing intestinal contact and absorption, hepatic metabolism, and enhancing excretion.

In considering the case of AFB1 as an example, the physical sequestration of the carcinogen has been implicated as the main mechanism for the reduced contact and absorption into the intestinal mucosa and metabolic transformation by the liver into mutagenic and carcinogenic metabolites. It was clearly shown that the effect

is not due to detoxification of the carcinogen, as nonviable heat and acid-treated LBGG and LC-705 still demonstrated carcinogen-binding properties (El-Nezami et al., 1998). It is believed that this binding involves bacterial cell surface carbohydrates. Further, new noncovalent or hydrophobic interactions were also found to be significant in the treated cells, as was demonstrated with the binding of the dietary mutagenic pyrolyzate, 3-amino-1,4-dimethyl-5-H-pyrido [4,3-b]indole (Trp-P-1) to a *Lactococcus* strain. Of minor significance is the electrostatic interactions produced by the presence of metal cations, especially with divalent cations, which are chelated by AFB1 and bound by bacterial cell walls to lessen bacterial AFB1 binding (Haskard et al., 2000).

Perhaps more germane to the current discussion is whether carcinogen binding demonstrated *in vitro* can be extrapolated to an *in vivo* situation. Bolognani et al. (1997) showed that while certain lactic acid bacteria are indeed able to effectively bind a range of dietary carcinogens *in vitro*, with differing species and carcinogen specificities, no reduction in *in vivo* mutagenicity was detected in animal studies. Thus, they concluded that binding of carcinogens to the fecal microbiota does not exert a significant influence on intestinal absorption, metabolic transformation, and distribution. They have offered explanations pertaining to the rise in pH between the stomach and the small intestine or changes in other relevant conditions that could have reversed binding *in vivo.* In addition, varying nutritional states prior to treatment may have contributed to disagreement among studies (Bolognani et al., 1997).

14.3.3 Modulation of Immune Response

The immune system consists of a complex series of interlinked mechanisms, which function in protection against infections (Perdigon et al., 1995) and uncontrollably growing tumor cells (Wollowski et al., 2001). The intrinsic properties of lactobacilli to modulate the immune system make them attractive for health applications. The mechanisms by which probiotics may inhibit colon cancer are not yet fully characterized; however, one mechanism by which this may occur is via modulation of the mucosal and systemic immune responses and by reduction in the inflammatory response to host flora.

Modulation of the immune system can occur through intrinsic adjuvance and cytokine-inducing properties of lactobacilli. Administration of lactobacilli can affect cytokine expression in specific and nonspecific manners. The ability to perform phagocytosis and kill microbes including bacterial pathogens is a major effector function of macrophages. Different strains of lactobacilli are able to activate macrophages and induce production of tumor necrosis factor-alpha (TNF-α), interleukins (IL), specifically viz. IL-1, IL-6, IL-12, IL-18 (Maassen, 2000), which increase the process of phagocytosis. The natural killer (NK) cells play a key role in protection against viral infections and tumor development.

Studies describing a probiotic-mediated increase in antitumor immunity via mechanisms including cytokine production and modification of T-cell function have been reviewed previously (Hirayama and Rafter, 2000; Rafter 2003). Recently, it has been demonstrated that lactic acid bacteria, particularly the cytoplasmic

fraction of *L. acidophilus* SNUL, *L. casei* YIT9029, and *B. longum* HY8001, were able to significantly reduce tumor proliferation *in vitro*, increase survival rate in mice injected with tumor cells, and promote antitumor activity via increased cellular immunity (Lee et al., 2004). Sun et al. (2005) have further demonstrated *in vivo* that peptidoglycan from a *Lactobacillus* species was able to dose-dependently reduce the growth of CT26 colon cancer cells in BALB/c mice via an increased level of apoptosis. Interestingly, peptidoglycan had no effect on tumor cell apoptosis *in vitro*, implying that the *in vivo* antitumorigenic activity may have been mediated by the immune response (Sun et al., 2005). Similarly, cell wall preparation of *B. infantis* was found to inhibit tumor activity in mouse peritoneal cells *in vitro* (Sekine et al., 1995), while cell wall preparation of heat-killed *L. casei* (LC9018) was found to induce immunity against tumor induction in a randomized, controlled, and comparative study involving 223 patients with stage III cervical cancer. The antitumor effects were found to be due to the activation of macrophage by LC9018 (Okawa et al., 1993).

A strain of *Lactococcus lactis* genetically engineered to produce the antiinflammatory cytokine, IL-10, has been demonstrated to reduce colonic inflammation in the dextran sulfate sodium model of colitis (Steidler et al., 2000). This study highlighted the potential for probiotics to be used as a delivery system for antiinflammatory or antitumorigenic substances that could assist in the prevention or treatment of colorectal cancer. A probiotic strain could potentially be engineered to produce other cytokines, such as transforming growth factor-b (TGF-b), which has been demonstrated to inhibit epithelial growth and promote apoptosis in the colon (Markowitz et al., 2000).

Prebiotic consumption has further been shown to convey an antitumorigenic effect via an enhancement of the immune response. Ghoneum et al. (2004) demonstrated that consumption of modified arabinoxylan rice bran (MGN-3/Biobran) was able to enhance the activity of NK cells and the binding of NK cells to tumor cells in aged C57BL/6 and C3H mice indicating potential benefits in the treatment of colorectal cancer.

Strengthening of tight junctions is another mechanism by which pro- and prebiotics may have the capacity to reduce colorectal cancer, as tight junction disruption and loss of intestinal barrier integrity are known features of the promotion stage of colon carcinogenesis. In support of this, a recent *in vitro* study demonstrated that pro- and prebiotic fermentation products led to an increased integrity of Caco-2 intestinal monolayers treated with the tumor promoter deoxycholic acid (DCA) (Commane et al., 2005). Synbiotic combinations have also shown a synergistic effect, greater than that of either the pro- or prebiotics administered individually. Roller et al. (2004) demonstrated that synbiotic combination of oligofructose-enriched inulin, *L. rhamnosus* and *B. lactis* conveyed an antitumorigenic effect via modulation of the intestinal immune system. This synbiotic treatment was also demonstrated to prevent azoxymethane-induced suppression of NK cell-like activity in Peyer's patches, an effect not observed in the individual pro- and prebiotic treatments.

14.3.3.1 Reduction of Intestinal Inflammation

Intestinal inflammation has been linked to the development of colorectal cancer, with inflammatory bowel disease (IBD) increasing the likelihood of colorectal cancer development later in life (Collins et al., 2006). Recently, probiotics have been shown to reduce intestinal inflammation in a number of animal models of IBD (Rachmilewitz et al., 2004) and in human patients with IBD (Bibiloni et al., 2005). This reduction in inflammation has the potential to lead to a reduced incidence of colorectal cancer. Some lactic acid bacteria, such as *Streptococcus thermophilus* TH-4, are bacterial strains with the capacity to produce high levels of folate, a compound with important DNA repair properties. *Streptococcus thermophilus* has been used successfully as a vehicle to deliver a source of folate to rats with chemotherapy-induced mucositis and reduce the proinflammatory response (Tooley et al., 2006). Similarly, Pompei et al. (2007) observed that administration of folate-overproducing bifidobacteria (*B. adolescentis* MB 227, *B. adolescentis* MB 239, and *B. pseudocatenulatum* MB 116) to Wistar rats produce folate *in vivo* and improved the folate status of rats. Future studies could also investigate the potential for folate-producing probiotics to reduce tumor development *in vivo*, as folate has been shown to protect against colorectal cancer (Van Guelpen et al., 2006).

14.3.4 Antioxidant Properties

Oxidative stress is a hallmark in the pathophysiology of various life-threatening human diseases including cancer (Halliwell, 2007). Oxidative stress is produced in cells by oxygen-derived species resulting from cellular metabolism and from interaction with cells of exogenous sources, such as carcinogenic compounds, redox-cycling drugs, and ionizing radiations. Oxidative stress is normally characterized by either higher production or lower clearance of reactive oxygen species. Reactive oxygen species (ROS) of various types are formed *in vivo* and many are powerful oxidizing agents, capable of damaging DNA and other biomolecules (Salim et al., 2008). Increased formation of ROS can promote the development of malignancy, and the "normal" rates of ROS generation may account for the increased risk of cancer development in the aged. Indeed, knockout of various antioxidant defense enzymes raises oxidative damage levels and promotes age-related cancer development in animals. In explaining this, most attention has been paid to direct oxidative damage to DNA by certain ROS, such as hydroxyl radical (OH•).

Various workers reported antioxidant effect of lactic acid bacteria and their fermented milk products (Grajek and Olejnik, 2005; Yadav et al., 2007, 2008). These studies show that lactic acid bacteria prevent the oxidative stress processes, which are considered to play a key role in the pathogenesis of cancer progression. Zommara et al. (1994) reported that whey collected from fermented milk was effective for suppressing the elevation of lipid hydroperoxide induced by bile duct ligation. Rats fed on milk whey and its fermented product exhibited lower levels of mitochondrial hydroperoxide activity compared with bile duct ligated

rats fed on the control diets. An elevation of serum hydroperoxide was also suppressed in rats fed on milk whey and its fermented products. Sanders et al. (1995) also reported that *Lactococcus lactis* demonstrated antioxidative superoxide dismutase (SOD) activity. Likewise, whey from cultured skim milk increased antioxidant enzymes in liver and RBCs of rats (Zommara et al., 1996). The activity of SOD in RBCs and the activity of catalase in liver were elevated on feeding cultured product diets compared with reference diets. In addition, the activity of glutathione peroxidase in RBCs was higher on diet containing *Lactobacillus acidophilus* compared to reference diet. The nonfermented whey diet was not effective in increasing antioxidant enzymes as with the fermented products. These results suggest that fermented milk exerts a specific effect on oxidative stress. In another study, Zommara et al. (1998) studied the antiperoxidative properties of a fermented bovine milk whey preparation in rats fed on a low vitamin E diet and identified the active principle in the preparation. They observed that fermented milk product exerted an antiperoxidative activity in these rats. An exogenous supply of either an amino acid mixture or lactic acid stimulated the unfermented whey proteins to prevent RBC hemolysis and to lower liver thiobarbituric acid reactive oxygen substances (TBARS). The supply of whey proteins, particularly β-lactoglobulin in the product resulted in an increase in liver reduced glutathione (GSH) and prevented iron-mediated lipoprotein peroxidation.

In addition, many workers identified more lactic acid bacteria exhibiting antioxidative activity. Lin and Yen (1999) identified five strains of *Streptococcus thermophilus* and six strains of *L. delbrueckii* ssp. *bulgaricus*. Likewise, Lin and Chang (2000) demonstrated antioxidant property of *L. acidophilus* ATCC 4356 and *B. longum* ATCC 15708. Terahara et al. (2000) studied the preventive effect of *L. delbrueckii* spp. *bulgaricus* on the oxidation of LDL *in vivo*. Recently, Kullisaar et al. (2003) reported that consumption of fermented goat's milk (made using *L. fermentum* ME-3) improved antiatherogenicity in healthy subjects, prolonged resistance of the lipoprotein fraction to oxidation, lowered levels of peroxidized lipoproteins, oxidized LDL, 8-isoprostanes, and glutathione redox ratio, and enhanced total antioxidative activity. Vibha (2004) and Kapila (2004) reported increased activity of antioxidant enzymes, specifically, catalase, SOD, and GPx, in RBCs of dahi, fermented milk, and probiotic cultures fed groups of rats. The levels of lipid peroxides in RBCs and liver were observed to be significantly lower in rats fed on fermented milk containing *L. casei* (Kapila et al., 2006). Moreover, Choi et al. (2006) demonstrated that heat-killed lactic acid bacteria cells and fractionations of such treated cells could suppress the viability of human cancer cells and inhibit the cytotoxicity associated with oxidative stress. They isolated soluble polysaccharides from *L. acidophilus* 606 and suggested that these polysaccharides may constitute a novel anticancer agent, which manifests a high degree of selectivity for human cancer cells and antioxidative agent in the food industry.

14.3.5 Desmutagenicity

Some investigations have also showed that cultured milk possesses desmutagenicity and this activity increases with increasing numbers of viable cells, indicating that probiotics play an important role in the inhibition of mutagenicity (Usman and Hosono, 1998). Thyagaraja and Hosono (1993) found that probiotic isolated from "idly," a traditional cereal pulse product of India could exert desmutagenicity on various spice mutagens, heterocyclic amines, and aflatoxins. Subsequent studies on the desmutagenicity properties of probiotics suggested that the desmutagenic substances may reside in the cellular envelope of the bacterial cell wall (Singh et al., 1997). Also, mutagens were suggested to be bound to the cell wall of probiotics. This has been supported by previous studies that have found binding properties by fractions of the cell wall skeleton of probiotics on mutagens (Zhang and Ohta, 1991) and the binding of heterocyclic amines by intestinal probiotics (Orrhage et al., 1994). In addition, whole cells of bifidobacteria have also been found to bind with the ultimate carcinogen methylazoxymethanol (Kulkarni and Reddy, 1994) and mutagen-carcinogen 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*] indole (Zhang and Ohta, 1993), thus physically removing it via feces and subsequently minimizing its absorption into the intestinal lumen.

14.4 CONCLUSIONS

Various *in vitro* and animal model studies proved the potential for and prebiotics to exert anticarcinogenic effects. Certain combinations of pro- and prebiotics (synbiotics) have revealed greater efficacy *in vivo* than either treatment alone, although studies in humans have been less definitive in colorectal cancer. Possible mechanisms by which pro- and prebiotics manifest anticancer activity include a change in gut pH, modulation of immune response, decreased colonic inflammation, antimutagenic properties, antioxidant properties, production of antitumorigenic compounds, and reduction of carcinogenic compounds. Further research is required to identify which probiotic, prebiotic, or synbiotic will be most efficacious.

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CHAPTER 15

Prebiotics and Probiotics in Infant Formulae

Günther Boehm, Richèle Wind, and Jan Knol

CONTENTS

15.1 INTRODUCTION

Human milk is the ideal nutrition for term infants because it provides all necessary nutrients for rapid growth and postnatal development. The quantity and quality of nutrients is adapted to the high nutritional requirement of rapid growth as well as to the functional maturation of the gastrointestinal tract and the metabolism of the infant. In addition, human milk contains components which are—partially or completely—resistant to intestinal digestion and provide functional capacity.[1,2] There is broad consensus that breastfed infants develop differently compared to infants with artificial feeding.[3] Breastfed infants, in comparison to formula-fed infants, have a reduced incidence of allergic or atopic diseases,[4–8] a reduced incidence of infections,[9–13] and a reduced incidence of diabetes mellitus type I.[14] This indicates a major impact of breastfeeding on the development of the immune system.[15–17] Better cognitive functions[18] and lower blood pressure[19] in later life have also been reported for breastfed infants.

The positive effects of breastfeeding are multifactorial. One of the physiologic aspects of the effects of breastfeeding is the establishment of a specific intestinal microbiota. There are many functions attributed to the intestinal microbiota found in breastfed infants. There is increasing evidence that the composition of the intestinal microbiota plays a key role in the postnatal development of the immune system,[20–23] but effects of bacterial fermentation products on the maturation of the immune system[24,25] are under investigation.

Because of the importance of the intestinal microbiota for the development of gut physiology and the immune system,[23] many attempts have been made to mimic the intestinal microbiota of breastfed infants also in bottle-fed infants.

The composition of the intestinal microbiota can be influenced either by administration of large amounts of living bacteria that survive the gastrointestinal tract to be active in the colon[26] or by the use of dietary ingredients that are nondigestible during the passage through the small intestine, reach the colon, and can selectively be used by health-promoting colonic bacteria.[27,28]

As a third opportunity, the combination of both principles as “synbiotics” is under discussion.[29]

This chapter summarizes the current knowledge of the influence of breastfeeding on the postnatal development of intestinal microbiota. The possibilities to mimic this function with prebiotics or probiotics are evaluated and the functional consequences of dietary manipulation of the composition of intestinal microbiota on the physiology of the host are discussed.

15.2 INFLUENCE OF BREASTFEEDING ON POSTNATAL DEVELOPMENT OF INTESTINAL MICROBIOTA

15.2.1 Postnatal Development of Intestinal Microbiota

Before birth, the infant's gut is sterile. During vaginal delivery, the natural colonization of the infant starts with bacteria mainly from the vaginal and intestinal microbiota of the mother. For the further development of the intestinal microbiota of the infant the diet plays an important role.[30] During breastfeeding, the composition of the gut microbiota changes within a short period and becomes dominated by bifidobacteria whereas infants fed formulas without prebiotics develop a flora of a more adult type with a lower total level of bifidobacteria.[31,32]

In healthy breastfed infants, many bifidobacterial species are found with the most dominant being *Bifidobacterium infantis*, *B. breve,* and *B. longum*. Formula-fed infants without prebiotics contain relatively more *B. adolescentis* and *B. catenulatum*.[30,33] Postnatal development of intestinal microbiota is furthermore influenced by mode of delivery, gestational age, infant hospitalization, and antibiotic use by the infant.[34] For example, in infants born by caesarean delivery and in preterm infants, the fecal colonization by bifidobacteria is delayed.[35–37]

15.2.2 Oligosaccharides as the Main Prebiotic Factor in Human Milk

The prebiotic effect of breast feeding was intensively investigated over the last century. Several so called "bifido-factors" have been identified as recently reviewed by Coppa et al.[38] Lactoferrin, lactalbumin, nucleotides, or urea were seen as specific substrates of intestinal microbiota or the low concentration of protein or phosphate in human milk might act as an environmental factor for bacterial growth. Consequently, the effect of human milk on the postnatal development of the intestinal microbiota cannot be attributed to a single ingredient. However, there is evidence that human milk oligosaccharides (HMOS) might play a key role in this matter.[39–44]

The fraction of oligosaccharides in human milk is characterized by an enormous structural diversity. Additionally, there are great variations in concentration and composition between individuals and during the course of lactation.[42,43] They appear as free structures or are conjugated to macromolecules as glycoproteins, glycolipids, and others. There is evidence that more than 1,000 distinct molecules in the HMOS fraction exist.[42–45]

Oligosaccharides appear in human milk at a concentration of up to 1 g/100 mL. As there are no enzymes in the human intestine to cleave the HMOS, they are resistant to enzymatic digestion during passage through the small intestine.[45–47] However, many intestinal bacteria express glycosidases to metabolize HMOS.[48–51] This clearly indicates the physiological role of HMOS as prebiotic components in breast milk. In particular, bifidobacteria possess several homologous genes to encode enzymes involved in the metabolism of numerous carbohydrates present in human milk.[51,52] This might be the reason for their large presence in the colon, reflecting a

specific adaptation to this highly competitive ecological niche, especially in breast-fed infants.[53]

All these data provide strong evidence that many HMOS are preferentially synthesized to be metabolized as prebiotic ingredients by intestinal microbiota rather than to be used as a nutritional substrate.

Apart from their prebiotic effects, there is also evidence that HMOS act as receptor analogues to inhibit the adhesion of several pathogens on the epithelial surface.[54] There are many different target structures of pathogens,[43] which might partially explain the great variety of structures of HMOS. On the other hand, the protection against adhesion of pathogens might open opportunities for interactions of commensal bacteria with the epithelial surface that seems to be of physiological importance.

15.2.3 Other Functions of HMOS

As carbohydrate compounds are a main part of structures on the cellular surface, HMOS can act as signaling molecules that might explain the great variety of functions attributed to HMOS.[38–43] The possibility that HMOS interact directly with immune cells is of particular interest. Such direct interactions have been reported with selectins,[55] dentritic cell-specific C-type lectin,[56] integrins,[57] and other target receptors.[58] In an *in vitro* study, particularly acidic HMOS demonstrated a direct effect on the number of activated or regulatory T cells.[59]

Because HMOS are resistant to digestion and the maturation of the gut is not fully developed,[60] they can pass the intestinal wall in smaller amounts (approximately 1 percent of intake).[46] It can be speculated that the appearance in the plasma and the distribution across the whole body might be one factor for a possibly direct systemic effect of HMOS on the immune system. However, this hypothesis needs further investigation.

15.2.4 Human Milk and Breastfeeding as the Source of Bacteria

For many years studies on the microbiology of breast milk have been restricted to transmission of pathogenic bacteria. This was mainly in relation to mastitis and contamination of breast milk related to its use in milk banks. Only a few studies are available in which bacteria from breast milk samples of healthy women were analyzed. These studies show that low amounts of bacteria are present in human breast milk. This may, however, be due to contamination or may originate from the ducts or areola of the breast. Bacterial strains isolated from breast milk included lactobacilli, *Streptococcus, Enterococcus, Peptostreptococcus, Staphylococcus,* and *Corynebacterium,* with sometimes *Escherichia* spp.[61–63] Recently, the presence of bifidobacteria has also been shown.[64,65] Bacterial numbers detected in breast milk range from lower than 1×10^3 to a maximum of 1×10^5 colony-forming units (cfu)/mL. Differences in bacterial numbers may be due to contamination and organisms residing in the ducts or on the areola of the breast.[61–63] Bacterial studies in breast milk, therefore, need to be repeated and their biological significance needs to be elucidated. It has been shown that transfer of bacteria through breastfeeding is one

of the ways that maternal microbes colonize the neonatal gut. Identical strains were found in bacterial isolates from mother and newborn pairs, which were not found on the breast skin.[61,66] Furthermore, it has been shown that breast milk contains a range of bacterial DNA signatures, as also found in maternal peripheral blood mononuclear cells.[63] These DNA signatures showed a larger biodiversity than observed by plating of breast milk samples. It was speculated that these signatures might program the neonatal immune cells as was shown in pregnant mice. As the impact of the bacteria transferred during breastfeeding on the colonization of the gastrointestinal tract is not completely clear, this topic is currently the subject of intensive research.

15.3 PREBIOTICS

15.3.1 Definition of Prebiotics

Prebiotics can be seen as food for the intestinal bacteria, which are mainly located in the colon. Gibson and Roberfroid, the pioneers in the developing of the prebiotic concept, defined prebiotics as dietary ingredients that are not digestible, reach the colon, and can be used by health-promoting colonic bacteria.[27]

More recently, the prebiotic concept was revised. The same authors now define prebiotics as dietary compounds, which have to be resistant against luminal digestion until they are fermented by the intestinal (i.e., not only colonic) microbiota. The balanced stimulation of bacterial growth and/or activity of the health-promoting bacteria in the gastrointestinal tract have to be demonstrated by performing studies in the target group.[28]

15.3.2 Characterization of Prebiotics

By using the example of human milk, several ingredients, such as lactoferrin, gangliosides, nucleotides, or urea, have been tested for their prebiotic activity. Among the prebiotic ingredients, carbohydrate structures have been identified as the most effective prebiotic compounds. Consequently, the majority of infant formulas on the market with a prebiotic claim contain carbohydrate structures as active ingredient.[53]

There is a wide range of molecule size distribution within the HMOS fraction.[43] Since 1980, oligosaccharides have been defined as carbohydrates with a degree of polymerization up to 10. However, recently the IUB-IUPAC Joint Commission on Biochemical Nomenclature stated that the borderline between oligo- and polysaccharides cannot be drawn too strictly. The term “oligosaccharide” is commonly used to refer to defined structures as opposed to a polymer of unspecified length. Thus, even though they have molecules with a degree of polymerization significantly larger than 10, the HMOS are all described as oligosaccharides. The same approach is used for oligosaccharides of nonhuman milk origin as long as they have defined structures.[67, 68]

Depending on the type, size, and structure as well as the source of oligosaccharides a variety of separation techniques and methods have to be applied for the

characterization of the molecules of interest. The most relevant methods have been recently reviewed by Boehm et al.[69]

For the analyses of the most widely used prebiotics like fructans (oligofructoses, inulin) and galacto-oligosaccharides, AOAC methods (Association of Official Analytical Chemists) have been recently published.

Because the structure of HMOS is so complex, such molecules are not yet available for the production of infant formulas. Thus, other sources of dietary oligosaccharides need to be identified. As alternatives, oligosaccharides from milk of domestic animals as well as several oligosaccharides of nonmilk origin are under investigation.

There are several structural and potentially functional similarities between HMOS and oligosaccharides from milk of domestic animals. The structure and function of oligosaccharides from domestic animals are extensively reviewed by Urashima et al.[70] The preparation of these compounds is difficult and, therefore, large-scale preparations have not been commercially available. Consequently, no clinical trial has been published so far using fractions of animal milk oligosaccharides as prebiotics.

The most important oligosaccharides already used as prebiotics in infant nutrition are galacto-oligosaccharides (GOS; derived from enzymatic synthesis from lactose) and fructans from inulin type (fructo-oligosaccharides, FOS; derived from extraction from plants and from enzymatic synthesis). But also palatinose/isomaltulose oligosaccharides (derived from enzymatic synthesis from sucrose), soy bean oligosaccharides (derived from extraction from soy beans), lactulose (derived from enzymatic synthesis from lactose), xylo-oligosaccharides (derived from enzymatic synthesis from, for example, corncob xylan), and galacturonic acid oligosaccharides (derived from enzymatic degradation of pectin) have already been tested in infant formulas.[53]

15.3.3 Physiological Effects of Prebiotics

The intestinal microbiota play an important role for the physiology of the intestinal tract. Not only does the direct contact between microbiota and the epithelial surface have to be considered, but also the physiological effect of bacterial fermentation products, such as short-chain fatty acids has to be considered. Theoretically, prebiotics might also get directly in contact with epithelial cells or the bacterial membrane. However, there are only few very preliminary data available to support this hypothesis. Thus, this chapter focuses on the effects of prebiotics mediated via their influence of the composition and metabolic activity of the intestinal microbiota.

15.3.3.1 Influence on the Intestinal Microbiota

In term infants, prebiotic effects during infancy have been investigated for several substances, such as short-chain GOS (scGOS),[71,72]scFOS,[73–81] inulin,[82,83] and lactulose.[84,85] Additionally, mixtures have been tested, such as a mixture of scGOS and lactulose,[86] scFOS with inulin,[87] galacturonic acid oligosaccharides in combination with scGOS/long chain FOS (lcFOS),[88,89] and a mixture of scGOS and lcFOS (IMMUNOFORTIS™)[90–112] (Table 15.1). In preterm infants, scFOS[113] and the mixture scGOS/lcFOS[114–117] have been tested (Table 15.2).

Table 15.1 Clinical Trials with Prebiotic Oligosaccharides in Term Infants (Nutritional Intervention During the First Year of Life)

Prebiotic Compound	Age (Months)	Target Group/ Completers in Prebiotic Group	Main Outcome	Ref.
scGOSL	0–6	Healthy infants/43	Increased counts of bifidobacteria and lactobacilli	Yahiro et al.[71]
scGOS	0–6	Healthy infants/69	Increased counts of bifidobacteria and lactobacilli, decreased fecal pH	Ben et al.[72]
scFOS	6–24	Infants with antibiotic treatment/57	Increased counts of bifidobacteria after antibiotic treatment	Brunser et al.[73]
scFOS	4–24	Healthy infants/63	Decreased severity of diarrhea diseases (no microbiology)	Saavedra et al.[74] Tschernis et al.[75]
scFOS	0–3	Healthy infants/58	No clear effect on counts of bifidobacteria, softer stools (dose dependent)	Euler et al.[76]
scFOS	4–12	Healthy infants/27	Softer stools, no effect on fecal pH (no microbiology)	Moore et al.[77]
scFOS	6–12	Healthy infants/239	No influence on clinical course and incidence of diarrhea, no effect on vaccination response (no microbiology)	Duggan C et al.[78]
scFOS	6–24	Healthy infants/10	Trend for higher counts of bifidobacteria and decrease in potential pathogens, no persistence after intervention	Waligora-Dupriet et al.[79]
scFOS	0–2	Healthy infants/35	Increased number of stools, no bifidogenic effect, no influence on fecal pH	Guesry et al.[80]
scFOS	0–4	Healthy infants/144	Safe and less complication (no microbiology)	Bettler et al.[81]
Inulin	2–6	Healthy infants/14	Increased counts of bifidobacteria and lactobacilli, softer stools	Kim et al.[82]
Inulin	5–12	Healthy infants/28	Tendency of increased short-chain fatty acid production, significant influence on mineral absorption (no microbiology)	Yap et al.[83]
Lactulose	0–6	Healthy infants/6	Increased counts of bifidobacteria, reduced fecal pH	Nagendra et al.[84]
Lactulose	1–36	Infants with allergic symptoms/12	Increased counts of bifidobacteria, improvement of symptoms	Rinne et al.[85]

continued

Table 15.1 Clinical Trials with Prebiotic Oligosaccharides in Term Infants (Nutritional Intervention During the First Year of Life) (continued)

Prebiotic Compound	Age (months)	Target Group/ Completers in Prebiotic Group	Main Outcome	Ref.
scGOS/lactulose	0–6	Healthy infants/150	Softer stools and increased stool frequency (no microbiology)	Ziegler et al.[86]
scFOS/inulin	0–12	Healthy infants/24	Increased postvaccination IgG measles antibody plasma levels	Firmansyah et al.[87]
AOS + scGOS + lcFOS	0–6	Healthy infants/31	Increased counts of bifidobacteria with GOS/FOS/AOS, decreased fecal pH	Fanaro et al.[88]
AOS + scGOS + lcFOS	0–6	Healthy infants/49	Bifidogenic, in particular if AOS are present	Magne et al.[89]
scGOS + lcFOS	0–5	Infants with constipation/20	Reduced hardness of stool (no microbiology)	Bongers et al.[90]
scGOS + lcFOS	0–4	Healthy infants/56	Increased counts of bifidobacteria and lactobacilli, decreased fecal pH, effect dose dependent	Moro et al.[91] Moro et al.[92]
scGOS + lcFOS	0–6	Healthy infants/28	Increased counts of bifidobacteria, softer stools	Schmelze et al.[93]
scGOS + lcFOS	0–6	Healthy infants/21	Increased counts of bifidobacteria and lactobacilli, dominance of *B. infantis*, short-chain fatty acid pattern like breastfed infants	Knol et al.[94] Harman et al.[95] Harman et al.[96]
scGOS + lcFOS	0–3	Healthy infants/34	Trend for higher counts of bifidobacteria, reduced counts of clostridia	Costalos et al.[97]
scGOS + lcFOS	9–12	Infants with minor gastrointestinal problems/604	Reduction of gastrointestinal problems (no microbiology)	Salvino et al.[98]
scGOS + lcFOS	9–12	Infants with minor gastrointestinal problems/55	Reduction of gastrointestinal problems (no microbiology)	Salvino et al.[99]
scGOS + lcFOS	4–12	Infants at weaning/10	Increased counts of bifidobacteria	Scholtens et al.[100]

scGOS + lcFOS	0–4	Healthy infants/19	Reduced fecal pH, increased fecal short-chain fatty acids, increased fecal sIgA; no bifidogenicity	Bakker-Zierikzee et al.[101] Bakker-Zierikzee et al.[102]
scGOS + lcFOS	0–6	Healthy term infants at risk for allergy/102	Increased counts of bifidobacteria, reduced incidence of atopic dermatitis, reduced incidence of infections, antiallergic serum antibodies	Moro et al.[103] Arslanoglu et al.[104] Garssen et al.[105] Arslanoglu et al.[106]
scGOS + lcFOS	0–6	Healthy infants/86	Increased counts of bifidobacteria, increased fecal sIgA	Alliet et al.[107]
scGOS + lcFOS	0–3	Healthy infants/14	Increased counts of bifidobacteria	Desci et al.[108]
scGOS + lcFOS	0–6	Healthy infants/8	Increased counts of bifidobacteria, *Bifidobacterium* microbiota close to breastfed infants	Rinne et al.[109]
scGOS + lcFOS	0–2	Healthy infants/20	Increased counts of bifidobacteria and lactobacilli	Penders et al.[110]
scGOS + lcFOS	0–12	Healthy infants/162	Decreased rate of infection (recurrent upper respiratory tract infection, diarrhea)	Bruzzese et al.[111]

Note: GOS, galacto-oligosaccharides; FOS, fructo-oligosaccharides; AOS, acidic oligosaccharides deriving from pectin; lc, long chain; sc, short chain.
Source: Adapted from Boehm and Moro, 2008.[53]

Table 15.2 Review Clinical Trials with Prebiotics in Preterm Infants (Nutritional Intervention During the First Year of Life)

Prebiotic Compound	Age	Target Group/ Completers in Prebiotic Group	Main Outcome	Ref.
scFOS	0–2	Healthy infants/36	Increased counts of bifidobacteria within 1 week of intervention	Kapiki et al.[119]
scGOS + lcFOS	0–2	Healthy infants/15	Increasing counts of bifidobacteria, reduction of hardness of stools, reduction of counts of fecal pathogens	Boehm et al.[114] Knol et al.[115]
scGOS + lcFOS	0–2	Healthy infants/10	Reduction of gastrointestinal transit time; reduction of stool viscosity (no microbiology)	Mihatsch et al.[116]
scGOS + lcFOS	0–2	Healthy infants/10	Statistically significant but small effect on reduction of gastric emptying time (no microbiology)	Indrio et al.[117]

Source: Adapted from Boehm and Moro, 2008.[53]
Note: GOS, galacto-oligosaccharides; sc: short chain; FOS, fructo-oligosaccharides; lc: long chain.

Related to the use of prebiotics during infancy the most experience exists for GOS and FOS. Most of the prebiotic infant formulas currently on the market contain these ingredients either as an individual compound or in various combinations. Therefore, the following section focuses on these two prebiotic ingredients.

15.3.3.1.1 Digestibility of Galacto-Oligosaccharides and Fructo-Oligosaccharides

As nondigestibility in the small intestine and selective fermentation by the intestinal microbiota are prerequisites of any prebiotic effect of dietary ingredients,[27,28] human studies have been performed to address this issue.

In a study in fructose-sensitive patients, no side effects of inulin could be detected demonstrating the low or absent digestibility of lcFOS.[118] In a study in adult patients with ileostoma focusing on pectin hydrolysates,[119] we could also demonstrate that scGOS are still detectable after passage through the small intestine (data not yet published). In a group of term infants fed with a prebiotic formula, the presence of the dietary scGOS and lcFOS could be detected in the feces.[92] The data clearly indicate that the studied prebiotics can reach the colon. This assumption is supported by the fact that the fecal pH and the concentrations of short-chain fatty acids could be significantly influenced by these prebiotics.[91,94,102,114] These findings are in line with results of fermentation experiments.[120–122] In addition, there is also evidence from such studies that the metabolic rate decreases with increasing chain length.[123]

15.3.3.1.2 Prebiotic Function of Galacto-Oligosaccharides and Fructo-Oligosaccharides

The counts of fecal bifidobacteria or the percentage of fecal bifidobacteria of the total bacteria are generally accepted measurements to detect a prebiotic effect. Using this marker, GOS and FOS can be classified as prebiotics.[124]

As demonstrated in Table 15.1 and Table 15.2, many authors use combinations of prebiotic oligosaccharides. There are several aspects favoring the use of mixtures of oligosaccharides instead of individual components. One principal aspect is the diversity and complexity of the HMOS,[39,41–44] which indicates that several structures and a wide range of molecule sizes[125] are necessary to provide the full functionality of HMOS.

In one study with the prebiotic mixture of scGOS/lcFOS, the counts of bifidobacteria were measured either with a conventional plating technique (measuring the living bacteria) or with a molecular biologic technique (measuring all bacteria). With both methods, an increase of bifidobacteria could be recorded. However, with increasing concentration of the prebiotics, this difference between the different methods disappeared.[126] This indicates that the counts of bifidobacteria as well as their metabolic activity have been stimulated by the prebiotics.

As the interaction between dietary components and the intestinal ecosystem is very complex, the matrix of the food might be important for the effect. Prebiotics

have also been successfully added to infant formula of different protein quality and quantity as well as to solid weaning food or cereals (recently reviewed by Boehm and Moro[53]). Prebiotic effects have also been seen with these compounds in adults when used as a supplement to a typical western diet.[127] Thus, there is evidence that the prebiotic effect can be independent of the type of food used as the basis for the nutrition.

The bifidogenic effect is often associated with a reduction of the stool pH and changes in the short-chain fatty acid pattern. Short-chain fatty acids are the fermentation products of bacteria in the colon. They are, therefore, an important characteristic feature of the intestinal microbiota.[128] As already mentioned, the profile of short-chain fatty acids depends considerably on the composition of the diet. Supplementing an infant formula with a mixture of scGOS/lcFOS resulted in a pattern of short-chain fatty acids in the feces that corresponded to the pattern found in the feces of breastfed infants.[94] Since the bifidobacteria produce only acetate and lactate, the short-chain fatty acid pattern reflects the metabolic activity of the entire microbiota and not only the activity of bifidobacteria. Thus, it can be assumed that short-chain fatty acid profiles similar to the profiles found in breastfed infants reflect similarities of the entire microbiota between breastfed infants and infants fed with a formula supplemented with the studied mixture.

There are several results available indicating that the short-chain fatty acid profile and pH influence the physiological role of intestinal cells. In particular, effects on mucin-2 synthesis and barrier integrity are described.[130] In addition, there was also an effect of the short-chain fatty acids pattern and pH on the growth of several pathogens.[131] This effect has a particular clinical relevance during infancy. In fact, the reduction of fecal pathogens could be demonstrated in a study in preterm[115] as well as term infants.[94]

There is evidence that early colonization with specific microbiota might be associated with the development of allergic symptoms later in life. Bjorksten et al.[132] found that allergic infants were less often colonized by lactobacilli and bifidobacteria than nonallergic infants. Additionally, it was found that allergic infants had more adultlike species in their fecal flora, including *B. adolescentis*, compared with healthy infants. In the latter, *B. bifidum, B. infantis,* and *B. breve* predominated.[133] Also in Japanese infants suffering from atopic dermatitis, similar findings have been reported.[134] This suggests that different bacterial species may have different functional effects on the immunological reaction of the host. In two studies using a mixture of scGOS/lcFOS as the prebiotic ingredient, it could be demonstrated that the prebiotic mixture promoted *B. infantis* and depressed *B. adolescentis*.[95,109]

In summary, the experimental data as well as the results of clinical trials prove that substances with a structure different from the structure of HMOS are able to influence the intestinal microbiota comparable to those found in breastfed infants.

15.3.3.2 Influence on Postnatal Development of the Immune System

There is accumulating evidence that the interaction between the intestinal microbiota and the gut plays an important role for the postnatal development of the immune system. However, the interactions between the intestinal epithelial and immune cells

and the different species of the intestinal microbiota are very complex and not fully understood.[20–23] The variability of the different layers of the human defense system[135] and the diversity of the intestinal microbiota[32] cause this complexity.

15.3.3.2.1 Results of Animal Studies

Following international recommendations,[136,137] studies in mice are recommended to substantiate conclusions related to immunological effects of dietary compounds. The available experimental data concerning the immune modulatory effect of prebiotics have been intensively reviewed by Vos et al.[58]

In mice, it could be shown that a prebiotic mixture (scGOS/lcFOS) was bifidogenic in a dose-dependent manner. This results in a reduction of the fecal pH and in a fecal short-chain fatty acid pattern as found in human infants, supporting the relevance of the animal data for the human situation.[138]

A mouse vaccination model adapted to investigate the effect of prebiotics was used to study the effect on prebiotics on the allergic reaction. It could be demonstrated that a prebiotic mixture (scGOS/lcFOS) significantly stimulated the vaccination response in a dose-dependent manner and modulated the immune system toward a Th1-dominated immune response.[138] The effect only occurred if the intervention with prebiotic nutrition started before the first vaccination. This indicated that the modulation of the immune system was mainly mediated by the developing intestinal microbiota. It might also indicate that the use of prebiotics for prevention is more relevant than for a treatment approach.

There are also data available concerning the effect of prebiotics on the allergic reaction in a mouse model using ovalbumin as antigen.[139] Feeding the animals with a prebiotic mixture (scGOS/lcFOS) significantly reduced the allergic reaction against ovalbumin as demonstrated by reduction of bronchial restriction after metacholine application, reduction of inflammatory cells in the bronchial lavage fluid, and reduction in the immunoglobulin E (IgE) concentration in plasma.[137]

In summary, the animal data allow the conclusion that prebiotics can positively modulate the immune system of the mice and provide preventive effects with regard to the development of infectious as well as allergic diseases. This effect seems mainly mediated by modulation of the intestinal microbiota.

15.3.3.2.2 Results of Human Studies

There is broad consensus that the intestinal microbiota are a physiological part of the gastrointestinal tract.[140–142] Therefore, it is a logical assumption that early inoculation by intestinal bacteria plays an important role in the development of the infant.

As the immune system is so complex, no individual biomarker can describe the whole immune system. Therefore, it is recommended that clinical studies focused on clinical outcome (incidence of infectious and/or allergic symptoms) and biomarkers representing the status of the immune system be performed.[136,137] Following this recommendation, the effect of prebiotics on the incidence and severity of diarrhea,

the response to vaccinations, and the effect of nongastrointestinal infections as well as allergic symptoms have been studied.

Saavedra et al.[74,75] reported that the supplementation of weaning food with scFOS was associated with a reduced rate of diarrheal episodes. However, no effects of the same prebiotics on the clinical course and incidence of diarrhea were found by Duggan et al.[78]

Firmansyah et al.[87] reported increased postvaccination IgG antibodies in plasma induced by a mixture of scFOS and lcFOS.

Moro et al.[103] reported a reduced cumulative incidence of atopic dermatitis diagnosed according to the international recommended diagnostic criteria.[143] The study was performed in a group of high-risk infants identified by familial history. This was accompanied by the development of an antiallergic immune globulin profile.[105] More recently, the 2-year follow-up data have been reported that further support the hypothesis that a prebiotic formula administered early in life modulates the development of the immune system.

In a study performed in a healthy population of 326 term infants,[111] the supplementation of a formula with a prebiotic mixture scGOS/lcFOS resulted in a reduced incidence of different infectious symptoms during the first year of life.

In summary, the available data from human trials are completely in line with the data derived from animal experiments demonstrating the immune modulatory effect of prebiotics. There is evidence that the effects are specific for each prebiotic ingredient. Thus, data obtained with a specific prebiotic compound cannot easily be transferred to all possible prebiotic oligosaccharides.

15.3.3.3 Influence on Gut Health

From animal studies it is known that short-chain fatty acids as products of bacterial fermentation play an important role in the regulation of intestinal motility mainly due to their interaction with the G-protein coupled receptor 43 (GPR43) and sequential release of serotonin. This receptor is also expressed in the human colon. Dietary intervention with the target to modulate the intestinal microbiota has demonstrated that this modulation influences interdigestive intestinal motility.[144,145] This is in line with observations in preterm infants. Prebiotic formulas (mixture of scGOS/lcFOS) fastened the gastrointestinal transit time[116] and significantly reduced the gastric emptying time.[117]

Feeding different prebiotics resulted in term infants in softer stools and/or increased stool frequency (see Table 15.1) also indicating an effect of prebiotics on gut physiology. Consequently, formula with prebiotics as the active ingredient has been designed to treat gastrointestinal symptoms like constipation and abdominal colics.[93,98,99]

There is first evidence that the mineral absorption during infancy can be positively influenced by prebiotics.

In summary, prebiotics might positively influence gut physiology. However, further clinical studies are necessary to evaluate the clinical relevance of these effects.

15.3.4 Safety in Infants

There are no known side effects when applying up to 1 g/100 mL of GOS. In theory, higher dosages could display osmotic effects. In clinical trials, concentrations higher than 1 g/100 mL have not been applied. Therefore, such side effects have not been described in infants.

At higher concentrations (>0.5 g/100 mL) FOS can cause flatulence. This is dose dependent and applies especially to scFOS (chain length of up to 10 monomers).[146] As a consequence, some commercially available infant formulas with prebiotic oligosaccharides especially use fractions of inulin with a chain length of more than 10 monomers at a relatively low concentration.[125] In this form, the prebiotic formulae could be used as treatment for intestinal symptoms.[93,98,99]

Based on the estimation of fecal excretion of *O*-linked oligosaccharides, Bruggencate et al.[147] reported increased bacterial translocation in adults by using FOS. However, the study design was not optimal; in particular, some other conditions in the study could cause the observed translocation. In a second study without this bias, the translocation could not be observed.[148] More recently, Barrat et al.[149] reported an increase bacterial translocation in artificially reared rats fed a prebiotic infant formula. Many questions related to the adequacy of the model as well as to methodology are still not solved. Thus, the consequences of these findings for human infant nutrition are not clear.

The Scientific Committee on Food of the European Union (EU) commented twice on applications from suppliers of infant nutrition.[150,151] The committee did not have any safety concerns with regard to a total concentration of 0.8 g/100 mL and a mixture of 90 percent GOS with 10 percent lcFOS. However, they stated in the second statement that this comment cannot simply be used as general safety statement for all prebiotics.[151]

Based on these comments, scGOS in connection with lcFOS (ratio 9:1; maximal concentration 8 g/L) has been included in the EU directive on infant formula and follow-on formula in 2006.[152]

15.3.5 Current Recommendations for Starter and Follow-On Formulae

There are no final recommendations available regarding prebiotics in infant formula. The most recent comment has been published by the ESPGHAN Committee on Nutrition in 2004.[153] Although the committee saw potential benefits, the data available at that time did not allow a general recommendation.

As demonstrated in Table 15.1 and Table 15.2, particularly for the mixture of scGOS and lcFOS, several new randomized, prospective, double-blind, and placebo-controlled studies have been published. The data indicate that these prebiotics can serve as an effective and safe tool to strengthen the immune system during infancy.

This might offer a new method for prevention of infections and allergy. Long-term follow-up studies are needed to provide insights whether these effects during infancy are relevant for the activity of the immune system during the whole life span.

Based on the experience from clinical trials and the composition of human milk, the concentration range of supplemented oligosaccharides should be between 0.4 and 0.8 g/100 mL. This is supported by the results of clinical studies: At a concentration of 0.8 g/100 mL of a GOS/FOS mixture, the concentration of bifidobacteria in feces was similar to that of breastfed infants.[91] The effect of prebiotic oligosaccharides depends on a constant supply. Therefore, the duration of the supplementation should follow the recommendations for breastfeeding.

15.4 PROBIOTICS IN INFANT FORMULAE

15.4.1 Definition

The intestine of a newborn is essentially sterile. It is inoculated with bacteria during birth and the first days of life. Thereafter, the gut microbial composition of infants is affected by infant feeding, mode of delivery, hospitalization, prematurity, and antibiotic use.[34] Several hundred to a thousand species of bacteria usually inhabit the human adult intestine. In the colon 10^{10} to 10^{13} microorganisms per gram feces are found. The microbiota in the intestine are involved in a number of metabolic and immunological processes. This may play a role in health and disease. To support beneficial microbiota, additional bacteria can be administrated to the infant as probiotics.

The term probiotic means "for life" and is currently used to name bacteria associated with beneficial health effects for humans and animals when consumed orally. Many definitions have circulated that have in common that the use of the word *probiotic* is restricted to products that contain live microorganisms and provide an adequate dose of probiotic bacteria in order to exert the desirable effects. Therefore, the WHO/FAO has adopted the definition of Guarner and Schaafsma:[154] "Live microorganisms which when administered in adequate amounts confer a health benefit on the host."[155,156] Since more and more studies are describing probiotic effects of nonviable bacteria and bacterial fragments, such as DNA, the ESPGHAN Committee on Nutrition broadened the definition to "microbial cell preparations or components of microbial cells with beneficial effect on the health and well being of the host."[157] In most cases, however, the WHO/FAO adopted definition is used.

15.4.2 Characterization of Probiotics

The number of probiotic products on the market has increased over the past years as well as the understanding of function, physiology, and biochemistry. Probiotic strains used in infant formula primarily belong to the genera of *Lactobacillus* and *Bifidobacterium*. Bifidobacteria are present as the predominant bacteria in the intestinal tract of breastfed infants[30,95,96,158] and are considered to contribute to the health

of infants. Good identification of the bacterial strains by means of fluorescent amplified fragment length polymorphism (FAFLP), repetitive DNA element (rep)-PCR fingerprinting, protein profiling, and 16S rDNA sequencing is highly desirable since many cases of misidentification in commercial probiotic products were reported.[159]. A number of criteria are used to select for probiotic strains based on safety, functional, and technological properties.[160,161] An effective probiotic must be nonpathogenic, nontoxic, and exert a beneficial effect on the host. Furthermore, they should be capable of surviving the passage through the gastrointestinal tract and remain viable during storage and use.

15.4.3 Physiological Effects of Probiotics

15.4.3.1 Influence on Intestinal Microbiota

Colonization with a probiotic bacterium depends on the interplay of multiple factors in the intestinal milieu, such as survival through the stomach–small intestine, presence of prebiotic factors, antibiotic treatment, and adherence to intestinal cells.[162] Probiotic bacteria can influence the intestinal microbiota by inhibition of other groups of bacteria via fermentative production of acids, such as acetate and lactate and secretion of antimicrobial components.[163]

In many of the clinical trials in which the benefit of probiotic bacteria on infants is examined, the primary health outcome is microbiota related. In most of these trials, the key groups of the gastrointestinal microbiota are determined: bifidobacteria, lactobacilli, streptococci, total anaerobes, clostridia, bacteroides, enterococci, and Enterobacteriacea group members. In some studies, the administrated probiotic strain is detected specifically. A large data set is available on the probiotic strains *B. animalis* subsp. *lactis* BB-12 (BB-12) and *L. rhamnosus* GG (LGG) (Table 15.3 and Table 15.4), which are discussed in more detail below. These studies show that administration of probiotic bacteria via infant formula can initiate a temporary increase in the administrated bacterial groups, such as bifidobacteria and lactobacilli, but only during the intervention period. These effects are clearly strain specific and depend on the dosages given. In healthy term infants, other major bacterial groups are in most cases not influenced. A decrease in the more pathogenic groups of bacteria can be found in infants who already have a disturbed microbiota at the start of the study as in preterm infants. A pH-lowering effect during the probiotic intervention was found in some cases; however, significant changes in short-chain fatty acid profiles were not detected in most cases.

15.4.3.1.1 Bifidobacterium animalis *subsp.* lactis *BB-12*

In healthy adult volunteers it was shown that the number of fecal bifidobacteria can increase significantly during the period of daily ingestion with viable BB-12.[164,165] Administration of BB-12 to term infants showed variable results on changes in microbiota. In a double-blind placebo-controlled trial, the percentage of bifidobacteria was not significantly increased by administration of BB-12 during the

Table 15.3 Clinical Trials with Probiotics in Term Infants (Nutritional Intervention During the First Year of Life)

Probiotic	Duration of Intervention	Target Group	Main Ooutcome	Ref.
Lactobacillus casei CRL431 and *Bifidobacterium lactis* Bb-12	12 months	119 infants with cow's milk allergy	Supplementation does not accelerate cow's milk tolerance	Hol et al.[268]
B. longum BB536 + *L. rhamnosus* LPR or *L. paracasei* ST11 + GOS/scFOS	4 months	284 healthy infants	Equivalent weight gain in all groups	Chouraqui et al.[269]
L. rhamnosus GG	7 months (start 4–6 weeks before expected delivery)	94 pregnant women/infants at risk	No reduction in incidence of AD nor altered severity, increased rate of recurrent episodes of wheezing bronchitis	Kopp et al.[270]
L. plantarum and FOS	7 days	31 healthy infants	Colonization of *L. plantarum*, increased bacterial diversity and Gram-positives, decreased Gram-negatives	Panigrahi et al.[271]
B. longum and LGG	6 months	37 infants	Increased bifidobacteria, decreased Enterobacteriaceae and *Bacteroides-prevotella*	Mah et al.[177]
L. acidophilus LAVRI-A1	0–6 months	178 infants at high risk	Colonization of lactobacilli No reduction of AD, increased senzitation to allergens until 12 months, no influence on FOXP3 expression, no influence on innate immune response, but effect on vaccine responses	Taylor et al.[272–275]
B. longum BB536 (combination with GOS/lcFOS)	0–7 months	138 infants without risk	No difference in growth, reduced constipation	Puccio et al.[229]
Four probiotic strains (combination with GOS)	0–6 months (start during pregnancy)	925 infants at high risk	Reduction of incidence atopic eczema	Kukkonen et al.[176]

L. reuteri ATCC 55730	0–12 months (start during pregnancy	188 infants at risk	No effect on incidence of atopic eczema, but less IgE-associated eczema at 2 years	Abrahamsson et al.[209]
L. rhamnosus GG	0–2 months	105 infants without risk	Increased growth (weight and length), softer stools	Vendt et al.[173]
L. reuteri ATCC 55730 or *B. lactis* BB-12	Start at 4 months of age, 4 weeks	95 infants without risk	Well tolerated, no effect on growth and stooling habits	Weizman et al.[184]
L. rhamnosus GG ATCC 53103	0–6 months (start during pregnancy)	132 infants with genetic risk	Well tolerated, some effects of occurrence of clostridia	Rinne et al.[172]
L. rhamnosus GG and *B. lactis* Bb-12	0–12 months	72 infants without risk	Increased number of cow's milk specific IgA secreting cells and increased production of sCD14 receptors	Rautava et al.[276]
Probiotics (four strains)	0–6 months	200 infants with genetic risk for diabetes mellitus type 1	No effect on prevalence of type 1 associated autoantibodies	Ljungberg et al.[277]
Four probiotic strains (not specified) combined with GOS	0–6 months (start during pregnancy	61 infants at risk	No effect on antibody responses to vaccination	Kukkonen et al.[278]
L. johnsonii La 1	15 weeks starting after 4 months	76 infants without risk	*L. johnsonii* La 1 colonized, but also fecal bifidobacteria were increased	Brunser et al.[279]
L. rhamnosus or L. GG	15 weeks starting after 4 months	50 infants with symptoms of AD	No clinical or immunological effect	Brouwer et al.[200]
B. animalis Bb-12	Until 8 months of age	38 infants without risk	No effect on metabolic effect of intestinal microbiota as well as on fecal sIgA	Bakker-Zierikzee et al.[101,102]
B. lactis Bb-12 or *L. reuteri* (ATCC 55730)	12 weeks starting at age 4–10 months	201 infants without risk in child care centers	Decreased incidence of episodes of fever and diarrhea (*L. reuteri* more effective than *B. lactis*)	Weizman et al.[228]
L. rhamnosus GG or mix (four strains)	4 weeks, starting at age <12 months	230 infants with symptoms of AD or cow's milk allergy	Probiotics induced low-grade inflammation	Viljanen et al.[280]

continued

Table 15.3 Clinical Trials with Probiotics in Term Infants (Nutritional Intervention During the First Year of Life) (continued)

Probiotic	Duration of Intervention	Target Group	Main Ooutcome	Ref.
L. rhamnosus GG	3 months, starting during pregnancy	96 infants without risk during breastfeeding	Increased counts of Ig-secreting cells in colostrums and in infants	Rinne et al.[171]
L. rhamnosus GG	2 weeks, starting age not specified	Infants without risk, number not specified	All levels of administration successfully colonized the intestine, no effect on stool characteristics	Petschow et al.[281]
B. lactis Bb-12 and *Streptococcus thermophilus* TH4	210 days	180 healthy infants	Formula was well tolerated and safe, adequate growth, less colic and antibiotic use	Saavedra et al.[227]
B. breve C 50 and *S. thermophilus* 065 in combination with fermentation products	5 months starting at 4–6 months of age	971 infants without risk	Reduced severity of diarrhea episodes, no effect on incidence	Thibault et al.[24]
L. rhamnosus GG or mix of four strains	4 weeks, age not specified	Infants suspected for cow's milk allergy	LGG raised interferon-gamma production but not the mix; the mix increased interleukin-4 secretion but not LGG	Pohjavuori et al.[282]
B. lactis Bb 12	5 months, starting before 8 months of age	90 infants younger than 8 months	Reduced duration of episodes of diarrhea and trend that the first onset occurred later	Chouraqui et al.[269]
LGG viable and heat-inactivated	7.5 weeks	35 infants with AD and cow's milk allergy	Decrease SCORAD in viable LGG group tended to be larger than placebo, adverse effects with heat-inactivated LGG	Kirjavainen et al.[170]
B. lactis Bb-12	At weaning	21 infants with early onset of AD with high risk for chronic allergic disease	Decrease in *Escherichia coli* numbers and protection against increase in *Bacteroides* during weaning	Kirjavainen et al.[205)]
L. fermentum VRI-033 PCC	8 weeks	53 infants 6–18 months with moderate to severe AD	Reduction in AD symptoms	Weston et al.[210]

L. rhamnosus GG	6 months, starting during pregnancy	132 infants at risk	Reduced incidence of AD	Kalliomaki et al.[197,202,204,206]
B. breve YIT4064	14 days, starting age not specified	19 infants without risk	Reduced rotavirus shedding and prevention of rotavirus infection	Araki et al.[283]
S. thermophilus and *L. helveticus*	0–8 weeks of age	20 infants without risk	Increased counts of bifidobacteria	Langhendries et al.[166]
B. bifidum Bb-12 and *S. thermophilus*	2–3 months	55 infants aged 5–24 months admitted to the hospital	Decreased incidence in diarrhea, decrease in rotavirus shedding	Saavedra et al.[189]

Note: AD, atopic dermatitis; GOS, galacto-oligosaccharides; FOS, fructo-oligosaccharides; lc, long chain.

Table 15.4 Clinical Trials with Probioticsin Preterm Infants (Nutritional Intervention During the First Year of Life)

Probiotic	Duration of Intervention	Target Group	Main Oootcome	Ref.
Lactobacillus acidophilus	0–2 months	37 preterm infants	37% of infants colonized, improved feeding tolerance, tendency to increased incidence of sepsis	Lee et al.[284]
Bifidobacterium lactis Bb-12	30 days	75 preterm infants	Higher bifidobacteria counts, decreased intestinal permeability (lactose mannitol test) and increased head growth	Stratiki et al.[192]
B. lactis Bb-12	0–3 months	69 preterm infants	No effect on colonization by antibiotic-resistant organisms, higher bifidobacterial numbers in probiotic group	Mohan et al.[167]
L. rhamnosus GG (Dicoflor)	0–3 months	80 preterm infants	Reduction of intestinal colonization of *Candida* species	Manzoni et al.[285]
B. breve M-16V (live but non viable)	0–4 weeks	19 preterm infants	Upregulation of TGF-β1 signaling	Fujii et al.[286]
L. acidophilus and *B. infantis* (Infloran)	During hospital stay, not specified	376 preterm infants (birth weight < 1,500 g)	Reduced incidence and severity of NEC	Lin et al.[287]
B. infantis and *Streptococcus thermophilus* and *B. bifidus*	During hospital stay, not specified	145 preterm infants (birth weight < 1,500 g)	Reduced incidence and severity of NEC	Connolly et al.[267]
B. breve M-16V	0–2 weeks of age	30 preterm infants (birth weight < 1,500 g)	*B. breve* was successfully colonized, no clinical outcome reported	Li et al.[288]
Saccharomyces boulardii	30 days during hospital stay	87 preterm infants (gestational age 28–32 weeks)	*S. boulardii* was successfully colonized, increased number of bifidobacteria, no effect on d-xylose or lipid absorption	Costalos et al.[289]
L. rhamnosus GG	21 days (birth weight < 1,500 g) or 7 days	71 preterm infants (39 <1,500 g birth weight; 21 1,500–1,999 g birth weight)	Colonization in 21% (birth weight < 1,500 g) or 47% in larger infants	Agarwal et al.[162]

L. rhamnosus GG	6 weeks during hospital stay	585 preterm infants with gestational age < 33 weeks	No significant effect on incidence of NEC or sepsis	Dani et al.[220]
Escherichia coli strain Nissle 1917	Not specified	61 preterm infants	In the successfully colonized infants (56%) increased specific IgA and nonspecific IgM in plasma	Cukrowska et al.[290]
B. breve M-16V	0–8 weeks of age (please control)	91 preterm infants (birth weight < 1,500 g)	Successful colonization in 73%, improved feeding tolerance and weight gain	Kitajima et al.[291]
L. rhamnosus GG	0–2 weeks of age	20 preterm infants	No effect on fecal short-chain fatty acids	Stansbridge et al.[175]
L. rhamnosus GG	2 weeks after starting with initiation of enteral milk feeding	20 preterm infants with gestational age < 33 weeks	Successful colonization, but no detectable clinical benefit	Millar et al.[147]
L. acidophilus	Duration not specified, starting within the first 72 hours of life	30 preterm infants (not specified by birth weight or gestational age)	No effect on intestinal microbiota	Reuman et al.[292]

Note: GOS, galacto-oligosaccharides; FOS, fructo-oligosaccharides; AOS, acidic oligosaccharides derived from pectin; lc: long chain; NEC, necrotizing enterocolitis.

first 4 months of life.[102] In this trial, no effects on short-chain fatty acid profiles were found in the infants receiving the BB-12 formula compared to the infants fed with the standard formula. The pH was significantly lower in the BB-12 formula group at day 10, but not at any of the other time points. In another trial administration, BB-12 was shown to increase the prevalence of colonization with bifidobacteria at 1 month of age similar to that of breastfed infants, which was significantly higher than in the standard control infant formula group.[166] In a group of preterm infants, receiving an infant formula with BB-12 from the first day after birth, effects were more pronounced as bifidobacterial numbers were significantly higher compared to the control group when analyzed by fluorescence *in situ* hybridization (FISH).[167] Earlier studies have shown that in general the intestinal bacterial colonization with beneficial bacteria like bifidobacteria and lactobacilli is delayed in preterm infants.[168] Administration of BB-12 was shown to affect the other major bacterial groups in preterm infants, as viable counts of Enterobacteriaceae and *Clostridium* spp. were significantly reduced.[167]

15.4.3.1.2 Lactobacillus rhamnosus *GG*

Previous studies in adults indicate that administration with LGG can enhance the bifidobacterial counts in gut microbiota.[169] Administration of LGG to allergic infants and infants at risk for allergy does not seem to induce significant changes in the major bacterial groups.[170–172] In a group of healthy infants, a more frequent colonization with lactobacilli was found in the LGG-administrated group during the first 6 months of life, but other major bacterial groups were not influenced.[173] Administration of preterm neonates with LGG was reported to give a colonization of 25 to 50 percent with LGG depending on the birth weight of the neonate. In this study, administration of LGG increased the total number of bacterial species significantly, mainly due to an increase in Gram (+) species and anaerobic spp. other than LGG. Only in the infants weighing less than 1,500 g, Millar et al.[174] and Stansbridge et al.[175] reported that 90 percent of the preterm infants were colonized with LGG, but simultaneous alterations in other bacterial groups were not observed. Colonization with LGG was not shown to give any significant increase in fecal short-chain fatty acids in preterm infants.[175]

Changes in microbiota by using a mixture of probiotic strains including LGG with or without prebiotics were more pronounced. Kukkonen et al.,[176] for example, showed that the probiotic group was more frequently colonized with lactobacilli and propionibacteria after administration of a mix of four strains and GOS in a group of infants at risk for allergy. Also fecal counts of bifidobacteria and lactobacilli were significantly higher at 3 and 6 months. It was shown that these microbiota changes were relatively short term because at 24 months no differences in these microbiota groups were no longer observed. Simultaneous administration of LGG with *B. longum* BB536 in a group of infants at risk for allergy showed a significant increase in bifidobacteria with a parallel decrease in Enterobacteriaceae and *Bacteroides-Prevotella* populations.[177]

15.4.3.2 Influence on Gut Health

The best-studied clinical outcome with probiotic bacteria in infants is the effect on acute infectious diarrhea. Diarrhea contributes significantly to infant morbidity and mortality, especially in developing countries. Evidence exists that probiotic bacteria are effective in treatment and prevention of acute infectious diarrhea in infants.[178,179] Results of recent clinical trials also suggest that probiotic bacteria reduce the risk of necrotizing enterocolitis (NEC) in preterm neonates.[180,181] Studies on the use of probiotic bacteria in prevention or treatment of other gastrointestinal diseases, such as irritable bowel syndrome, inflammatory bowel disease, and constipation in infants, are scarce and provide inadequate evidence so far.[182]

15.4.3.2.1 Acute Infectious Diarrhea

Acute diarrhea is most often caused by bacterial and viral infections; bacterial infections are mostly found in early infancy whereas from the age of 6 months to 2 years rotavirus infections account for most cases.[183] Evidence exists that probiotic bacteria are effective in the treatment and prevention of acute infectious diarrhea in infants. In a meta-analysis of 34 masked, randomized, placebo-controlled trials on prevention of acute diarrhea (all causes), it was shown that in children the overall reduction was 57 and in adults 26 percent.[179] No significant differences were found among the probiotic strains used including *L. rhamnosus* GG (LGG), *L. acidophilus,* and *Saccharomyces boulardii*. Effectiveness in prevention of acute diarrhea was also shown for *L. reuteri* ATCC 55730 and *B. animalis* BB-12.[184] The number and duration of diarrheal episodes was reduced using *L. reuteri* ATCC 55730 or BB-12 compared to the control formula in a group of infants 4 to 10 months old in childcare centers. In another meta-analysis, it was shown that *L. rhamnosus* GG had a consistent effect in treatment of acute infectious diarrhea in infants and children especially reducing the risk of diarrhea lasting longer than 3 days.[185] It was calculated that four patients need to be treated with LGG to avoid one case of diarrhea lasting more than 3 days. The results on prevention of acute diarrhea in various trials with LGG were more heterogeneous. It was, however, shown that LGG in one study significantly reduced the incidence of diarrhea.[179] On a mechanistic level, it has been shown that LGG influences intestinal mucosa by the upregulation of MUC-2. This might result in increased inhibition of bacterial translocation,[186] in addition to inhibition of pathogen adhesion to intestinal mucus.[187,188] In infants admitted to a hospital, the supplementation of infant formula with *B. bifidum* (BB-12) and *Streptococcus thermophilus* was shown to reduce the incidence of acute diarrhea and rotavirus shedding.[189] A fermented infant formula with *B. breve* C50 and *S. thermophilus* 065, containing no live bifidobacteria after production, reduced severity of acute diarrhea but not the incidence and duration of diarrheal episodes.[24]

Five commercially available probiotic preparations were recently compared in a randomized trial for treatment of acute diarrhea in infants and children aged 3 to 36 months.[190] The five preparations tested were: LGG, *Saccharomyces boulardii*,

Bacillus clausii, a mix of four lactic acid bacteria (*L. delbrueckii* var. *bulgaricus*, *Streptococcus thermophilus*, *L. acidophilus*, and *Bifidobacterium bifidum*), and *E. faecium* SF68. From the five preparations tested only LGG and the mix of four strains were shown to be effective in reducing the duration of the diarrheal episodes, whereas the other preparations were found ineffective in the target group tested. The data for *Saccharomyces boulardii* were unexpected because in previous trials it was shown to be beneficial in children and infants.[191]

Overall, it can be concluded that the individual effects on infants might be modest, reducing the duration of diarrhea 17 to 30 hours. However, the larger effects on the population may be significant. In a recent review, it was shown that effects are strain specific, with LGG the most effective; dose-dependent (larger effect with doses > 10^{10} cfu/day); and most helpful for watery diarrhea (rotaviral) and viral gastroenteritis, but not for invasive bacterial diarrhea.[182]

15.4.3.2.2 Necrotizing Enterocolitis

NEC is the most commonly occurring gastrointestinal disease in preterm infants. The disease results from an activation of the inflammatory cascade leading to high expression of proinflammatory mediators caused by certain changes in microbiota. The incidence is highest in infants with less than 1,500 g birth weight and mortality rates approach 30 percent.[180] The intestinal complications occur mostly in the first weeks of life, suggesting that immaturity of the intestinal epithelial barrier function and absorptive capacity play a role. In a recent study, probiotic bacteria were shown to decrease intestinal permeability of preterm infants as measured by using the sugar absorption test.[192] *Bifidobacterium animalis* subsp. *lactis* BB-12 was shown to lower the lactose/mannitol ratio (a marker for intestinal permeability) significantly compared to the control group at day 30.

Results of recent clinical trials suggest that probiotic bacteria reduce the risk of NEC in preterm neonates with less than 33-week gestation. Seven trials were included in a meta-analysis using various probiotic strains (*B. breve* M-16V, LGG, *Saccharomyces boulardii*, BB-12, or a mix of strains). This showed an overall reduced risk of developing NEC and a reduced risk of death due to all causes in the probiotic group.[181] If a larger well-designed trial, taking into account short-term and long-term safety of probiotic bacteria in preterms, confirmed these results, this would make a strong case for the routine use of probiotic bacteria in neonates. The dose, duration, and type of probiotic agents (species, strain, single or combined, live or killed) used for supplementation should be investigated in more detail.[181]

15.4.3.3 Influence on Postnatal Development of the Immune System

Probiotic bacteria have been shown to induce changes in gut barrier function and immune responses in *in vitro* and *in vivo* animal studies. These are now also being documented in human studies in adults, children, and infants. Effects include responses of the innate nonspecific immune system like promotion of mucin production, inhibition of pathogens, decrease in gut permeability, enhancement of

natural killer cell activity, macrophage activation, and phagocytosis. There are also responses of the adaptive immune system like an increase in IgA-, IgG-, and IgM-secreting cells, an increase in total and specific secretory IgA in serum and intestinal lumen, and modulation of inflammatory gut responses.[193] Most clinical benefits in infants are reported on prevention and treatment of allergy and infections, which are described in more detail in the next two sections.

15.4.3.3.1 Allergy

The incidence and prevalence of allergic diseases in many western countries have increased during the past 40 to 50 years. It is estimated that around 20 percent of the population in Western countries suffers from some form of allergy. Development of asthma and other allergic diseases is strongly influenced by genetic components but studies suggest that environmental factors through a decreased immune stimulation also play an important role.[194,195] The concept of probiotics as a possible means for antiallergic therapy emerged out of indirect evidence linking the composition of the intestinal microbiota and the incidence rate of allergies in several studies.[133,134,196–202] Björksten and colleagues compared infants from Sweden, a country with a high prevalence of allergies, with infants from Estonia, a country with a low prevalence of allergies. In these studies it was found that allergic infants in both Estonia and Sweden were less often colonized with lactobacilli and bifidobacteria, whereas they were more often colonized with aerobic pathogenic microorganisms.[132,196] A reduced level of bifidobacteria in infants with atopic dermatitis was also reported in children in Japan.[134] Moreover, the reduced level of bifidobacteria has been shown to precede the development of the atopic disease in infants from Finland.[202–204] The composition of bifidobacteria in allergic infants has been reported to be more adultlike with more *B. adolescentis*, whereas in healthy infants, *B. bifidum, B. infantis,* and *B. breve* predominated.[133,198]

A limited number of probiotic strains were tested for their efficacy in treatment and prevention of allergy in infants. A standard scoring system for SCORing Atopic Dermatitis (SCORAD) as developed by the European task force for atopic dermatitis is applied in most clinical trials.[143] In a small trial ($n = 27$) the addition of *B. animalis* subsp. *lactis* BB-12 or *L. rhamnosus* GG was found to reduce SCORAD ($p = 0.01$) in a group of infants who manifested atopic eczema during breastfeeding.[206] Other clinical trials on treatment of allergy using LGG showed a trend in decrease on SCORAD ($n = 43$)[170] or no effects on SCORAD ($n = 50$).[200,201] LGG was effective in prevention of early atopic disease in infants at high risk ($n = 132$); the frequency of atopic eczema in the LGG group was half that of the placebo group ($p = 0.008$).[197] In this study, mothers were prenatally administrated with LGG capsules in addition to administration of LGG to their infants up to an age of 6 months. It was suggested that probiotic bacteria increased the immunoprotective potential of breast milk as shown by the increase of transforming growth factor (TGF)-β2 in the milk of mothers receiving LGG.[203] The preventive effect extended to the age of 4 years.[20] The study cohort was also reexamined after 7 years showing that the overall risk for developing eczema during the first 7 years of life was significantly decreased.[206] However,

allergic rhinitis and asthma tended to be more common in the probiotic group after 7 years indicating that more longer-term studies are needed. Administration of heat-inactivated LGG was found ineffective.[170] Administration of *L. reuteri* ATCC 55730 in combination with *L. rhamnosus* 19070-2 to treat infants and children (1 to 13 years) with atopic dermatitis did not change the total SCORAD score; SCORAD was, however, significantly reduced in the allergic patients with elevated IgE levels or at least one positive skin prick test ($p = 0.02$).[207] In the same study, it was shown that the intestinal mucosal barrier was impaired in the children with atopic dermatitis as measured by a positive association between the lactulose-to-mannitol ratio and the severity of eczema.[208] After probiotic treatment, the lactulose-to-mannitol ratio was lower compared to the control, which might indicate a stabilization of the intestinal barrier function. *Lactobacillus reuteri* ATCC 55730 reduced IgE-associated eczema significantly in a group of infants from 188 families with a history of allergy ($p = 0.02$).[209] Occasionally, other strains were found effective.[210]

15.4.3.3.2 Infection

In vitro it has been shown that probiotic bacteria are able to inhibit pathogenic bacteria through a blockade of epithelial access,[187,211] production of antimicrobials, and production of acids.[212] Also in animal models probiotic bacteria have been shown effective in preventing infections.[213,214] In adults, studies have shown a beneficial effect of probiotic bacterial strains on prevention of infectious complications; a significant reduction of infection rates in patients with abdominal surgery, liver transplantation, and acute pancreatitis was reported.[215–217] In patients with severe acute pancreatitis, probiotic prophylaxis with a mix of six different strains was associated with increased mortality and did not reduce the risk of infectious complications.[218] Therefore, the use of probiotics in critically ill patients and patients at risk for nonocclusive mesenteric ischemia is currently under debate.

In infants, most data are available on treatment and prevention of acute infectious diarrhea by using probiotic bacteria (see above). Evidence for a modest effect of some probiotic strains preventing gastrointestinal and respiratory infections in healthy infants was provided in a limited number of clinical trials.[184] LGG showed a modest but significant effect in reduction in incidence of respiratory infections and their severity among children in daycare.[219] The administration of either *B. animalis* BB-12 or *L. reuteri* ATCC 55730 to infant formula for infants in child care centers was not shown to affect respiratory illnesses, but the number of days with fever was significantly reduced as well as the number of clinical visits, child absences, and antibiotic prescriptions.[184] In this trial the incidence of diarrheal episodes was reduced as well as their duration. In preterm infants, the administration of 7 days of LGG was not shown to be effective in reduction of sepsis, urinary tract infections, and NEC.[220] Bacterial sepsis was more frequent in the LGG group, but the difference was not significant.

15.4.4 Safety in Infants

Various committees and expert groups have published reports and made recommendations on the issues that should be addressed to prove the safety of a probiotic strain in food.[155–157,221–224] At this moment there is no consensus document on a European level.[225]

Recommendations are described in very general terms: the documents lack guidance on how certain issues should be addressed, what kind of experiments should be conducted, and how the obtained results should be interpreted. Most of the reports produced by these different authorities have in common that the following possible side effects should be addressed: systemic infections, deleterious metabolic activities, excessive immune stimulation, antibiotic resistance, and gene transfer.

In the United States, probiotic bacteria used in food can be classified as an additive. Additives can either be approved by the Food and Drug Administration (FDA) on the basis of their safety and efficacy dossiers or they can be generally recognized as safe (GRAS). The GRAS status can be achieved when microorganisms have a history of safe use dating before 1958 or have been recognized by experts as safe under the conditions of intended use.[225] For example, *B. animalis* subsp. *lactis* BB-12 and *S. thermophilus* TH-4 have GRAS status since 2002 for specific use in infant formula. Currently, the European Food Safety Authority (EFSA) is introducing a similar approach. The qualified presumption of safety (QPS) concept provides a generic assessment system for internal use within EFSA that in principle can be applied to all requests received for the safety assessments of microorganisms deliberately introduced into the food chain.[226]

Several clinical trials within healthy infant groups with existing probiotic lactobacilli and bifidobacteria have shown that the products are safe and well tolerated by this age group.[227–229] It is, however, currently unknown if consumption in early life could lead to longer-term adverse effects. A double-blind, randomized, placebo-controlled tolerance and safety study addressing consumption of infant formula containing probiotic bacteria by healthy infants aged 3 to 24 months did not show any adverse effects.[227] The formulas were supplemented with *B. animalis* subsp. *lactis* BB-12 and *S. thermophilus* and resulted in adequate growth, reduced reporting of colic or irritability, and a lower frequency of antibiotic use.

The particular use of probiotic bacteria in infant formula has been addressed by the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN).[157] In the ESPGHAN Committee on Nutrition, experts in the field of infant nutrition are represented. The committee is concerned that available data are not sufficient to support the safety of probiotic bacteria in healthy newborn infants, very young infants with immature defense systems, infants with immunocompromised systems, premature infants, and infants with congenital heart disease. Infant formula with added bacteria in these target groups should be marketed only if a full evaluation of safety and benefits has been performed. The use of probiotic bacteria in premature infants is advised against, due to the current lack of information in that target group.[157] Indeed, no clinical trials were found in which the safety in preterm

infants was specifically assessed. Clinical trials in preterm infants were mainly focused on reducing the risk of NEC, in general showing no serious adverse events and no difference in risk of sepsis between the probiotic and control group.[230] In a small trial, the clinical safety of *L. casei* Shirota in critically ill children aged 0.5 to 15.9 years was assessed. There was no evidence of either colonization or bacteremia and the preparation was well tolerated with no apparent side effects.[231] In another study, in which the effect of *L. rhamnosus* GG to reduce the incidence of nosocomial infection in pediatric intensive care was studied, a trend toward an increase in infection was seen and therefore the study was terminated prematurely.[232] Indeed, more studies would be needed to study safety of probiotic bacteria in pediatric critical care. The committee has fewer concerns on follow-on formulas designed for infants older than 5 months because there is a more mature immune response, an established intestinal colonization, and a history of exposure to organisms from the environment.

15.4.4.1 Systemic Infections

Lactobacilli and bifidobacteria are generally regarded as safe, they are supposed to have low pathogenicity, and they are seldom detected in blood culture. Bifidobacteria are among the first microbes to colonize the gastrointestinal tract of newborn infants. They are usually transmitted by the mother and the surrounding environment.[233,234] Bifidobacteria are present as the predominant bacteria in the intestinal tract of breastfed infants[31,95,158] and are considered to contribute to the health of infants. Cases of infections by bifidobacteria are considered extremely rare and are mostly resolved by antibiotic treatment.[235–237] Lactobacilli are natural commensals of the gastrointestinal tract and are used worldwide as starter cultures for dairy products. Lactobacilli have been associated with isolated cases of clinical infections, such as bacteremia and endocarditis, mostly in sick people with underlying conditions. The species *L. casei* and *L. rhamnosus* are most commonly isolated from infection sites.[238] A possible epidemiological link between probiotic consumption and rise in clinical isolates of lactobacilli could not be made.[239,240] Increased probiotic use of *L. rhamnosus* GG has not led to an increase in *Lactobacillus* bacteremia.[248] However, several reports can be found in which infections are directly linked to consumption of probiotic products mostly using *L. rhamnosus* GG.[240–245] Two pediatric patients, one 6-week-old term patient and one 6-year-old patient receiving probiotic lactobacilli, subsequently developed bacteremia and sepsis attributable to *Lactobacillus* species. The isolates were indistinguishable from LGG as determined by rep-PCR.[246] Two other cases of *Lactobacillus* septicemia were reported in two infants of 34- and 36-week gestation with short bowel syndrome. Both infants were treated with Culturelle®, containing *L. rhamnosus* GG, for its antidiarrheal effects. In one infant it was confirmed by DNA fingerprinting (PFGE) that the supplemented strain was indistinguishable from the blood culture isolate.[243,245] These cases show that although the beneficial effects of probiotic agents for infants are well documented, probiotic therapy may be associated occasionally with adverse effects, such as bacteremia,

sepsis, or endocarditis, for a select subset of patients, such as immunocompromised or severely debilitated hosts.

The safety of *S. boulardii* is still under discussion since *Saccharomyces* has been described as an emerging fungal pathogen, with *S. boulardii* accounting for 40 percent of the *Saccharomyces* infections reported in literature.[247] Also in newborns and infants cases of fungemia with *S. boulardii* have been described.[248,249]

15.4.4.2 Antibiotic Resistance and Gene Transfer

It is generally recommended that bacteria that contain transmissible drug resistance genes should not be used in food.[155–157,221–225,250] Therefore, probiotic strains should be assessed for their phenotypical antibiotic resistance and potential to transfer resistance genes. A draft text proposal for future IDF/ISO international standard on antibiotic susceptibility testing of nonenterococcal lactic acid bacteria is currently under review.[250] Further research would be needed on characterization of acquired resistance mechanisms and transferability of resistance genes, and on methods for determining transferability.

Some of the probiotic strains currently used in infant formula are known to possess acquired antibiotic resistance genes. *Lactobacillus reuteri* ATCC 55730 contains two antibiotic resistance genes: the tetracycline resistance gene *tet(W),* residing on a plasmid, and the lincosamide resistance gene *lnu(A)* (formerly *LinA*).[251] *Bifidobacterium animalis* subsp. *lactis* BB-12 carries an antibiotic resistance (*tetW* gene) against tetracycline which is chromosomally located.[251–254] So far, Kastner et al.[251] failed to show transferability of the *tet*(W) gene from *L. reuteri* ATCC 55730 to *E. faecalis* and *Lactococcus lactis*. However, transferability of *tet* and other antibiotic resistance genes is possible as was shown *in vitro*[255] and *in vivo* animal models.[256,257] *In vivo* transfer of wild-type antibiotic resistance plasmids from *Lactobacillus plantarum* to *E. faecalis* was shown in gnotobiotic rats.[256] An interesting approach for currently used probiotic bacteria with acquired transferable genes would be to eliminate antibiotic resistance by selective removal or curing of plasmids coding for antibiotic resistance.[251]

15.4.4.3 Deleterious Metabolic Activities: D-Lactic Acid

D-Lactic can be produced by certain species of lactobacilli that are currently applied in infant formulae. L(+)-Lactic acid is naturally present in the human body and is easily degraded. D(–)-Lactic acid, however, is present in the human body by bacterial production and/or via ingested food. D-Lactic acid in adults is degraded at a 30 percent lower rate compared to L-lactic acid via the enzyme D-2-hydroxy acid dehydrogenase.[258,259] It has been suggested in the literature that newborn infants may fail to completely metabolize D-lactate because of liver immaturity;[260] however, currently very little information is available on D-lactic acid metabolism in infants. D-Lactic acid is taken up from the colon and secreted in the urine. This is in general no problem except for in infants with specific diseases, like short bowel syndrome, and the kidneys can easily be overloaded. Too much D-lactic acid results

in a decrease of the blood pH and acidosis, which has been reported by several investigators for infants and children.[261–263] In the Codex Alimentarius (FAO/WHO food standards) and the EU directive on Additives 95/2/EC, it is stated that only L-lactic acid (E270) or bacteria that produce only L-lactic acid are allowed in infant products.[264,265] In a toxicological evaluation of the FAO/WHO on D- and L-lactic acid in adults and infants, it was concluded that neither D-lactic acid nor DL-lactic acid should be used in infant foods.[266] Only one study was found with the primary end point being the evaluation of D-lactic acid levels in infants administrated with D-lactic acid producing microorganisms. No adverse effects were found from the administration of *L. reuteri* ATCC 55730 regarding its D-lactic acid production in a small subgroup of 24 infants.[267] It was shown that there was no elevation of D(–)-lactic acid in the blood of infants given *L. reuteri* ATCC 55730 at a dose of 10^8 cfu/day from birth daily for 12 months. Larger studies would be needed to confirm the safety of using D-lactic acid producing bacteria in infant formula.

15.4.5 Current Recommendations for Starter and Follow-On Formulae

Addition of probiotic bacteria to infant formula has shown promising benefits in treatment and prevention of allergy, prevention of NEC, and treatment and prevention of acute infectious diarrhea. Effects are clearly strain specific, depending on the dosage given, but also specific for a target group with a given clinical condition. Therefore, probiotic bacteria should always be tested for safety and efficacy in the target population of end use in its final product composition. Pediatricians should choose bacterial preparations based on these effectiveness data. Illustrative is the study of Canani in which five commercially available probiotic preparations were tested to treat acute infectious diarrhea in infants of which only two preparations were shown effective.[190] Adequate doses need to be defined for each strain and each product independently. The importance of dose was emphasized by the FAO/WHO committee, which recommended definition of probiotic bacteria as "live microorganisms which when administrated in adequate amounts confer a health benefit on the host."[155–157] However, dose–response studies are lacking in infants. Furthermore, safety of probiotic bacteria in pediatric critical care needs more attention. Finally, good identification of the strains used in commercial products is highly desirable since many cases of misidentification in commercial probiotic products have been reported.[159]

15.5 SUMMARY AND FUTURE DEVELOPMENTS

The fact that diseases later in life can be influenced by nutrition during infancy has raised a completely new perspective regarding the judgment of infant formulae. The consequences of prebiotics or probiotics on the development of the immune system are current examples, which have been extensively reviewed in this chapter. However, the starting point of the development was the current requirements of the infant rather than the prevention of later diseases. Therefore, the composition of

human milk served as an example. However, the question still remains why there is such a huge structural variety of the oligosaccharide fraction of human milk. It may be assumed that a great variety of structures are associated with a large number of functions. Better understanding of the relation between oligosaccharide structure and function will be a field of intensive research in the future.

There is also evidence that probiotics offer beneficial effects on the host. The great variability of the intestinal microbiota might indicate that different bacteria play different roles in the symbiosis between the microbiota and the host. Modern techniques for identification and quantification of intestinal bacteria will provide further insights in this complex ecosystem.

In summary, the possibility of modulating the immune system and intestinal resistance offers the opportunity for prevention of infectious and immune-related diseases. If finally confirmed, the modulation of the developing immune system will be a completely new approach to prevent allergic diseases during an entire life time.

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CHAPTER 16

Probiotics and Prebiotics in Elderly Individuals

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CONTENTS

16.1 GUT FUNCTION AND MICROBIOTA OF ELDERLY INDIVIDUALS

16.1.1 Aging and Gastrointestinal Tract Function

Aging affects the gastrointestinal (GI) tract in many ways. The aging-associated physiological changes influence the GI tract microbiota both directly and indirectly. First, physiological changes affecting a person's eating behavior include increased taste and smell thresholds, decreased muscle strength for chewing, and loss of teeth, which all can lead to a very selective consumption of foods and consequently to an altered nutritional status or even to malnutrition. Further, difficulties in swallowing may narrow the range of consumed products. In addition, hypochlorhydria due to atrophic gastritis or due to use of proton pump inhibitors or H_2-antagonists

is common among elderly people and may lead to small intestinal bacterial overgrowth, chronic diarrhea, and malabsortion.[1,2] Atrophic gastritis may also affect the bioavailability of calcium, ferric iron, and vitamin B_{12} and contribute to the deficiency of these minerals and vitamins. Also colonic transit may slow with aging, but the individual variation is high. Constipation, which is a common symptom in elderly people, may be partly explained by the decreased intestinal motility. Another factor linked with constipation is low fecal weight, which has been reported among the elderly people. The slow intestinal transit has been associated also with increased bacterial putrefaction and, consequently, increased levels of ammonia and phenols in the gut. The immune system is often adversely affected by the aging process and the resistance to diseases may be decreased. More detailed description about the aging-related physiological and functional changes in the GI tract can be found elsewhere.[3,4] The microbiological changes in the GI tract due to aging have been characterized and are discussed in detail below after the short introduction on modern microbiota assessment techniques. Further in this chapter, we discuss the possibilities of counteracting the aging-associated changes in the GI tract with probiotics and prebiotics. There is experimental and clinical evidence that they may support antibacterial and barrier-enhancing actions, have antiinflammatory effects, as well as enhance immunity.[5,6]

16.1.2 Assessment of Microbiota

The knowledge on intestinal microbiota has been gained over the years by using various microbiological techniques. Although early studies relied entirely on cultivation, today molecular biological techniques complement the culturing techniques and also allow us to study the microbiota in a culture-independent way.[7]

Cultivated colonies can be identified by genetic fingerprinting by, for example, ARDRA, RAPD, or PFGE (amplified ribosomal DNA restriction analysis, randomly amplified polymorphic DNA, pulsed field gel electrophoresis, respectively). Whereas the above-mentioned techniques usually require some in-house optimization and standardization, fingerprinting by automated ribotyping and (partial) 16S rDNA sequencing are user-friendly choices. The major advantage of PFGE is that it has the highest discriminatory power, and the advantages of RAPD and ARDRA include easy performance and relatively low cost. For the phenotypic molecular typing of cultivated bacteria cell membrane fatty acid profiling, the so-called FAME (fatty acid methyl ester identification) analysis is a popular and well-standardized technique.

Entire bacterial communities can be profiled directly from samples in a culture-independent manner by using techniques, such as PCR-TGGE or PCR-DGGE and T-RFLP (polymerase chain reaction coupled with temperature or denaturing gradient gel electrophoresis and terminal restriction fragment length polymorphism, respectively). Construction of 16S rDNA libraries by PCR and subsequent cloning and the sequencing of clones have also been extensively used. Specific microbial groups can be traced by using specific primers and probes in PCR and fluorescent *in situ* hybridization (FISH), respectively. Recently, major methodological improvements for the microbiota analysis have been achieved by the development of bacterial high-density

microarrays.[8,9] The arrays consist of thousands of 16S rRNA gene-targeted oligonucleotide probes selectively recognizing different taxonomic groups or species of bacteria. Today, methods of array design and analysis are still imperfect and evolving, but it is already evident that they provide us with a powerful high-throughput tool.

16.1.3 Aging and Gut Microbiota

The microbial colonization of the gut is thought to start at birth when the newborn comes into contact with the mother's microbiota and that of the environment. During the microbiota development toward the complex adult microbiota, several abrupt shifts occur in the population structure, but the shifts are not necessarily linked to any specific age or event.[9] The transition to an adult-like profile happens gradually after the introduction of solid foods and, at the age of 1 to 2 years, microbiota resembles that of adults.[9,10] Some geographic and demographic factors may influence the microbiota composition. In adulthood, the gut microbiota is highly complex, individual-specific, and stable.[8,11,12] The highly complex and stable normal microbiota functions in maintaining host health in providing colonization resistance against invading pathogens, by providing energy in the form of short-chain fatty acids (SCFA), and by producing vitamins K and B_{12}.

When a people age, their individual microbiota also "ages" in an individual manner. Although the interindividual variations of the microbiota composition are great, general overview on the age-related changes can be obtained with large study groups. During aging, no significant change is observed in the total number of anaerobic bacteria, but the total number of facultative anaerobes increases.[13,14] Shifts in dominant species within bacterial groups are common. Table 16.1 compiles changes occurring in the GI tract microbiota during aging at the microbial group level as assessed by comparing the gut microbiota of healthy young adults and elderly individuals.[13] Some general trends are particularly notable. Bacteroides, prevotellas, bifidobacteria, and lactobacilli decline, while fusobacteria and propionibacteria rise. Clostridia increase particularly in antibiotic-treated elderly people. These changes have been observed in culture-based studies and have also been verified by studies based on molecular techniques.[15–18]

The defining factors in microbiota composition and fluctuation in old age have not been identified. The multiple physiological and functional changes related to aging (see above) are likely to contribute. Decreased secretion of mucus (major sources of nutrients for gut microbes) is a possible factor causing changes in the microbiota, which is supported by the recent finding that mucin-degrading bacterium *Akkermansia muciniphila* or *A. muciniphila*-like bacteria decrease significantly in elderly individuals.[19]

Antibiotic use causes changes in the intestinal microbiota in all age groups and in elderly individuals some of the age-related changes are fortified with ongoing antibiotic treatment.[13,15] The antibiotic-treated elderly people have markedly decreased bifidobacteria and increased clostridia including prevalence, numbers, and species diversity as compared to the healthy elderly individuals. Propionibacteria increase and prevotella further decrease both in prevalence and numbers. Enterococci are

Table 16.1 Changes in the Fecal Microbiota in Healthy Elderly Individuals as Assessed by Culturing And Fame-Identification

	Change		
Microbial Group	**Prevalence**	**Numbers**	**Species Diversity**
Bifidobacteria	= or ↓	= or ↓	↓
Bacteroides	↓	↓	↓
Prevotellas	= or ↓	= or ↓	↓
Fusobacteria	↑	↑	↑
Propionibacteria	= or ↑	= or ↑	= or ↑
Clostridia	= or ↓	=	↓
Eubacteria	↑	↑	↓
Lactobacilli	↓	↓	↓
Enterobacteria	= or ↑	↑	↓
Enterococci	↓	↓	↓
Streptococci	↑	↑	↑
Staphylococci	↑	↑	↑
Candida albicans	↑	↑	

Note: = no change, ↑ increased or ↓ decreased as compared to the healthy young adults.
Source: Compiled from Woodmansey et al., 2004.[13]

increased in numbers and diversity as compared to the healthy adults, whereas lactobacilli are increased in diversity but not in numbers. The numbers and overall diversity of staphylococci, enterobacteria, and eubacteria are at the same level in antibiotic-treated elderly and healthy adults, but different species often prevail.

The changes in the microbiota composition bring along functional changes. Changes in the microbial metabolites, such as decreased concentration of SCFA and increased concentrations of branched SCFA and l-lactate in feces, have been reported.[14] Collectively, the balance in the complex cross-feeding network and the metabolic activity of the microbiota can be altered. As a consequence, putrefaction can be increased and amylolytic activity decreased. Decreased concentrations of SCFA indicate decreased energy supply to the mucosa in the form of butyrate[14] and supposedly decreased energy supply to the host in general. The transformation of bile acids is increased, leading to metabolites potentially harmful for the host. Colonization resistance is weakened due to the less stable microbiota. The decline of *Bifidobacterium* numbers and the reduced stability is considered to have a negative impact on gut health. The *Bifidobacterium* decline may be related to reduced adhesion to the intestinal epithelium, which can also result in lowered responsiveness of the gut-associated immune system. Elderly individuals have increased susceptibility to GI tract infections,[20–22] which does not seem surprising, taking together the less stable microbiota, the reduced *Bifidobacterium* numbers, and usage of antibiotics.

It is suspected that intestinal microbiota changes may produce a more proinflammatory signal to the mucosal immune cells and that such inflammatory activation could contribute to systemic inflammation**.** Mucosa-associated bacteria are suspected to have, because of their location, more influence on the immunological and inflammatory parameters of the host than bacteria in the intestinal lumen (fecal bacteria). However, knowledge on the mucosa-associated bacteria is scarce in comparison to that of fecal bacteria and in particular the changes mucosa-associated microbiota related to aging have not been characterized adequately. Several findings indicate that aging may bring significant compositional changes to the mucosa-associated microbiota. First, bifidobacterial strains isolated from the feces of elderly people are bound worse to the intestinal mucus than those isolated from healthy adults, indicating a shift to a *Bifidobacterium* population with reduced adhesive abilities.[23] Second, the numbers of the mucin-degrading *A. muciniphila*-like bacteria have been found to decline in elderly individuals.[19]

16.2 PROBIOTICS AND PREBIOTICS FOR ELDERLY INDIVIDUALS

16.2.1 Probiotics and Prebiotics

Probiotics are defined as viable microbes, which through oral administration produce health benefits to the host. Probiotics act by functioning as members of the healthy gut microbiota and by balancing the microbiota. Most studied probiotic strains belong to the genus *Lactobacillus* or *Bifidobacterium*. The health benefits of probiotics are reviewed in Chapters 12 through 16 of this book.

Prebiotics act through promotion of specific groups of bacteria, which are considered to be essential in maintaining and enhancing gut health. Naturally, the prerequisite for the prebiotic activity is that bacteria to be stimulated are already present in the gut. Most prebiotic components have been shown to enhance the *Bifidobacterium* microbiota, but different prebiotic oligosaccharides have different microbiota-modifying properties. When assessing the efficacy of a prebiotic substrate it should be considered that it might also enhance the levels of unknown microbes in human gut and thus potentially facilitate undesirable effects. This emphasizes the importance of proper microbiota analysis at an adequate level of accuracy during clinical interventions.

The objective of developing probiotic and prebiotic products for elderly people is essentially the same as for other age groups with special emphasis to counteract the microbiota changes related to aging, to improve bowel function (alleviate constipation and diarrhea), and to enhance immunity and thereby to improve general well-being and health. Selected recent clinical trials on probiotics and prebiotics for elderly people are compiled in Table 16.2 and Table 16.3.[6,24–30]

16.2.2 Efficacy of Probiotics

The particular challenges related to the probiotic research include the right selection criteria for probiotic strains for elderly individuals. Each probiotic strain has its

Table 16.2 Selected Recent Probiotic Clinical Trials with Elderly Individuals

Probiotic	Subjects, Study Groups, and Doses[a]	Study Design[b]	Effects	Ref.
Bifidobacterium lactis HN019 in skim milk	Age 60–87 (mean 69.5 years) Placebo: $n = 20$ Low dose: 6.5×10^7/day, $n = 20$ Medium dose: 1×10^9/day, $n = 20$ High dose: 5×10^9/day, $n = 20$	2 week run-in, 4-week feeding with three different doses, 2-week wash-out	Increase of fecal bifidobacteria, lactobacilli, and enterococci and decrease of enterobacteria with all three probiotic doses	Ahmed et al.[24]
B. longum 46 and 2C or *B. lactis* Bb12 in fermented oat drink	Age 61–102 years (mean 84), nursing home residents Placebo: $n = 51$ Group 1: *B. longum* 10^9/day, $n = 46$ Group 2: *B. lactis* 10^9/day, $n = 82$	3 months run-in, 6-month feeding period	Normalizing effect on bowel movements (relief of constipation, diarrhea, and other intestinal problems) in both probiotic groups	Pitkälä et al.[25]
Lactobacillus johnsonii La1 in fermented milk	Age 75–96 years (mean 85), hospitalized, enterally fed Placebo: $n = 12$ Probiotic: 10^9/day, $n = 12$	12 weeks run-in, 12-week feeding period	Decrease in the percentage of days with infections, increased blood Hb	Fukushima et al.[26]
L. casei DN-114001, *Streptococcus thermophilus,* and *L. bulgaricus* in yogurt drink	Mean age 74 years, hospitalized, taking antibiotics Placebo: sterile milkshake, $n = 66$ Probiotic: 10^{10}/day of each strain, $n = 69$	Feeding during a course of antibiotics and 1 week after the course finished	Decrease in the incidence of antibiotic and *C. difficile*-associated diarrhea	Hickson et al.[27]
L. rhamnosus GG and Lc705 and *P. freudenreichii* JS in cheese	Age 70–100 years Placebo: n = 140 Probiotic: 5×10^8/day of each strain, n = 136	3 week run-in, 16-week feeding period	Reduced the risk of high yeast counts and hyposalivation	Hatakka et al.[28]

[a] Unless otherwise indicated, the placebo was the same product without the probiotic.
[b] All trials were randomized, double-blind, and placebo-controlled.

Table 16.3 Selected Recent Prebiotic and Synbiotic Clinical Trials with Elderly Subjects

Prebiotic or Synbiotic	Subjects, Study Groups, and Doses	Design	Effects	Ref.
Fructo-oligosaccharide (FOS)	Age 84 ± 7 years, malnourished or at risk of Placebo: liquid nutrition supplement (LNS), *n* = 37 Intervention: LNS with FOS min 1.95 g to max 3.9 g / day, *n* = 37	Randomized, double-blind, placebo-controlled; 12-week feeding	Diminished levels of TNF-α and IL-6 mRNA in blood leukocytes, decreased serum levels of sCD14, no change in fecal bifidobacteria, lactobacilli, bacteroides, or Enterobacteriaceae, slight increase in *C. perfringensis,* no change in nutritional parameters	Schiffrin et al.[6]
Short-chain fructo-oligosaccharides (scFOS)	Age 69 ± 2 years, scFOS 8 g / day, *n* = 12	Single study group; 3-week basal period, 4-week intervention (scFOS), 4-week follow-up period	Increase in fecal bifidobacteria, increase in fecal cholesterol concentration, decrease in fecal pH, increased frequency of flatus and bloating with mild symptoms	Bouhnik et al.[29]
Inulin enriched with oligofructose and *B. bifidum* BB-02 and *B. lactis* BL-01	Placebo: age 63–85 years (mean 71), 12 g malto-oligosaccharides/day, *n* = 9 Intervention: age 68–90 years (mean 73), 12 g inulin + 7×10^{10} of each strain/day, *n* = 9	Randomized, double-blind, placebo-controlled; 4-week feeding period	Increase in fecal bifidobacteria numbers and diversity (including *B. lactis* and *B. bifidum*), increase in lactobacilli	Bartosch et al.[30]

specific properties, which have to be evaluated prior to application. Salminen and Ouwehand with their co-workers isolated and selected probiotics strains *B. longum* 2C and 46, which adhere well to intestinal mucus from elderly individuals.[31,32] In a clinical trial, these strains showed efficacy in normalizing bowel movements of institutionalized elderly people,[25] modulating the fecal *Bifidobacterium* microbiota and inducing potentially beneficial immunological changes.[33] In the study by Ahmed et al.[24] with *B. lactis* HN019, an increase of fecal bifidobacteria, lactobacilli, and enterococci and a decrease of enterobacteria were observed showing the potential of probiotics to counteract the age-related microbiota changes. Recently, prevention of antibiotic- and *Clostridium difficile*-associated diarrhea by consumption of a yogurt drink containing *L. casei* DN-114001, *Streptococcus thermophilus,* and *L. bulgaricus* (undefined strains) was demonstrated in elderly hospitalized patients.[27] Likewise, the intake of *L. acidophilus* and *B. bifidum* (undefined strains) in capsules reduced the incidence of *C. difficile*-associated diarrhea in elderly people receiving antibiotics.[34] Previously, the treatment of relapsing *C. difficile* diarrhea with *L. rhamnosus* GG has been reported.[35] Beneficial microbiota modification in elderly individuals and efficacy in reducing the extent of microbiota disruption due to antibiotic treatment has been obtained with several probiotics.[36–39]

Concerning the immunoenhancing potential of probiotics in elderly people, perhaps the most convincing data of all have been obtained with the strain *B. lactis* HN019.[40–42] The clinical trials reported that consumption of *B. lactis* HN019 resulted in stimulation of phagocytic activity of mononuclear cells and natural killer (NK) cell activity, increased size of T- and NK-cell populations, and enhanced production of interferon-alpha (IFN-α) from stimulated PBMC in culture. The probiotic strain *L. rhamnosus* HN001 increased polymorphonuclear cell phagocytic activity and NK-cell activity in elderly subjects.[42,43] Indication of the improvement of the immunological status by probiotics in elderly people in the form of decreased incidence of infections was recently reported.[26] In a Japanese study, decreased incidence of infections in response to feeding with *L. johnsonii* La1 was observed in hospitalized, enterally fed subjects.

An interesting new application of probiotics is the prevention of oral candidosis.[28] Hatakka et al.[28] demonstrated that a probiotic cheese containing a mixture of *L. rhamnosus* GG and Lc705 and *Propionibacterium freudenreichii* JS decreased the prevalence of high salivary yeast counts in elderly subjects.[28]

Taken together, it is apparent that specific probiotics can provide measurable and clinically relevant benefits to elderly people in counteracting the age-related changes in gut microbiota, enhancing immunity, and promoting intestinal health. However, one should bear in mind that probiotic properties are strain specific and results cannot be extrapolated to apply other strains even if they are of the same or closely related species.

16.2.3 Efficacy of Prebiotics

Traditionally, prebiotic components have been aimed at fortifying the indigenous *Bifidobacterium* microbiota in the intestine. The bifidogenic effect of inulin

and fructo-oligosaccharides (FOS) in elderly subjects has been demonstrated in clinical trials.[29,44,45] Prebiotics may have adverse side effects, such as abdominal discomfort, bloating, and increased frequency of flatulence, when consumed in high doses, and therefore it is important to determine the appropriate daily doses in order to avoid the undesired side effects. For FOS the bifidogenic effectiveness could not be demonstrated with a daily dose of approximately 4 g or less,[6] but an 8 g-dose per day yielded increased fecal counts of bifidobacteria and was well tolerated although increased frequency of flatus and bloating with mild symptoms occurred.[29] Feeding of 8 or 15 g per day of galacto-oligosaccharide (GOS) did not show any bifidogenic effect in adults,[46–48] but a constipation-relieving effect was observed with 9 g daily intake of GOS in the elderly individuals.[49] The constipation-relieving effect in elderly subjects was also demonstrated for inulin.[45]

Potentially adverse effects of prebiotics have been revealed in experimentation with animals. Ten Bruggencate et al.[50,51] revealed that inulin and FOS disturbed the intestinal barrier in rats and increased the translocation of *Salmonella*. In humans, however, the daily consumption of 20 g FOS did not affect the intestinal permeability, although increased flatulence and intestinal bloating were observed indicating for excessive dosage.[52]

Changes in the microbial metabolism including decreased concentrations of fecal SCFA have been reported for elderly people.[14] It has been considered that prebiotics could redirect the microbial metabolism to a favorable course. However, clinical studies with adults did not detect any effect of FOS or GOS on the fecal concentration of SCFA.[48,53] Likewise, Kleessen et al.[45] did not detect any change in the concentration fecal SCFA in response to inulin or lactose in elderly subjects, but a slight trend toward higher molar ratios of acetate to butyrate was observed. Other interesting findings from prebiotic trials include the possible change in cholesterol metabolism, which could possibly be related to decreased cholesterol bacterial transformation.[29] The potentially beneficial change in cholesterol metabolism requires further studies.

Few studies with prebiotics have focused on the possibility of improving the low noise inflammatory process frequently observed in elderly subjects.[6] The rationale behind the assumption is that intestinal microbes may contribute to the inflammatory status in elderly people and that prebiotics could affect at the level of the composition of the gut microbiota including the mucosa-associated microbiota. Previously it was shown that prebiotic carbohydrates can change the composition of the mucosa-associated microbiota by increasing the bifidobacteria, lactobacilli, and eubacteria populations.[54] In a prebiotic trial with FOS, a decreased level of proinflammatory gene activation—tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) mRNA—and decreased serum levels of sCD14, a product shed by activated macrophages, were measured.[6] Guigoz et al.[44] reported similar decrease in IL-6 mRNA in blood leucocytes and decreased phagocytic activity of granulocytes and monocytes in response to FOS. Thus, specific prebiotics may influence the inflammatory condition of elderly individuals. It should be emphasized that the obtained results are preliminary, but this interesting area of research certainly warrants further research.

It can be concluded that, analogous to the strain specificity of the probiotic properties, the prebiotic effects are specific to the components used.

16.2.4 Synbiotics

Products containing both probiotics and prebiotics have been termed synbiotics. A synbiotic is a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the GI tract, by selectively stimulating the growth and/or activating the metabolism of one or limited number of health-promoting bacteria, and thus improving host welfare.[55] Trials demonstrating the application of synbiotics in elderly people are limited. Bartosch et al.[30] performed a study with a synbiotic product containing two *Bifidobacterium* strains (*B. lactis* BL-01 and *B. bifidum* BB-02) and inulin-based prebiotic. The consumption of the symbiotic product increased the size and diversity of fecal bifidobacteria and increased lactobacilli numbers. The characterization of bifidobacterial species revealed that the rise of total bifidobacterial numbers was most likely due to the consorted effect of both ingested probiotic strains and the stimulation of indigenous bifidobacteria by prebiotic compounds.

16.3 SUMMARY AND CONCLUDING REMARKS

Our understanding of microbiota has improved stage by stage along with the methodological improvements. In the future, large-scale studies with detailed microbiota descriptions will become possible and this will tremendously increase our knowledge on the human intestinal microbiota including the microbiota in elderly individuals and the possibilities to modulate it. Probiotic and prebiotics are aimed at modulating the intestinal microbiota, promoting intestinal health, enhancing immunity and thereby improving general well-being and quality of life. The results obtained so far, particularly with probiotics, are encouraging and further clinical trials seem justified to establish the place of probiotic and prebiotic supplements in elderly subjects. Research on the elucidation of mechanisms of probiotic and prebiotic actions proceeds rapidly. In the future, a better understanding of the mechanisms of host–microbiota cross-talk and of the role of the human and probiotic genomes as well as the whole microbiota genome (microbiome) will help to select optimal product components for elderly people.[56] The future probiotic, prebiotic, and synbiotic products thus will be more tailored to meet the requirements of this specific target group. Carefully selected combinations of probiotics and prebiotics—synbiotic products—may offer optimal means for creating and maintaining a healthy microbiota, functioning intestinal tract, and good nutrition in all age groups.

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CHAPTER 17

Prebiotics and Probiotics in Companion Animal Nutrition

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CONTENTS

17.1 INTRODUCTION

The companion animal industry continues its robust growth with a global market value of approximately US$60 billion for pet food and pet care products. The United States is the single largest market with approximately 36 percent of world sales (estimated to be US$26.1 billion by 2011). Dog and cat food make up over 70 percent of the market. Market drivers include an increase in pet ownership, humanization of pets by owners, an increased popularity of dry foods, and an increased desire by owners for very high-quality pet diets containing functional ingredients.[1]

Prebiotics and probiotics could play a major role in the development of new pet foods now and in the future. As regards the future market outlook, two factors in particular relate to the potential increased usage of prebiotics and probiotics. First, owners are particularly concerned about the health of their pets, and this will drive demand for high-quality foods. Both prebiotics and probiotics and their combination (synbiotics) relate well to health concerns as they have been shown to affect a

number of biomarkers of health status in humans and animal models. Second, and partly as a result of the health message expressed above, niche diets will be formulated to appeal to consumers with demands for high quality. New types of foods and treats will emerge for dogs and cats, and they will be of near human grade quality. Again, the pre-, pro-, and synbiotics will be viewed as important components of these health-enhancing diets.

It is the intent of this chapter to provide a comprehensive review of the research that has been conducted in the dog and cat related to use of pre-, pro-, and synbiotics. Several outcome variables have been measured to test efficacy of these compounds in pet animals, but relative to the research reported on rodents, humans, livestock, and poultry, it becomes clear that much less research is available on this topic for pets than for any of the other animal species just mentioned.

17.2 PREVIOUS REVIEW OF PREBIOTIC OLIGOSACCHARIDE USAGE IN COMPANION ANIMAL NUTRITION

The use of prebiotics in companion animal nutrition was reviewed comprehensively by Swanson and Fahey.[2] As regards research conducted from 1992 through 2004, 23 canine and 4 feline prebiotic publications were reported in the literature. Of those, most reported the effectiveness of fructans of varying degrees of polymerization (dp), including chicory (a natural source of long-chain fructans), inulin (up to 60 dp), oligofructose (OF; 8 to 9 dp), and scFOS (3 to 5 dp). Other oligosaccharides evaluated in canine diets included yeast cell wall (YCW), a source of mannanoligosaccharides, α-galacto-oligosaccharides (GOS), isomalto-oligosaccharides (IMO), lactosucrose, lactulose, maltodextrin-like oligosaccharides (MD), transgalacto-oligosaccharides (TGOS), and xylo-oligosaccharides (XOS). Inulin, lactosucrose, OF, and scFOS have been tested in the limited number of published reports involving felines.

Studies evaluating prebiotics have utilized several outcome variables to assess efficacy in canine and feline diets, including (1) food intake, (2) fecal output, (3) stool consistency, (4) macronutrient digestibility (ileal and total tract apparent digestibility), (5) fermentative end-products, (6) immune indices, and (7) intestinal microbial populations. Stool consistency and quantity of fecal output are important in companion animal nutrition and are, therefore, important criteria to measure in prebiotic studies. Furthermore, for a nondigestible carbohydrate to be considered a "prebiotic," it must modulate the activity of one or a select number of microorganisms, another important experimental outcome to measure when conducting prebiotic studies. Swanson and Fahey[2] discussed each of these outcome variables in their review. A brief summary of that review follows.

In canine studies, inclusion of prebiotics at 1 to 2 percent of the diet resulted in few effects on food intake. Prebiotic supplementation can lead to greater wet fecal weight and decreased fecal dry matter (DM) percentage. This may prove beneficial in preventing and treating constipation. Total tract macronutrient digestibility—organic matter (OM) and crude protein (CP)—sometimes decreased with prebiotic

supplementation, but was dependent on dose. It also was noted in many studies that there was an increase in fecal nitrogen (N) due to increased bacterial protein synthesis in the large bowel.

Decreases in CP digestibility are indicative of more protein reaching the large bowel to be either excreted or fermented. Bacteria act as N sinks in the colon, thereby utilizing the undigested protein for protein synthesis. Therefore, if the excess N is used for bacterial protein synthesis, N will not be utilized for energy, which is related to putrefactive compound formation.

Fermentative end-products of both carbohydrate and protein fermentation were evaluated in nine and seven canine experiments, respectively. Short-chain fatty acids (SCFA; acetate, propionate, and buyrate) and lactate were those most commonly measured. In 50 percent of experiments conducted, increases in fecal acetate, propionate, total SCFA, and lactate concentrations were reported, while data on butyrate showed no clear trend. It also was noted that prebiotic supplementation increased intestinal length, weight, and surface area, colonic blood flow, and small intestinal carrier-mediated glucose uptake; however, the data set was limited (two studies). These increases may be due to the increased production of SCFA, which can lead to intestinal hypertrophy.

Amino acid fermentation, often considered detrimental, is responsible for fecal odor as well as being potentially harmful to intestinal epithelia. Fecal ammonia and branched-chain fatty acids (BCFA) were not affected by prebiotic supplementation. Phenol and indole concentrations, however, decreased in four of the seven experiments.

Mixed results were noted in the three studies evaluating immune indices in the canine. Although all studies reported significant effects of prebiotic supplementation on immune cell populations, results were conflicting and no clear trends were found. Given the influence of bacterial protein on gut-associated lymphoid tissue (GALT) and disease states in dogs and cats, it was suggested that more thorough experimentation on the effects of prebiotics on immune function were warranted.

Modulation of intestinal bacteria is necessary for a compound to be termed a prebiotic. This outcome was evaluated in 14 canine experiments, including evaluation of changes in *Lactobacillus* and *Bifidobacterium* spp., which are considered beneficial, as well as changes in potentially pathogenic bacteria, such as *Escherichia coli* and *Clostridium* spp. Approximately 50 percent of the canine studies reported increased bifidobacteria and lactobacilli and decreased *Clostridium* spp. with prebiotic supplementation.

Due to the limited data set (four studies) on prebiotic supplementation of felines, few trends were noted. Similar to dogs, wet fecal weight, decreased fecal DM percentage, and softer feces were noted in cats. Decreased CP digestibility and increased fecal N concentrations also were noted. Furthermore, greater fecal SCFA concentrations and decreased fecal protein catabolite concentrations were noted, and beneficial modulation of the microbiota (increased bifidobacteria and lactobacilli and decreased clostridia), as was noted in dogs, have been reported in feline studies.

17.3 UPDATED REVIEW OF PREBIOTIC OLIGOSACCHARIDE USAGE IN COMPANION ANIMAL NUTRITION

The literature published since the Swanson and Fahey[2] review contains 11 studies on prebiotic supplementation, with only 1 study using the cat. These experiments are outlined in detail in Table 17.1 and are summarized briefly in the following paragraphs.

The recent study evaluating prebiotic supplementation of cats tested the effect of OF on urea metabolism (using ^{15}N-labeled urea) and fecal odor components.[3] After a 3-week adaptation period to canned test diets (control vs. OF), samples were collected for 5 consecutive days from four adult cats in a cross-over design. The treatment diet was supplemented with 3.11 percent OF. Trends similar to those reported by Swanson and Fahey[2] were noted in this study. Fecal output and moisture tended to increase with FOS supplementation. Fecal N excretion also tended to increase. Fecal bacterial N, expressed as a percent of N intake, increased during OF supplementation. There also was a trend for urinary ^{15}N excretion to decrease and fecal ^{15}N excretion to increase when cats were supplemented with OF. No differences were noted in fecal odor components.

More feline research clearly is warranted in the prebiotic area. There are several anatomical and nutritional differences between cats and dogs, and although studies have noted similar trends between the species when prebiotics were supplemented, little is known of the full extent to which prebiotics may be utilized by the cat. Given that the cat is an obligate carnivore, dosage and type of prebiotic that are most efficacious remain unknown.

Studies recently published using dogs have reported utilization of molecular techniques to better assess microbial populations, have evaluated new outcome variables, such as blood metabolite concentrations and insulin sensitivity, and have evaluated novel prebiotics. Furthermore, dogs in a diseased, or immunocompromised, state also have been evaluated.[4,5] Of the 10 published studies in dogs, fructans were still the major prebiotics evaluated (7 studies); 3 studies evaluated inulin, 5, scFOS, and 1, OF (some studies evaluated more than one prebiotic source). Three additional YCW studies were published. Novel prebiotics that had not previously been evaluated in dogs included high-molecular-weight pullulan (1 study) and γ-cyclodextrin (1 study).

Prebiotic usage in hypoallergenic, hydrolyzed protein diets was evaluated.[6] Hydrolyzed protein diets are fed to dogs with food allergies. The proteins were enzymatically degraded prior to inclusion in the diet and, therefore, were less likely to result in an immunological reaction upon consumption by the animal. Fecal DM decreased with the addition of inulin. Apparent total tract CP digestibility decreased while bacterial protein percentage increased in dogs fed the intact protein + inulin diet. Immunoglobulins in the blood and feces were not affected by treatment. While this study evaluated a diet meant for dogs with food allergies, the dogs tested were healthy. Evaluation of dogs suffering from actual food allergies might yield differing results with addition of inulin to a hypoallergenic diet.

Table 17.1 *In Vivo* Experiments (In Chronological Order Beginning in 2005), Reporting Effects Of Prebiotics in Cats and Dogs

Ref.	Outcome Variables Quantified	Animals/ Treatment (Age, initial BW)	Dietary Information; Time on Treatment	Daily Prebiotic Dose; Source	Major Findings
Hesta et al., 2005[3]	Urea metabolism Fecal odor components	4 female cats (>7 yr of age; initial BW between 2.2 and 4 kg)	Basal diet fed at ME requirement of ideal BW Chemical composition: 29% CP 37% Crude fat 1% CF Time on treatment: 3 wk	0% supplementation 3.11% OF (Raftilose, Orafti, Belgium), DMB	OF: ↑ Fecal moisture* (6%) ↑ DM fecal output* (27%) ↑ Fecal N excretion* (36%) ↓ Urinary N excretion* (48%) ↑ Fecal bacterial N* (% of N intake; 125%)
Jeusette et al., 2005[7]	Plasma leptin Plasma insulin Plasma ghrelin Blood glucose	12 obese dogs (between 1 and 9 yr of age; 21.9 ± 0.8 kg BW)	Basal diet (Obesity Veterinary Diet, Royal Canin, France) fed to promote weight loss Chemical composition: 34% CP 10% Fat 19.8% TDF 1% scFOS Time on treatment: Until ideal BCS obtained (5 out of 9)	1% scFOS in control diet Control diet + 2% scFOS (Beghin-Meiji Industrie, France)	No effect of scFOS supplementation on leptin, insulin, ghrelin, or glucose

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Table 17.1 *In Vivo* Experiments (In Chronological Order Beginning in 2005), Reporting Effects Of Prebiotics in Cats and Dogs (continued)

Ref.	Outcome Variables Quantified	Animals/ Treatment (Age, initial BW)	Dietary Information; Time on Treatment	Daily Prebiotic Dose; Source	Major Findings
Spears et al., 2005[13]	Food intake Apparent ileal and total tract nutrient digestibilities Microbial populations Fecal characteristics	5 purpose-bred adult dogs (3.7 yr of age; 28.9 kg BW)	Basal diet: 400 g Hill's Prescription Diet d/d- Rice and Duck Chemical composition: 17% CP 14% Fat 4.0% TDF Time on treatment: 14 d	1. No supplement 2. 2 g high-molecular-weight pullulan 3. 4 g high-molecular-weight pullulan 4. 2 g γ-cyclodextrin 5. 4 g γ-cyclodextrin	Linear ↓ in food intake (352 g control versus 305 g on 4 g/d treatment) with increasing γ-cyclodextrin* Linear ↑ in ileal bifidobacteria (9.46 cfu/g feces DM in control vs. 10.12 cfu/g feces DM) and lactobacilli (9.12 cfu/g feces DM in control vs. 10.02 cfu/g feces DM on 4 g/d treatment) with increasing pullulan* Quadratic effect of ileal bifidobacteria (control: 9.46, 2 g/d: 10.20, 4 g/d: 9.83 cfu/g feces DM) and lactobacilli control: 9.12, 2 g/d: 10.11, 4 g/d: 9.42 cfu/g feces DM) due to increasing γ-cyclodextrin* ↑ γ-cyclodextrin quadratically decreased fecal *Clostridium perfringens* (control: 9.75, 2 g/d: 9.44, 4 g/d: 9.76 cfu/g feces DM)**

Vanhoutte et al., 2005[10]	Fecal microbiota population banding patterns (DGGE)	7 adult dogs (Propst et al., 2003[37])	Basal diet: 250 g/d Chemical composition: 32.7% CP 23.5% fat Time on treatment: 10 d	Baseline 4.5 g/d oligofructose 5.6 g/d inulin	↑ *Streptococcus lutetiensis*
Gouveia et al., 2006[4]	Number of leukocytes, neutrophils, and lymphocytes Fecal enteropathogenic bacteria	8 dogs (2 to 6 mo of age) all suffering from gastroenteritis	Basal diet not provided Time on treatment: 10 d	0 g supplementation 2 g MOS (Bio-Mos, Alltech, Nicholasville, KY)	Elimination of *E. coli* in 85.71% of animals
Verlinden et al., 2006[6]	Nutrient digestibility Fecal characteristics Hematology Serum and fecal IgA, IgG, IgE, IgM	4 adult beagle dogs (2–11 yr of age; 6–15 kg BW)	Basal diets: Hydrolyzed protein diet (Hill's z/d ultra, allergen-free); intact protein source (Hill's d/d with duck and rice) Chemical composition: Hydrolyzed protein 18% CP 3% TDF Intact protein 14% CP 5% TDF Time on treatment: 21 d	0% supplementation 3% Inulin (Raftifeed ®ips, DP 2-60; Orafti, Tienen, Belgium)	↓ Fecal DM (12%)*** ↓ Apparent CP digestibility in intact protein + inulin diet (4.6%)*** ↑ Estimated bacterial protein content in feces (% fecal DM, 16%; and % CP intake, 33%) in intact protein + inulin diet***

—*continued*

Table 17.1 *In Vivo* Experiments (In Chronological Order Beginning in 2005), Reporting Effects Of Prebiotics in Cats and Dogs (continued)

Ref.	Outcome Variables Quantified	Animals/ Treatment (Age, initial BW)	Dietary Information; Time on Treatment	Daily Prebiotic Dose; Source	Major Findings
Adogony et al., 2007[8]	Mammary, nasal, and blood immunoglobulin concentrations Diarrhea incidence in puppies	8 primiparous female beagles (10–12 kg BW)	Control diet: 31% CP 16% Crude fat 7.8% TDF 0.12% scFOS Test diet: 30% CP 15% Crude fat 6.6% TDF 0.91% scFOS Time on treatment: Gestation d 35 through weaning	0% supplementation 1% scFOS (Profeed, Béghin-Meiji, France)	↑ IgM in colostrum and milk (40%)** ↑ blood IgM concentrations (60%)* ↑ *Bordetella bronchiseptica*-specific IgM immune response in puppies from dams fed scFOS*
Apanavicius et al., 2007[5]	Food intake Gastrointestinal tract histopathology Body temperature after infection Ileal and colonic nutrient and ion transport Microbial populations	6 hound-cross puppies (12 wk of age)	Control diet 32% CP 19% fat 3% TDF scFOS diet 32% CP 19% fat 3% TDF Inulin diet 30% CP 19% fat 5% TDF Time on treatment: 14 d	0 % supplementation 1% scFOS 1% inulin	**scFOS and inulin:** ↓ Change in food intake following infection (26%)** ↓ Enterocyte sloughing severity (9%)** Maintenance of ileal Na^+-dependent glucose transport (400% ↓ in control, no change in supplemented puppies)**

					Inulin diet: ↑ change from baseline in fecal acetate (control: –37.7 µmol/g vs. inulin 85.5 µmol/g) and SCFA concentrations (control: –53.5 µmol/g vs. inulin: 145.5 µmol/g)** ↑ Change in fecal lactobacilli concentrations (7%)**
Middelbos et al., 2007[11]	Nutrient digestibility Fecal microbial populations Fecal fermentative end-products Immunological indices	6 purpose-bred adult female dogs (4.5 yr of age; 23 kg BW)	350 g/d 1. Control- no supplemental fermentable carbohydrate 2. Control + 2.5% cellulose 3. Control + 2.5% beet pulp 4. Control + 1.0% cellulose + 1.5% scFOS 5. Control + 1.0% cellulose + 1.2% scFOS +0.3% YCW 6. Control + 1.0% cellulose + 0.9% scFOS + 0.6% YCW Time on treatment: 14 d	scFOS (Nutraflora P-95, GTC Nutrition, Golden, CO) YCW (Safmannan, LeSaffre Yeast Corp., Milwaukee, WI)	Supplemented diets ↓ CP digestibility (15%)** ↑ Fecal bifidobacteria concentrations (14%)** ↑ Fecal lactobacilli concentrations (8%)* ↑ Fecal butyrate concentrations (67%)**

—*continued*

Table 17.1 *In Vivo* Experiments (In Chronological Order Beginning in 2005), Reporting Effects Of Prebiotics in Cats and Dogs (continued)

Ref.	Outcome Variables Quantified	Animals/ Treatment (Age, initial BW)	Dietary Information; Time on Treatment	Daily Prebiotic Dose; Source	Major Findings
Middelbos et al., 2007[12]	Apparent ileal and total tract nutrient digestibility Serum IgA, IgM, and IgG Fecal microbial populations	5 purpose-bred adult female dogs (4 yr of age; 23 kg BW)	280 g basal diet Chemical composition: 30% CP 21% fat 4% TDF Time on treatment: 14 d	0 g supplementation 0.07 g YCW/d 0.35 g YCW/d 0.63 g YCW/d 0.91 g YCW/d (Safmannan, Lesaffre Yeast Corporation, Milwaukee, WI)	↑ ileal nutrient digestibility (10% DM, 11% CP)* Linear ↓ in monocyte counts (control: 1.0 thousands/μL vs. 0.65% supplementation: 0.7 thousands/μL)** Linear ↓ in fecal *E. coli* populations (control: 9.1 cfu/g fecal DM versus 0.65% supplementation: 8.2 cfu/g fecal DM**)
Respondek et al., 2008[9]	Euglycemic hyperinsulinemic clamp Adipose tissue gene expression	8 beagle dogs (6.5 years; 12.8 ± 1.3 kg) Obese dogs	Basal diet chemical composition: 29.4% CP 18.6% fat Days on treatment: 6 wk	1% w/w of DM intake (Profeed, Béghin-Meiji, Marckolsheim, France)	↑ rate of glucose infusion (7.8 vs. 4.7 mg/kg/min)** ↑ Adipose tissue UCP 2 gene expression (~39%)** ↑ Adipose tissue CPT1 gene expression (~32%)*

Note: BCS, body condition score; BW, body weight; CPT1, carnitine palmitoyl transferase; CF, crude fiber; cfu, colony-forming units; CP, crude protein; DGGE, denaturing gradient gel electrophoresis; DM, dry matter; FOS, fructo-oligosaccharide; IgA, immunoglobulin A; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; MOS, mannanoligosaccharides; N, nitrogen; SCFA, short-chain fatty acids, scFOS, short-chain fructo-oligosaccharides; TDF, total dietary fiber; UCP2, uncoupling protein 2; YCW, yeast cell wall.

* $P < 0.10$.

** $P < 0.05$.

*** $P < 0.001$.

Two studies evaluated the use of prebiotics in diseased or immunocompromised animals.[4,5] Gouveia et al.[4] evaluated 16 dogs experiencing gastroenteritis and supplemented with MOS (Bio-Mos, Alltech, Nicholasville, KY) for 10 days. The dogs were divided into two groups: T1, dogs receiving treatment + MOS; and T2, dogs receiving treatment for the disease only. By day 10 of the study, *E. coli* was eliminated from 85.7 percent of the dogs on T1 and only 25.0 percent on T2. The authors suggested that the presence of *E. coli* would lead to an intensification of the symptoms of gastroenteritis.

A second study evaluated weanling puppies, which are considered to be in an immunocompromised state due to the stress of separation from the mother and to a change of diet, some of which were challenged with *Salmonella typhimurium*.[5] Puppies were fed a control diet, control + 1 percent scFOS, or control + 1 percent inulin. All dogs decreased their food intake at day 15 following oral gavage of either *S. typhimurium* or saline. Dogs fed the diets containing a prebiotic had less of a decrease in food intake. Enterocyte sloughing was higher in control puppies that were infected; however, there were no differences in sloughing when puppies were fed either prebiotic. Furthermore, puppies fed the prebiotics were able to maintain ileal glucose transport, while puppies fed the control diet and that were infected with *Salmonella* experienced low glucose transport.

These two studies indicate a protective effect of prebiotic supplementation for dogs that are immunocompromised. This has often been speculated, yet these are the first studies to report such findings. Further work in this area would be beneficial for several other disease states (e.g., inflammatory bowel disease, small intestinal bacterial overgrowth).

Jeusette et al.[7] evaluated obese dogs during weight loss fed a control diet (1 percent scFOS) or supplemented diet (control + 2 percent additional scFOS). Food-restricted blood samples were analyzed for total ghrelin, insulin, leptin, and glucose at the beginning and end of this period of weight loss. Ghrelin is a peptide that influences satiety. It is considered an orexigenic hormone, leading to increased food intake. Leptin is produced by the adipose tissue and increases in the circulation as body adiposity increases. It is an anorexigenic hormone leading to decreased food intake and increased energy expenditure. The authors noted no differences in any blood metabolites due to prebiotic supplementation. While these authors noted no changes, these criteria will be of interest in future studies. Dogs utilized in this study were obese and fed to lose weight. The changes due to weight loss may have overshadowed any changes due to diet. Further investigation of the effects of prebiotics on these blood metabolites is warranted.

Blood immunoglobulin concentrations often are used as an indicator of beneficial effects of prebiotic supplementation. Adogony et al.[8] measured immunoglobulins in colostrum and milk of bitches fed either a control diet or one supplemented with 1 percent scFOS. Higher concentrations of immunoglobulin-A (IgA), IgG, and IgM in the colostrum would be considered a beneficial response, as these would be transferred to the offspring. Colostrum and milk IgM were higher in dogs supplemented with scFOS. This increase in IgM was noted to have a beneficial effect on puppies as well, as they tended to have a higher *Bordetella bronchiseptica*-specific

IgM immune response. No effects were noted with IgG and IgA, similar to previous findings that these immunoglobulins were not affected by prebiotic supplementation of dogs.

Recently, Respondek et al.[9] evaluated the effects of scFOS on insulin sensitivity and adipose gene expression in obese and lean adult beagles. Dogs were fed a control diet or the control + 1 percent scFOS (DM basis). In obese dogs, the rate of glucose infusion was increased in dogs supplemented with scFOS during the euglycemic hyperinsulinemic clamp, suggesting a greater insulin sensitivity compared to the obese dogs fed the control diet. Supplementation with scFOS also led to increases in adipose tissue gene expression, including uncoupling protein 2 and carnitine palmitoyl transferase 1. Both genes play an active role in fatty acid metabolism, and the authors suggested that these increases may have contributed to the increased insulin sensitivity noted. Outcome variables measured in this study are unique in prebiotic supplementation research and demonstrate the need for further testing. Obesity is a growing problem in both dog and cat populations throughout the developed world. Finding dietary mechanisms that may ameliorate diseases associated with obesity would be beneficial to those populations.

Modern technology now allows for a more thorough analysis of microbial changes in the gut due to prebiotic supplementation. One of the first of these studies conducted was by Vanhoutte et al.[10] and evaluated fecal samples from healthy, adult dogs fed a control diet, control + 4.5 g/day OF, or control + 5.6 g/day inulin. Utilizing denaturing gradient gel electrophoresis (DGGE), researchers evaluated population diversity of microbial species. The DGGE analysis revealed a band that appeared or became more prominent after fructan supplementation. This band then was excised and sequenced. The sequencing determined the band was *Streptococcus lutetiensis*. To date, the role of *S. lutetiensis* in the dog remains unclear.

Use of DGGE during prebiotic supplementation was evaluated further by Middelbos et al.[11,12] These researchers evaluated six diets: (1) control—no supplemental fermentable carbohydrate; (2) control + 2.5 percent cellulose (poorly fermentable fiber source); (3) control + 2.5 percent beet pulp (moderately fermentable fiber source); (4) control + 1.0 percent cellulose + 1.5 percent scFOS; (5) control + 1.0 percent cellulose + 1.2 percent scFOS + 0.3 percent YCW; and (6) control + 1.0 percent cellulose + 0.9 percent scFOS + 0.6 percent YCW. Decreased total tract apparent CP digestibility and increased fecal butyrate concentrations with prebiotic supplementation were noted. By using DGGE and quantitative real-time PCR, changes in fecal bacterial species were noted. An increase in fecal bifidobacteria and a trend for increased lactobacilli were noted in dogs fed the prebiotic-supplemented diets.

Middelbos et al.[12] compared qPCR analysis to the more conventional method of plating for microbiota enumeration. In this study, comparisons of differing doses of YCW supplementation were evaluated in healthy, adult dogs. Using the plating techniques, fecal *E. coli* decreased linearly and *Clostridium perfringens* responded cubically to increasing YCW supplementation. Using q-RT-PCR, *E. coli* and lactobacilli tended to respond cubically to increasing YCW supplementation. The authors indicated that the differences in results obtained from the techniques to measure

bacterial populations may be due to the fundamentals of the two procedures. Plating measures those bacterial species that are alive at the time of plating, whereas qPCR measures bacterial DNA, thereby measuring those bacteria from dead as well as living organisms. scFOS is rapidly fermented in the proximal colon, and that is the area where bacterial cells utilizing these substrates will proliferate. It is possible that these cells die prior to reaching the distal colon and, therefore, qPCR likely results in a more accurate representation of the number of bacteria in the proximal large bowel.

Finally, two novel carbohydrates were evaluated in dogs by Spears et al.[13] High-molecular-weight pullulan is a slowly hydrolyzed carbohydrate, while γ-cyclodextrin is a cyclic oligosaccharide in which a portion is able to escape enzymatic digestion and thereby become available for fermentation. Increasing concentrations of pullulan and γ-cyclodextrin tended to increase ileal bifidobacteria and lactobacilli. Increasing concentrations of γ-cyclodextrin resulted in a quadratic decrease in fecal *C. perfringens* concentrations. These novel carbohydrates responded similarly to other prebiotic oligosaccharides fed to dogs.

Although several novel outcomes were reported in the studies discussed previously, it is clear that the trends noted by Swanson and Fahey[2] were consistent with those reported here. These new experiments add ever-growing evidence of the beneficial effects of feeding prebiotics to pets. They also begin to fill in the gaps in the research, most notably, measuring the effects of prebiotics in immunocompromised animals. These studies, however, by no means complete the research needed on prebiotic supplementation. As mentioned previously, work in cats is lacking compared to other species and more research is warranted. Despite the more extensive research in dogs, little is known regarding optimal dosage, and/or the effects on immune characteristics. Although some research has evaluated blends of prebiotics,[11] more research in this area is warranted, especially in cats that have unique nutritional needs.

17.4 EVALUATION OF PROBIOTICS IN DOGS AND CATS

The most common microbial species evaluated and utilized as probiotics in the pet include *L. acidophilus* and *Enterococcus faecium*. Three studies evaluated the effects of probiotics *in vitro*.[14–16] *In vivo* work includes 5 studies evaluating *Enteroccoccus* spp.,[17–21] 10 studies evaluating *Lactobacillus* spp.,[22–31] and 1 study evaluating *Bacillus* spp.[32] Of these, only 2 studies evaluated the use of probiotics in cats.[21,27] These studies are described in detail in Table 17.2 and are summarized briefly in the following paragraphs.

Because probiotic usage in pet nutrition is still a relatively new concept (literature dates to 1998), many studies reported only the ability of the probiotic to survive in the gastrointestinal tract of dogs and cats. Furthermore, many of them were prospective studies to determine if a bacterial strain had probiotic effects. Because of this, very little information is available regarding the dosage that is most appropriate. A difficulty with pet foods containing probiotic strains is the fact that most ingredients are extruded, using high heat and pressure for short periods of time.

Table 17.2 *In Vivo* Experiments (In Chronological Order), Reporting Effects of Probiotics In Cats and Dogs

Ref.	Outcome Variables Quantified	Animals/ Treatment (Age, Initial BW)	Dietary Information; Time on Treatment	Daily Prebiotic Dose; Source	Major Findings
Biourge et al., 1998[32]	Fecal bacilli concentrations during and after removal of treatment Nutrient digestibility	5 female dogs (5-10 yr of age; 24 ± 3 kg BW)	RCCI M25, Royal Canin, Aimargues, France Chemical composition: 25% CP 12% fat 6.5% CF Time on treatment: 0–7 d delay of appearance; 3 wk disappearance study	1.5 × 10^8 cfu/g diet of *Bacillus* CIP 5832 (Paciflor, Pasteur Institute)	No changes in nutrient digestibility *Bacillus* spp. present in feces within 24 h No detection of *Bacillus* after 3 d of removal of probiotic treatment
Pasupathy et al., 2001[22]	Nutrient digestibility Growth Fecal characteristics Fecal microbial populations	4 mongrel puppies (10 wk of age; 5.3 kg BW)	Basal diet: 33% CP 13% Crude fat 4% CF Time on treatment: 9 wk	2 ml of 1 x 10^7 cfu/ml *Lactobacillus acidophilus*	↑ Fecal lactobacilli counts (11%)** ↑ coliform counts (8%)* ↓ CF digestion (16%)*

Weese and Anderson, 2002[23]	Presence of probiotic after feeding Fecal colonization of probiotic	32 healthy, adult beagle dogs *n* = 4 for control and group 4 treatments *n* = 8 for groups 1, 2, and 3 treatments	Basal diet not provided Time on treatment: 5 d	Control: no supplementation Group 1: 1×10^9 cfu Group 2: 1×10^{10} cfu Group 3: 5×10^{10} cfu Group 4: 5×10^{11} cfu *L. rhamnosus* strain GG (LGG)	LGG present after 24 h in dogs in groups 2 (25%), 3 (50%), and 4 (100%) LGG present in 1 dog after 72 h of removal ↑ Fecal LGG levels in group 4 (~129%)***
Swanson et al., 2002[24]	**Study 1** Fecal characteristics Fecal metabolites Fecal microbial ecology Total tract nutrient digestibility	5 adult pointers (6.25 yr of age; 23 kg BW)	600 g basal diet Chemical composition: 24% CP 18% fat 6% TDF Time on treatment: 28 d	1. Control- no supplementation 2. 4 g scFOS 3. 2×10^9 cfu *L. acidophilus* 4. 4 g scFOS + 2×10^9 cfu *L. acidophilus*	*L. acidophilus:* ↑ Hydrogen sulfide (39%) and methanethiol (40%) concentrations at 24 h**

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Table 17.2 *In Vivo* Experiments (In Chronological Order), Reporting Effects of Probiotics In Cats and Dogs (continued)

Ref.	Outcome Variables Quantified	Animals/ Treatment (Age, Initial BW)	Dietary Information; Time on Treatment	Daily Prebiotic Dose; Source	Major Findings
	Study 2 Fecal characteristics Fecal metabolites Fecal microbial ecology Total tract nutrient digestibility	5 adult pointers (2.2 yr of age; 21 kg BW)	600 g basal diet Chemical composition: 24% CP 18% fat 6% TDF Time on treatment: 28 d	1. Control- no supplementation 2. 4 g scFOS 3. 2×10^9 cfu *L. acidophilus* 4. 4 g scFOS + 2×10^9 cfu *L. acidophilus*	*L. acidophilus:* ↑ Fecal bifidobacteria (5%)* ↑ Dimethyl sulfide concentrations (38%) at 24 h** ↑ DM digestibility (2%)** ↑ CP digestibility (2%)*
Benyacoub et al., 2003[17]	Fecal IgA Plasma IgG and IgA Blood lymphocytes	7 puppies (8 wk of age)	Basal diet: Friskies Alpo® Complete dry dog food; Nestlé Purina Petcare, Glendale, CA Chemical composition: 22% CP 10% fat Time on treatment: 44 wk	Control- no supplementation Test- 5×10^8 cfu/d *Enterococcus faecium* (strain NCIMB10415; SF68; Cerbios-Pharma, Barbengo, Switzerland)	↑ Fecal IgA (~50%)* ↑ Plasma IgA wk 18–56 (~50%)** ↑ Response to CDV vaccination ↑ CDV-specific IgA (~50%) and IgG (~97%) ↑ proportion of mature B cells (39% wk 31; 73% wk 44)** ↑ MHCII molecule surface expression in monocytes (62%)**

Vahjen and Männer, 2003[18]	*Salmonella* spp., *Campylobacter* spp., and *Clostridium* spp. population counts	12 dogs (4.6 ± 2.6 yr of age; 30.7 ± 20.5 kg BW)	Basal diet fed to maintain BW Fed a dry or canned commercial diet Time on treatment: 18 d	2 g/dog (9.2×10^9 cfu) *E. faecium* (NCIB 10415, Enteroferm)	↓ *Clostridium* spp. in 10/12 dogs**
Baillon et al., 2004[25]	Presence in fecal matter Fecal microbial ecology WBC analysis Serum biochemical profile	15 adult dogs (7.1 ± 2.5 yr of age; 28.8 ± 4.0 kg BW)	Basal diet fed to maintain BW Chemical composition: 33% CP 20% fat 3% CF Time on treatment: 4 wk	7.1 z 10^6 CFU/g of *L. acidophilus* DSM 13241	Presence detected in feces, disappeared after 2 wk cessation ↓ Fecal *Clostridia* spp. (approximately 83%)** ↑ Serum IgG (18%) ** ↓ Erythrocyte fragility (45%) and nitric oxide 281%) *** ↑ RBC count (9%) and hematocrit (11%)*** ↑ WBC count (6%)* and monocyte number (53%) *** ↓ B cell counts (20%)*
Manninen et al., 2006[26]	Presence in jejunal chyme DGGE	5 fistulated beagles (4–8 yr of age)	Basal diet Chemical composition: 23% CP 13% fat 3% fiber Time on treatment: 7 d	$1.4–5.9 \times 10^7$ cfu/mL/d mixture of lactic acid bacteria (LAB) *L. fermentum* LAB8 *L. salivarius* LAB9 *Weissella confusa* LAB10 *L. rhamnosus* LAB11 *L. mucosae* LAB12	LAB detected in jejunal chyme 7 d after cessation, no LAB in chyme Reduced indigenous LAB in 4/5 dogs

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Table 17.2 ***In Vivo*** **Experiments (In Chronological Order), Reporting Effects of Probiotics In Cats and Dogs (continued)**

Ref.	Outcome Variables Quantified	Animals/ Treatment (Age, Initial BW)	Dietary Information; Time on Treatment	Daily Prebiotic Dose; Source	Major Findings
Marciňáková et al., 2006[20]	Colonization and survival of probiotic Blood lipids, proteins, and cholesterol	11 dogs (aged 2–7 yr)	Fed to maintain BW Basal diet not indicated Time on treatment: 7 d	1×10^9 cfu/mL 2–3 mL administered, depending on BW of dog *Enterococcous faecium* strain EE3	↓ Blood lipids in 8/11 dogs ↓ Total protein in blood of 6/11 dogs ↓ Pseudomonas-like spp.*** Survival of *E. faecium* EE3 through 3 months after cessation of probiotic
Marshall-Jones et al., 2006[27]	Fecal quality Fecal bacterial populations (FISH enumeration), pH, ammonia, and hydrogen sulfide concentrations WBC count Serum biochemical analysis Serum IgA, IgM, and IgG	15 adult domestic short hair cats (4.5 ± 0.4 yr of age; 3.6 ± 1.1 kg BW)	Fed to maintain BW Basal diet not indicated Time on treatment: 4.5 wk	4.1×10^9 cfu/kg diet *L. acidophilus* DSM13241 Daily intake between 1.2×10^8 cfu and 2.8×10^8 cfu	↓ Bacterial culture of clostridia (9.5%)**, coliforms (14%)***, and enterococci (31%)*** ↓ Fecal pH (2%)** ↑ *Lactobacillus* spp. (4%)***, *L. acidophilus* (0.47 log/g baseline vs. 7.25 log/g treatment)*** ↓ *Enterococcus faecalis* (66%)*** ↑ Fluorescence intensity of granulocytes (30%)*** ↓ Plasma endotoxin concentrations (>250 U/mL baseline vs. <50 U/mL treatment)***

Sauter et al., 2006[28]	Duodenal and colonic cytokine gene expression Fecal microbial populations	10 placebo, 11 test dogs diagnosed with food responsive diarrhea (28 ± 4 mo of age)	Basal diet- elimination diet with novel protein source Purina® Canine LA (limited antigen) Diet, St. Louis, MO Chemical composition (as is): 28% CP 17% crude fat 2% CF Time on treatment: 4 wk	1 g/d probiotic cocktail (1×10^{10} cfu) Probiotic cocktail two *L. acidophilus* stains (NCC2628, NCC2766) One *L. johnsonii* strain (NCC2767)	↓ Duodenal IL-10 mRNA levels (38%)* ↑ Total *Lactobacillus* spp. (29%)*
Strompfová et al., 2006[29]	Fecal microbial populations Blood total protein, total lipid, cholesterol, glucose, aminotransferase, and urea	15 healthy adult dogs (0.5–3 yr of age)	Basal diet provided at 20 g/kg BW APORT Ideal Adult (Tekro s.r.o., Žitňany, Slovakia) Time on treatment: 7 d	3 mL (1×10^{9} cfu/mL) *L. fermentum* AD1	↑ *Enterococcus* spp. (25%)*** ↑ *Lactobacillus* spp. (55%)*** ↑ Total blood protein (21%)*** and total lipids (33%)** ↓ Blood glucose (11%)** Biagi et al., 2007[30]

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Table 17.2 *In Vivo* Experiments (In Chronological Order), Reporting Effects of Probiotics In Cats and Dogs (continued)

Ref.	Outcome Variables Quantified	Animals/ Treatment (Age, Initial BW)	Dietary Information; Time on Treatment	Daily Prebiotic Dose; Source	Major Findings
Biagi et al., 2007[30]; Veir et al., 2007[21]	Fecal microbial populations Fecal *C. perfringens* enterotoxins and *C. difficile* toxin A or B CBC, serum biochemical profiles, non-specific immune response Fecal, sera, and saliva IgG and IgA Serum FHV-1-specific IgG, FHV-1-specific IgA, FCV-specific IgG and FPV-specific IgG	10 kittens (7 wk of age)	Basal diets chicken and rice dry kitten growth formula Time on treatment: 20 wk	0.25–0.28 g (5×10^8 cfu/d) of dry probiotic powder *E. faecium* SF68 (NCIMB10415, LBC ME5 PET, Cerbios-Pharma SA, Switzerland)	*E. faecium* detected in 7/9 treated kittens ↑ $CD4^+$ lymphocytes (~36%)**

Pascher et al., 2008[31]	Frequency of defecation Fecal quality Total tract nutrient digestibility Fecal *C. perfringens*, *Escherichia* spp., lactobacilli, and bifidobacteria	6 adult German shorthair pointers with nonspecific dietary sensitivity (4.5 yr of age; 30.8 ± 2.0 kg)	Dry kibble diet Main dietary ingredients: Poultry meal Cereals Rice Vegetables Chemical composition: Control diet 27.2 % CP 8.8 % crude fat 2.0 % CF Probiotic diet 28.1 % CP 8.8 % crude fat 1.8 % CF Time on treatment: 12 wk	*L. acidophilus* DSM 13241 (6×10^6 cfu/g dry dog food) Added post-extrusion	Improved frequency of defecation (~70% 1–2 defecations per d vs. 50% 1–2 defecations per d no probiotic), fecal consistency (~70% fecal score 3 (ideal) vs. ~45% fecal score 3 no probiotic), and fecal DM (11.8%)** Numerical increases in lactobacilli (6.2%) and bifidobacteria (6.6%) Numerical decreases in *C. perfringens* (4%) and *Escherichia* spp. (1.4%)

Note: BCS, body condition score; BW, body weight; CBC, complete blood count; CDV, canine distemper virus; CF, crude fiber; cfu, colony-forming units; CP, crude protein; DGGE, denaturing gradient gel electrophoresis; DM, dry matter; FCV, feline calicivirus; FHV, feline herpes virus; FISH, fluorescence in situ hybridization; FPV, feline panleukopenia virus; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IL-5, interleukin-5; IL-10, interleukin-10; LAB, lactic acid bacteria; LGG, *Lactobacillus rhamnosus* strain GG; RBC, red blood cells; TDF, total dietary fiber; WBC, white blood cell.

* $P < 0.10$.

** $P < 0.05$.

*** $P < 0.001$.

All canned diets must undergo retort. Both processes kill the majority of bacteria in the food, including probiotic strains. Additionally, most pet foods are guaranteed to have a shelf life of up to 1 year. Probiotics may not survive for this length of time, thus no label guarantee can be made.

Weese and Arroyo[33] evaluated 19 commercial pet foods claiming to contain probiotics. Of those 19 pet foods, 13 were for dogs and 6 were for cats. All diets were evaluated prior to their indicated expiration date. None of the tested diets contained all organisms listed on the ingredient label. Of the 19, 10 (53 percent) diets had at least one microorganism listed on the ingredient label; 5 (26 percent) products had no probiotic bacteria present. Some diets allegedly contained bacterial fermentation products without the bacteria itself listed as an ingredient, but still claimed to contain a probiotic.[33] The need for proper ingredient labeling, oversight of claims, and guidelines for probiotics in pet foods is obvious.

Although there are some difficulties still to overcome, probiotics have been noted to have positive effects both *in vitro* and *in vivo. In vitro* research has, to date, evaluated only various strains of lactobacilli. The first study measured the effects of a probiotic cocktail (three *Lactobacillus* spp.) on mRNA inflammatory cytokine expression in intestinal samples from dogs suffering from chronic enteropathies compared with healthy dogs.[14] The ratio of regulatory to inflammatory cytokines was improved following the addition of probiotics, suggesting that this may be of use *in vivo* to decrease inflammation in the intestinal tissue.[14] A second study isolated and evaluated *L. murinus* as a potential probiotic in dogs.[16] After isolation, the probiotic was tested to determine its ability to survive in different pH and bile salt conditions, to inhibit growth *in vitro* of *E. coli* and *C. perfringens*, and to adhere to glass and intestinal mucus. All criteria were met with *L. murinus* and, therefore, it may be capable of surviving the gastrointestinal tract of the dog and lead to beneficial effects in the host.[16]

Results *in vivo* appear to be positive, but some conflicting results occur. Due to the varying doses and mode of administration, it is difficult to quantify trends occurring due to probiotic supplementation. Overall trends suggest that probiotic bacteria, administered at a sufficiently high dose, will lead to increases in gut probiotic bacterial species, as well as a decrease in potentially pathogenic bacteria. During feeding of a probiotic, most studies (79 percent) indicated the presence of, or a significant increase in, the probiotic species in fecal matter. Four studies indicated a decrease in fecal *C. perfringens* or *Escherichia coli,* which often are considered potentially pathogenic bacteria when allowed to grow above normal levels. One problem with probiotic supplementation is that bacteria disappear shortly after cessation of supplementation. Therefore, these changes are not lasting, indicating that probiotic bacteria are likely not attaching and colonizing within the gastrointestinal tract. Biourge et al.[32] indicated no detection of probiotic species (*Bacillus* CIP 5832) after 3 days of probiotic cessation, and Weese and Anderson[23] noted *L. rhamnosus* probiotic present in only one dog after 72 hours of removal. This was contrary to Marciňáková et al.[20] who found survival of *Enterococcus faecium* EE3 after a 3-month cessation of probiotic treatment. The authors indicated that *E. faecium* EE3 is a strain that has

adhesive capability in human and canine mucus (human: 7.3 percent, canine: 7.4 percent adhesion).[20]

Total tract apparent macronutrient digestibility does not appear to be influenced by probiotic supplementation. One study found a decrease in crude fiber digestion,[22] while another found a tendency for increased DM and CP digestibilities.[24] The authors of the first study, who utilized young puppies 10 weeks of age, indicated that the decrease in crude fiber digestibility was negligible.[22] The subsequent study found only a tendency to increase DM and CP digestibility in one of two identical experiments in adult dogs.[24] Other studies evaluating digestibility found no differences due to probiotic supplementation.

Studies reporting the effects of probiotic supplementation on immunological changes are limited. Benyacoub et al.[17] noted an increase in fecal and plasma IgA in puppies fed an *E. faecium* strain. Furthermore, the authors indicated an increased response to canine distemper virus, an increased proportion of mature B cells, and increased MHCII molecule surface expression in monocytes. Given the stressful time period of weaning, this increased immune response would be beneficial. In healthy adult dogs, increased serum IgG, decreased erythrocyte fragility, and increased white blood cell (WBC) and monocyte number were noted.[25] Only one study to date has evaluated immune characteristics in weanling kittens supplemented with a probiotic. An increased $CD4^+$ lymphocyte concentration, but no changes in IgG, IgA, WBC counts, or response to vaccination were noted.[21] Based on these findings, further investigation into the effects on pets suffering from gastrointestinal diseases is warranted.

Although this area of research is rapidly expanding, more clear trends are necessary to make specific recommendations. It is clear, however, that probiotic supplementation appears to positively influence gut health of dogs and cats. Finding optimal doses as well as combinations of probiotics that may work synergistically will be of great importance in advancing the field.

17.5 EVALUATION OF SYNBIOTICS IN DOGS AND CATS

The idea of combining probiotics and prebiotics to create a synergistic effect is not a novel concept, but a paucity of information currently exists on this topic in canine and feline nutrition. In an *in vitro* study, Tzortizis et al.[34] synthesized α-galacto-oligosaccharides from *L. reuteri* (canine origin). These researchers then evaluated the fermentative properties of galacto-oligosaccharides compared to other fermentable carbohydrate sources in combination with *L. acidophilus* and *L. reuteri*. Utilizing an oligosaccharide in conjunction with the bacterial strain it was created from *L. reuteri* led to the most beneficial changes in microbial ecology.[35] The galacto-oligosaccharide + *L. reuteri* increased bifidobacteria and lactobacilli concentrations more than any oligosaccharide mixture alone, or the oligosaccharide + *L. acidophilus* combination. Additionally, clostridia decreased after 24 hours in the galactose + *L. reuteri* and galactosyl melibiose mixture. *Escherichia coli* also decreased throughout 24 hours of fermentation in melibiose, fructo-oligosaccharide,

galactosyl melibiose mixture, and galactose + *L. reuteri* groups. This concept of creating synbiotics formulated to work with each other is likely to lead to many important future studies in the canine and feline nutrition field.

The ability of three lactobacilli strains, *L. mucosae*, *L. acidophilus*, and *L. reuteri*, to work synergistically with carbohydrate sources to produce antagonistic compounds against *E. coli* and *Salmonella enterica* (serotype Typhimurium) was studied.[36] The authors noted that each of the lactobacilli strains were able to produce antimicrobial compounds when grown in sugar mixtures (consisting of α-glucosidases, dp 1–4), indicating a synergistic effect.[36] Results of this study can be used to design *in vivo* experiments to test these synergistic effects, for the purposes of warding off gastrointestinal pathogens.

Only one study has evaluated synbiotic usage in dogs, while no studies have been reported in cats. In this study, dogs were randomly assigned to one of four treatments: control, scFOS alone, *L. acidophilus* (1×10^9 cfu/day) alone, or 2 g scFOS + 1×10^9 cfu/day *L. acidophilus*.[24] A synergistic effect was noted in decreasing putrefactive compounds (biogenic amines, BCFA, phenols, and indoles) in the feces. These decreases were greater than for either scFOS or *L. acidophilus* alone. This result, however, was noted in only one of two replicated experiments. Evaluation of synbiotics *in vivo* is needed to determine the ability of these mixtures to modify gut microbial populations and influence gut health in companion animals.

17.6 CONCLUSION

The use of prebiotics and probiotics in companion animal nutrition is continuing to increase in popularity. Although much knowledge has been gained in recent years on this topic, more research is needed in several areas. Appropriate dosages to maximize response while maintaining reasonable diet costs may be the most immediate need for prebiotic and probiotic studies in the future. Also, testing both prebiotics and probiotics in more disease states is warranted. Further research specific to cats is needed. Finally, increasing the database on synbiotics may aid in creating economical, yet effective, dietary supplementation programs for dogs and cats at several physiological states in addition to those that are health compromised.

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CHAPTER 18

Probiotics
Potential Pharmaceutical Applications

Indu Pal Kaur, Anurag Kuhad, Amita Garg, and Kanwaljit Chopra

CONTENTS

18.1 INTRODUCTION

Historically, Charaka Samhita supposedly wrote a treatise on ayurvedic medicine around 1000 BCE in which he referred to the beneficial microbial flora of the gastrointestinal tract (GIT) as "jataragni" (fire in the stomach), the sustaining force of all living beings, and referred to "takra," that is, fermented milk, as "amrita" or elixir. It has now been established that the *Lactobacillus* strain stabilizes the healthy intestinal flora and destroys the pathogenic strains present therein. More than 100 years ago, Elie Metchnikoff (1907) was the first to propose a scientific rationale for the role of lactobacilli in maintaining health and longevity.[1] The term probiotic dates to 1965 when Lilly and Stilwell first used it to describe any substance or organism that contributes to the intestinal microbial balance, and Fuller in 1989 further emphasized its role in health.[2,3] A probiotic is defined as a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract (Figure 18.1).[4–8] The most commonly used probiotics mainly come from two genera: *Lactobacillus* and *Bifidobacterium* (Table 18.1). At present, probiotics are almost exclusively consumed as fermented dairy products, such as yogurt or freeze-dried cultures, but in the future they may also be found in fermented vegetables and meats.[9] Novel modes of therapeutic and prophylactic interventions may include the consumption of probiotics either alone or in combination with prebiotics.

A prebiotic is defined as a nondigestible food that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon.[5] Modification of the intestinal microflora by prebiotics leads to the predominance of health-promoting bacteria, especially, but not exclusively, lactobacilli and bifidobacteria. Nondigestible oligosaccharides in general and fructo-oligosaccharides in particular are prebiotics. These are found naturally in onions, garlic, leeks, chicory, artichokes, beans, and peas, as well as in some cereals.[10] A synbiotic or eubiotic is a mixture of probiotics and prebiotics, which beneficially

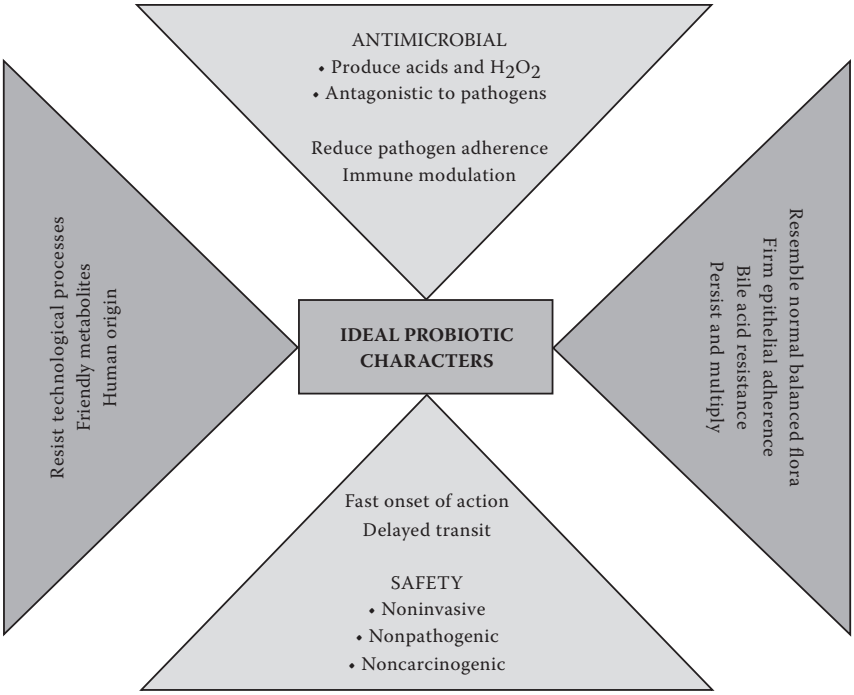

Figure 18.1 Desirable and ideal characteristics of probiotics.

affects the host by improving the survival and implantation of live microbial dietary supplements in the GIT and, thus, improving host health and well-being.[5]

18.2 MECHANISM OF PROBIOTIC ACTION

The usefulness of probiotics has been implied in a host of human diseases ranging from a wide variety of GIT-related problems, to allergies, cancer, AIDS, respiratory and urinary tract infections, aging, fatigue, and autism. Newer claims indicate their role in reducing the risks of osteoporosis, obesity, and possibly type 2 diabetes.[11] Probiotics have been proposed to exert therapeutic effects via several mechanisms (Figure 18.2).[4,12,13] Various theories of their action have been put forth for consideration:

1. Receptor competition, whereby probiotics compete with microbial pathogens for limited number of receptors present on the surface of the intestinal epithelium.[12,14]
2. Probiotics release antimicrobial compounds, such as organic acids, free fatty acids, hydrogen peroxide, and bacteriocins, which may induce an antagonistic action against pathogenic organisms.[14,15] Furthermore, the accumulation of such metabolites can reduce the pH of the surrounding environment, which may directly inhibit the growth of harmful organisms. The best characterized probiotic with

Table 18.1 A Comprehensive List of Probiotic Strains and Their Sources Reported in the Literature

Commercial Strains	Sources
1. *Lactobacillus*	
L. acidophilus NCFM	Rhodia, Inc. Madison, WI
L. acidophilus LB	Lacteol Laboratory, Houdon, France
L. acidophilus DDS-1	Nebraska Cultures, Inc. Lincoln, NE
L. rhamnosus LB21	Essum AB, Umea, Sweden
L. plantarum 299v	Probi AB, Lund, Sweden
L. crispatus	Gynelogix, Colorado, USA
L. rhamnosus 271	Probi AB, Lund, Sweden
L. fermentum RC14	Urex Biotech, Canada
LGG	Valio Dairy, Helsinki, Finland
L. acidophilus R0011	Institut Rosell, Monterol, Canada
L. paracasei F19	Arla Dairy, Sweden
L. rhamnosus R0052	Institut Rosell, Monterol, Canada
L. plantarum	Arla Dairy, Stockholm, Sweden
2. *Bifidobacterium*	
B. lactis FK120	Fukuchan milk, Japan
B. lactis HN019 DR10	New Zealand Dairy Board
B. longum	Snow Brand Milk Products Co. Ltd., Japan
B. infantis HN019 DR10	New Zealand Dairy Board
B. lactis Bb-12	Chr. Hansen, Horsholm, Denmark
B. breve Yakult	Yakult, Tokyo, Japan
B. longum BB536	Morinaga Milk Industry Co. Ltd., Japan
B. lactis LKM512	Fukucha milk, Japan
3. Miscellaneous	
Enterococcus faecalis SF68	Cerbios Pharma, Switzerland
Streptoccocus thermophilus 1131	Kenko-dontokoi, Japan
S. thermophilus F2	Danlac, Canada

these properties is *L. casei* strain GG, reclassified as *LGG.*[12,16] Lactic acid bacteria also release antimicrobial substances reuterin and bacteriocins.[12] This is the most widely accepted theory.

3. Increased induction of mucin secretion, which results in enhanced binding of probiotics to the intestinal mucosa. This action blocks enteropathogen binding to epithelial receptors.[14,17] Studies demonstrate that *L. acidophilus* and *L. casei* adhere to Caco-2 cells at the expense of enteropathogens, such as *Salmonella typhimurium*, *Yersinia enterolytica,* at a relatively high number.[18]
4. Competition for nutrients in the GIT.[4]

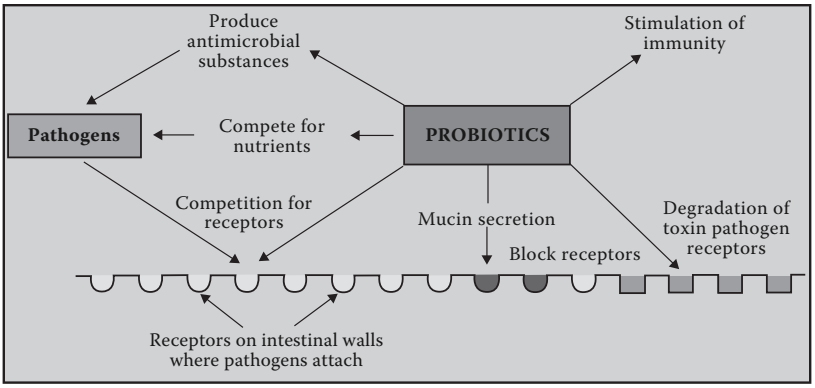

Figure 18.2 Mechanism of action of probiotics.

5. Possible modification of toxin receptors and blockage of toxin-mediated pathology by probiotics.[12,19] *Saccharomyces boulardii* degrades *Clostridium difficile* toxin receptors in the rabbit ileum[20] and blocks cholera-induced secretion in rat jejunum by the production of polyamines.[21]
6. Possible promotion by probiotics of nonspecific stimulation of the host immune system, including immune cell proliferation, enhanced phagocytic activity of macrophages, and increased production of secretory immunoglobulin A (IgA) and IgM.[12,22] Probiotics have also been reported to stimulate the production of interferon gamma (IFN-γ), interleukin 2 (IL-2), IL-12, and IL-18.[23,24] IL-12 may in turn downregulate the Th2 response, thereby decreasing IL-4 and IgE production, which would explain the role of probiotics in allergy prevention.[24]
7. Stabilization of intestinal permeability barrier, which restricts colonization by pathogens, eliminates foreign antigens, which have penetrated the mucosa, and regulates the antigen-specific immune responses.[23]
8. Probiotic bacterial "priming" of gut-associated lymphoid tissue (GALT) and immunomodulation of gut-associated lymphoid and epithelial tissue response.[4,24]

18.3 SALVAGE OF VARIOUS DISORDERS THROUGH PROBIOTIC THERAPY

18.3.1 Intestinal Disorders

Intestinal homeostasis relies on the equilibrium between absorption (nutrients, ions), secretion (ions, IgA) and barrier capacity (to pathogens and macromolecules) of the digestive epithelium. Disturbance of this homeostatic control results in inflammation, diarrhea, and various intestinal diseases. To better understand the beneficial effects of probiotics in digestive diseases, it is important to take into account the mechanisms involved in the derangement of epithelial functions (Figure 18.3), such as (1) dysregulation of ion-coupled nutrient absorption and (2) an abnormal stimulation of ion secretion, in turn driving water losses.[25,26] Water movements are

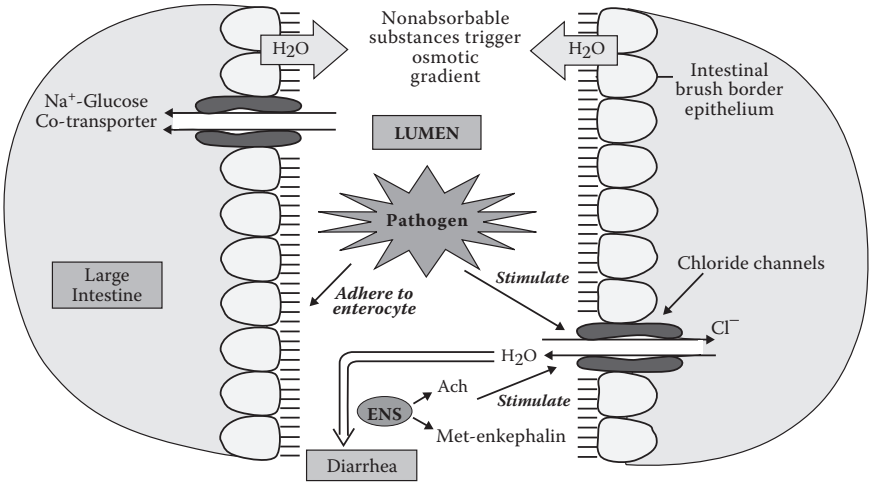

Figure 18.3 Factors affecting intestinal homoeostasis resulting in diarrhea. ENS, enteric nervous system; Ach, acetylcholine; Met, methionine.

mainly generated by the sodium-solute cotransport systems (Na^+-glucose) or chloride (Cl^-) secretion across the apical membranes of intestinal epithelial cells. Any luminal serosal factor affecting the sodium absorption (driven by sodium-glucose cotransport leads to net water absorption) and chloride secretion (drives water secretion in the intestinal lumen) transport systems will also affect electrolyte and water movements. Pathogenic bacteria can adhere to brush border membranes of the enterocyte, inducing epithelial dysfunction, such as lesions of the brush border membrane, and release of enterotoxins, which stimulate Cl^- secretion (diarrhea), or cytotoxins disrupting epithelial integrity.[25,27] Osmotic diarrhea can also be induced when a nonabsorbable compound (e.g., lactose in case of lactase deficiency) reaches the intestinal lumen. Abnormal stimulation of the underlying immune system (mast cells, phagocytes, lymphocytes) may as well lead to the release of inflammatory mediators capable of altering epithelial function.[27] The use of probiotics and prebiotics as therapeutic agents for gastrointestinal disorders is rapidly moving into "mainstream" therapy.[26,28]

18.3.1.1 Antibiotic-Associated Diarrhea (AAD)

The incidence of AAD differs with the type of antibiotic and may occur in almost 15 to 25 percent of patients receiving antibiotics. Most cases of AAD are directly or indirectly caused by alterations of gut microflora by the antibiotics resulting in functional disturbances of intestinal carbohydrate or bile acid metabolism.[29] Lactobacilli, especially *LGG,* have been reported to be beneficial in AAD.[26] The incidence of diarrhea was reduced from 25 percent in the placebo-treated group to 8 percent in *LGG*-treated group.[30] A fermented multistrain probiotic milk drink prevented four of five cases of AAD in adult hospitalized patients.[31] Madden et al.[32] reported that

probiotic supplementation modulates the response of the intestinal microflora to the effects of antibiotic therapy. *LGG* has been shown to reduce the risk of AAD by approximately 75 percent in children in studies carried out in the United States and Finland.[33] A meta-analysis summing the results of nine controlled trials indicates that both *Lactobacilli* and *S. boulardii* are effective in preventing AAD.[34]

Very recently, a double-blind, placebo-controlled study with 87 patients treated with antibiotics was carried out. Patients were administered fermented milk drink containing *LGG*, La-5, and Bb-12 ($n = 46$) or placebo with heat-killed bacteria ($n = 41$) randomly for a period of 14 days. Of the 63 patients who completed the study, 2 patients (5.9 percent) in the treatment group and 8 (27.6 percent) in the placebo group developed AAD ($P = 0.035$). The relatively low risk of developing AAD (0.21; 95 percent confidence interval: 0.05 to 0.93) indicates that a fermented multistrain probiotic milk drink may prevent four of five cases of AAD in adult hospitalized patients.[35]

18.3.1.2 Radiotherapy-Induced Diarrhea

Radiotherapy is an important aspect of multimodal cancer therapy, but radiation-induced acute intestinal injury is a common and serious problem. Disruption of morphologic mucosal integrity and normal bacterial microflora after abdominal radiation leads to malabsorption and bacterial translocation. Probiotic lactic acid-producing bacteria are an easy, safe, and feasible approach to protect patients with cancer against the risk of radiation-induced diarrhea.[36] Probiotics added as substrates can be given by an oral or enteral route to patients, who undergo radiotherapy to prevent radiation-induced enteritis, diarrhea, and related malnutrition.[37] In patients undergoing abdominal irradiation, the prevention of intestinal diarrhea (side effect) was obtained by the administration of live *L. acidophilus* cultures[55] or *L. rhamnosus* in a double-blind trial design.[38]

In a double-blind, placebo-controlled trial, 490 patients who underwent adjuvant postoperative radiation therapy after surgery for sigmoid, rectal, or cervical cancer were assigned to either the high-potency probiotic preparation VSL#3 (one sachet three times a day) or placebo starting from the first day of radiation therapy. Placebo patients had higher incidence of radiation-induced diarrhea than VSL#3 patients (124 of 239 patients, 51.8 percent, and 77 of 243 patients, 31.6 percent; $P < 0.001$), and patients given placebo suffered grade 3 or 4 diarrhea compared with VSL#3 recipients (55.4 percent and 1.4 percent, $P < 0.001$).[36]

18.3.1.3 Clostridium difficile-Associated Diarrhea

Clostridium difficile is a classical example of the opportunistic proliferation of an intestinal pathogen after breakdown of colonization resistance due to antibiotic administration and is the cause of ~20 to 40 percent of AAD cases.[39,40] In fact, this microorganism is the major identifiable cause of nosocomial diarrhea in the United States, infecting 15 to 20 percent of adult hospitalized patients.[3]

In the case of recurrent *C. difficile* colitis in humans, a successful treatment was obtained using *LGG*, both in adults and children in prospective, randomized,

placebo-controlled trials with standard antibiotics.[41] Probiotics, such as *S. boulardii,* in combination with standard antibiotics were demonstrated more effective than antibiotics alone in the treatment of recurrent *Clostridium* infection.[42] In a placebo-controlled study, McFarland et al.[42] examined standard antibiotic therapy (metronidazole or vancomycin) with concurrent *S. boulardii* or placebo in 124 adult patients, 64 patients with an initial episode of *C. difficile* disease, and 60 patients with a history of at least one prior episode of *C. difficile* disease. It was found that *S. boulardii* significantly inhibited further recurrence of disease.

18.3.1.4 Traveler's Diarrhea

Traveler's diarrhea is a common health complaint among travelers. The incidence of diarrhea in travelers to foreign countries varies from 20 to 50 percent depending on the origin and the destination of the traveler, as well as the mode of travel.[43,44] Although various infectious agents cause traveler's diarrhea, enterotoxigenic *Escherichia coli* is the most common. Several probiotics have been examined for their ability to prevent traveler's diarrhea, including *Lactobacillus*, *Bifidobacterium*, *Streptococcus,* and *Saccharomyces.*[43,45] Several probiotics (*Saccharomyces boulardii* and a mixture of *L. acidophilus* and *B. bifidum*) had significant efficacy.[43] Three double-blind, randomized, controlled trials have suggested some preventive efficacy of *LGG* and *S. boulardii*. In a recent study, *LGG* was found to provide 49 percent protection against traveler's diarrhea.[46] However, an effective medically recommended probiotic therapy for traveler's diarrhea is not fully established.

18.3.1.5 Infantile Diarrhea

Rotavirus is a very common cause of infantile diarrhea, and is characterized by increased intestinal permeability and a higher serum level of β-lactoglobulin-containing immune complexes. Rotaviruses are a significant cause of infant morbidity and mortality, particularly in developing countries.[47] Investigators have demonstrated that the duration of infantile diarrhea may be significantly shortened (from 2.4 to 1.4 day) in infants receiving *LGG*.[48] Treatment with *LGG* was associated with an enhancement of IgA-specific antibody-secreting cells to rotavirus and of serum IgA antibody level during convalescence.[49] Saavedra et al.[50] have shown that supplementing an infant formula with *B. bifidum* and *Streptococcus thermophilus* can reduce the incidence of acute diarrhea and rotavirus shedding in infants admitted to the hospital. Another randomized trial in young children showed that yogurt feeding was associated with a clinically relevant decrease in stool frequency and duration of diarrhea, especially in children with carbohydrate malabsorption.[51] Very recently, in a randomized, double-blind, placebo-controlled trial, administration of Lakcid L® (*LGG*) to 87 children (age range: 2 months to 6 years) having infectious diarrhea, the duration of rotaviral diarrhea was markedly reduced.[52]

18.3.1.6 HIV/AIDS-Associated Diarrhea

Diarrhea is a very serious consequence of human immunodeficiency virus (HIV) infection.[8] The etiology is unknown and effective therapy is not available. However, *Saccharomyces boulardii* has been reported to treat 33 HIV patients with chronic diarrhea.[53] A randomized, double-blind, controlled trial with 77 HIV-infected children (2 to 12 years), divided into two groups: one receiving probiotics (formula containing *B. bifidum* with *Streptococcus thermophilus* -2.5×10^{10} colony forming units) and the other, a standard formula (control group), for 2 months. The CD4 counts (cells mm^{-3}) were collected at the beginning and end of the study. The quality and number of stools were assessed by a questionnaire (watery to normal stool consistency). There was an increase in the mean CD4 count in the probiotics group (791 cells mm^{-3}) and a small decrease in the control group (538 cells mm^{-3}). The change from baseline in mean CD4 cell count was +118 cells mm^{-3} versus –42 cells mm^{-3} for children receiving the probiotic formula and control formula, respectively ($p = 0.049$). A similar reduction in liquid stool consistency in both the groups ($p < 0.06$), with a slight enhancement in the probiotics group, was observed, but without significant difference ($p < 0.522$). The incidence of loose-soft stools showed a small decrease in both groups ($p < 0.955$) and there was an increase in the incidence of normal stool consistency in both the groups ($p < 0.01$). This study showed that probiotics have immunostimulatory properties and might be helpful in the treatment of children infected with HIV.[54]

18.3.1.7 Enteral Feeding-Associated Diarrhea

Patients receiving nasogastric tube feeding frequently develop diarrhea.[8] The investigators postulate that the enteral feeding causes changes in normal flora that result in altered carbohydrate metabolism and subsequent diarrhea. Two separate studies (both placebo controlled and double blind) demonstrated a significant reduction in diarrhea in these patients when they were administered *Saccharomyces boulardii*.[55,56]

18.3.1.8 Persistent or Chronic Diarrhea

Persistent diarrhea is diarrhea that starts acutely but lasts for at least 2 weeks. A beneficial effect of feeding yogurt versus milk was shown in children with persistent diarrhea.[27] In a controlled, randomized, single-blind clinical trial, treatment of children with chronic diarrhea with a probiotic (Lactipan®) promoted complete remission of intestinal disorders.[57] Feeding fermented milk in children with postgastroenteritis syndrome eliminates the disease in 4 days, and was even more beneficial in patients with malnutrition.[57]

18.3.1.9 Sucrase Isomaltase Deficiency

Sucrase isomaltase deficiency is an inherited condition that leads to malabsorption of sucrose. The resulting bacterial fermentation of sucrose leads to an accumulation

of hydrogen in the colon, producing diarrhea, abdominal cramps, and bloating. A sucrose-free diet causes disappearance of symptoms.[8] Harms et al.[58] administered *Saccharomyces cerevisiae* along with sucrose to treat eight children with sucrase isomaltase deficiency. An improvement in their hydrogen breath test as well as gastrointestinal symptoms was observed. The investigators postulated that S. *cerevisiae* was supplying the missing isomaltase enzymes.

18.3.1.10 Lactase Deficiency

Lactose maldigestion occurs frequently and is due to insufficient activity of lactase in the human gut and causes various degrees of abdominal discomfort, such as cramps, bloating, diarrhea, and nausea.[59] Probiotic bacteria such as *L. acidophilus* and bifidobacteria produce β-d-galactosidase (bacterial lactase), which autodigests lactose and improves tolerance to lactose. It was observed that in the lactase-deficient people, lactose is absorbed much better from yogurt than from milk probably due to intraluminal digestion of lactose by the lactase released from yogurt microorganisms.[60] Bile salts in the GIT cause the lysis of yogurt bacteria resulting in a rapid release of lactase. Other probiotics like *L. acidophilus* may also be rich in lactase, but are less efficient, because of their resistance to bile.[61,62] Another explanation for this improved tolerance could be the slowing of the gastrointestinal transit of yogurt compared with milk, which may facilitate prolonged contact between residual lactase on enterocyte and lactose in the lumen.[63] Savaiano et al.[60] have demonstrated that yogurt is superior to cultured buttermilk or pasteurized yogurt in enhancing the digestion of lactose as pasteurization may destroy the β-galactosidase activity.[64]

18.3.1.11 Inflammatory Bowel Disease (IBD)

IBD is a collective term used to describe Crohn's disease (CD), ulcerative colitis (UC), and nonspecific colitis.[66] These diseases, although each with distinct features, are characterized by inflammation of the GIT that can lead to pain, diarrhea, and bleeding.[66] The exact etiology responsible for initiation and perpetuation of these processes is unknown but it is proposed to be related to disturbance in the endogenous intestinal microbial flora and/or a defective mucosal barrier.[67,68] The distal ileum and the colon are the areas with the highest luminal bacterial concentration and represent the major sites of inflammation in IBD.[69,70]

Probiotics seem to represent an effective and safe approach for the maintenance treatment of patients with chronic CD, suggesting their potential role in IBD therapy.[69] The different therapeutic modifications of gut flora, which can be useful in IBD, are discussed in Table 18.2. A double-blind comparison of an oral probiotic (*Saccharomyces boulardii*) preparation and mesalazine in maintaining remission of UC showed that the probiotic treatment was as effective as mesalazine in the maintenance therapy.[27,71] A combination of Balsalazide and VSL#3 (a combination of three species of bifidobacteria, four strains of *Lactobacillus,* and one strain of *Streptococcus salivarius* spp. *thermophilus*) was found to be a very good choice in the treatment of active mild-to-moderate active UC versus balsalazide or mesalazine alone.[72]

Table 18.2 Reported Studies on the Use of Probiotics in the Remission of Inflammatory Bowel Diseases

Disease State	Probiotic Product Used	Details of Study	Clinical Effect	Ref.
IBD	*Bifidobacterium bifidum* BGN4	Conventional diet containing only skim milk or a diet containing skim milk with 0.3 percent (w/w) BGN4 for 4 weeks	BGN4 supplemented diet could be helpful for the control of aberrant immune responses in the intestinal tissue	172
	Lactobacillus rhamnosus GG (LGG), *Streptococcus thermophilus* TH-4 (TH-4), *Bifidobacterium lactis* Bb12 (Bb12), and *Lactobacillus fermentum* BR11	1×10^{10} CFU/mL orally twice daily for 14 days	*Lactobacillus fermentum* BR11 was most effective at reducing colitic symptoms	173
	Bifidobacterium, Lactobacillus, and *Enterococcus*	1,260 mg/d three times daily for 4 weeks	Administration of live combined *Bifidobacterium, Lactobacillus*, and *Enterococcus* improved the symptom of irritable bowel syndrome	174
	A dietary integrator (IBS Active), composed of l-tryptophan, inulin, angelica, vegetal charcoal, vitamin PP, group B vitamins (B_1, B_2, B_6) and probiotics (*Lactobacillus sporogenes, Lactobacillus acidophilus, Streptococcus thermophilus)*	IBS Active (440 mg twice daily) over a mean period of 6 months	IBS Active led to a significant improvement in pain symptoms, abdominal distension, and regulation of bowel movement	175
	A symbiotic consisting of a probiotic, *Bifidobacterium longum* W11, and the short-chain oligosaccharide prebiotic Fos Actilight	3 g/day for at least 36 days	Product can increase stool frequency in patients with constipation-variant IBS and reduce abdominal pain and bloating	176
Crohn's disease	*Saccharomyces boulardii*	In combination with mesalamine	The combination was found to be more effective in the maintenance treatment of inactive Crohn's disease	177

Mucosal alkaline sphingomyelinase activity is reduced in the intestine of IL-10 knockout mice with colitis and in humans with UC. VSL#3 probiotic therapy upregulates mucosal alkaline sphingomyelinase activity.[73] It was found in a clinical trial that oral administration of VSL#3 showed a relapse of chronic pouchitis in only 15 percent as compared to 100 percent relapse in placebo group.[6] Although more convincing results are needed to confirm the advantages of using probiotics in IBD, a trend toward the beneficial effects of bacterial supplementation as an adjuvant to treatment is fast emerging.[65] However, two studies indicate the absence of effect of *L. johnsonii* LA1 and *LGG* in controlling CD.[74,75]

18.3.1.12 Helicobacter pylori *Infection*

Helicobacter pylori infection is a major cause of chronic gastritis and peptic ulcer and a risk factor for gastric malignancies.[76] Although antibiotic therapy for gastritis is quite often effective, eradication is not always achieved and reinfection may occur. Several reports suggested that supplementation of anti-*H. pylori* therapy with probiotics could be effective in increasing the eradication rates of *H. pylori*.[77] *In vivo* models demonstrate the pretreatment with a probiotic can markedly reduce an existing *H. pylori* infection and thus can be used as a prophylactic therapy for *H. pylori* infections. *Lactobacillus reuteri* effectively suppressed *H. pylori* infection in humans and decreased the occurrence of dyspeptic symptoms.[78] Ingestion of *L. acidophilus*, *L. salivarius*, or *L. johnsonii* reduced the activity of *H. pylori* in the stomach and attenuated *H. pylori* indicating an effect in the stomach as well. Two randomized, controlled trails have reported that a fermented dairy product containing the strain *L. johnsonii* LA1 or a heat-killed *L. acidophilus* could help to decrease the gastric colonization by *H. pylori*. The activity of 63 dairy starter cultures (single/mixed), grown in skim milk, against five strains of *H. pylori* showed that skim milk culture strongly inhibited this pathogen; although acids produced by the dairy lactics were only partly responsible for the inhibitory action.[79]

18.3.2 Nonintestinal Disorders

18.3.2.1 Allergy

Allergy, manifested in atopic diseases like atopic eczema, allergic rhinitis, and asthma, currently represents a chronic disorder affecting 20 percent of the world population especially in developed countries. It is the most common chronic disease of childhood.[80] The hygiene hypothesis of allergy, the most appropriate explanation for the rising frequency of atopic diseases, supposes that rapid increase in atopy is related to reduced exposure to infections early in life, when the immune responder phenotype is consolidated.[81] At the time of birth, the GIT of the newborn is sterile and the gut-associated lymphatic tissue (GALT), the most important organ of the adaptive immune system, is in the development stage (Figure 18.4). Concomitant to the development of GALT, during the first months and years of life, an adult-type pattern of stable indigenous gut microflora is established.[82,83] All infants are initially

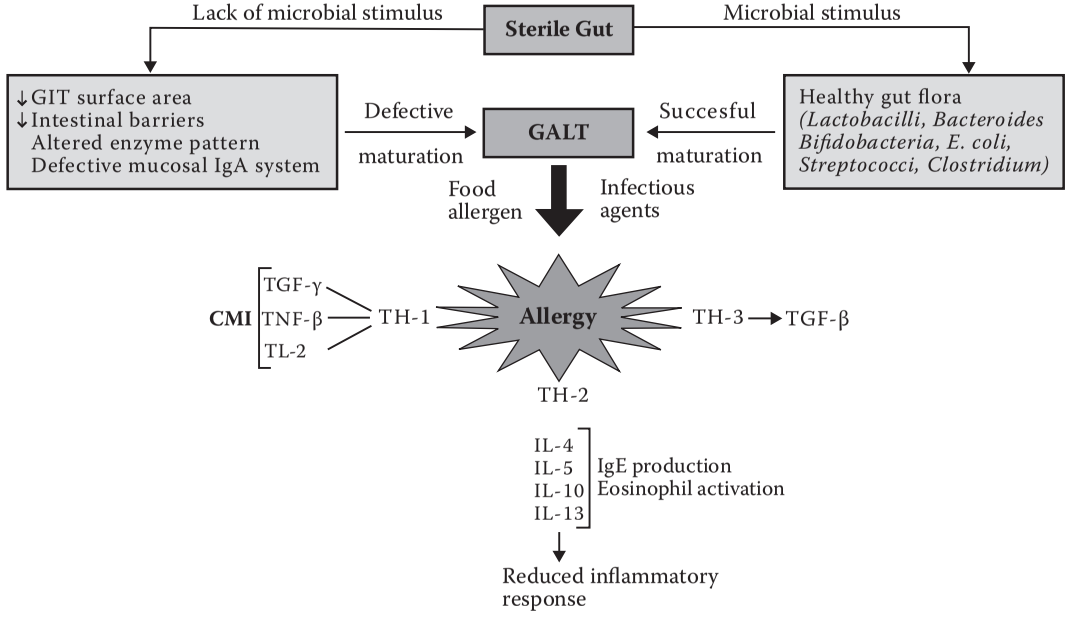

Figure 18.4 Development of GALT and mechanism of allergy development. IL, interleukin, TNF, tumor necrosis factor, TGF, transforming growth factor; CMI, cell mediated immunity; TL, T lymphocytes.

colonized by *E. coli* and streptococci followed by the establishment of the anaerobic genera *Bacteroides, Bifidobacterium,* and *Clostridium* by the end of the first week of life.[27,83] Even though the intestinal mucosa is efficient in assimilation of antigens encountered by the enteric route, it has been reported that in the absence of a healthy gut flora antigen transport is increased.[82,83] Successful maturation of the gut mucosal immune system requires constant microbial stimulus from the developing gastrointestinal microflora. The lack or inadequacy of such a stimulus results in a decreased intestinal surface area, altered mucosal enzyme patterns, defects in the intestinal barriers, reduced capacity for antiinflammatory responses, defective mucosal IgA system,[83] and deficient oral tolerance induction.[84] The exposure to infectious agents generally leads to stimulation of TH-1 lymphocytes and to the release of cytokines such as IFN-γ. In the absence of such stimulation of the immune system by infectious agents triggering TH-1 responses, the main defense mechanism for parasitic infection, that is, TH-2-type cytokine secretion and IgE antibodies, is still present, but it may be redirected against environmental substances, such as food or respiratory antigens. A TH-2 skewed immune system has been shown to be characteristic of allergic inflammation.[80]

Certain probiotics and microbial products have been indicated to be potentially useful in allergy prevention and therapy, by targeting the Toll-like receptor network.[84,85] Also, probiotics have been found to trigger the innate immune system and thus help protect against and treat allergies.[85,86] The reduced exposure of neonates to microbial stimuli leads to skewed immune response favoring TH-2 versus a TH-1 cytokine profile.[86] The precise mechanism by which probiotics induce immune modulation is still largely unknown, although adhesion to the intestinal mucosa is thought to be important.[87] Close contact of probiotics with intestinal mucosa and possibly some benign translocation may lead to an enhanced interaction of probiotics and the intestinal immune system. This interaction stimulates naive T cells to differentiate to TH-1 cells under the influence of IFN-γ, IL-2, and IL-12, while the development of TH-2 cells is downregulated under the influence of IL-4. The result of the generation of counterregulatory TH-1- and TH-3-type immune responses is a reduced production of IgE and an increased secretion of IgA,[88] which leads to a reduced allergic response. Administration of probiotic strains early in life may provide an opportunity for an early interaction with host cell (Toll) receptors resulting in their apical attachment on epithelial and mucosal surfaces, thus, in turn establishing an autochthonous (permanent) condition. Probiotics, in general, are said to constitute the allochthonous (transient) microflora considering their ability to persist only during periods of dosing and for a short time after feeding is halted.[89]

A recent clinical study demonstrates a highly significant reduction in the frequency of atopic eczema in 2-year-old children who as newborns were nursed by their mothers and received a *Lactobacillus* supplement.[90] The first sensitizing antigens are frequently derived from food. There are data to suggest that infants manifesting cow's milk allergy in early infancy have an increased risk of multiple food allergy and asthma.[91] Gut microflora of allergic infants has an atypical composition with reduced levels of bifidobacteria, mainly *B. adolescentis*; and increased levels of clostridia.[92] Pro/prebiotics have been shown to increase levels of bifidobacteria.[93]

A study indicates usefulness of probiotic therapy in prevention or long-term reduction in allergy and also management of atopic eczema and cow's milk allergy in infants.[82] Major factors that can sensitize atopic individuals include genetic susceptibility, aberrant barrier functions of the skin epithelium and gut mucosa, and dysregulation of antigen-specific IgE pattern.[80] Programming of initial microbe exposure is suggested by Reid et al.[94] as an effective means of repressing atopic dermatitis and reducing the risk of other diseases.

18.3.2.2 Immunity

The immune response to a particular pathogen must induce an appropriate set of effector functions that can eliminate the disease agent or its toxic products from the host. Two $CD4^+$ TH cell subpopulations, designated TH-1 and TH-2, can be distinguished *in vitro* by the cytokines they secrete. The TH-1 subset is responsible for many cell-mediated functions and production of IgG antibodies. The TH-2 subset stimulates eosinophil activation and differentiation, provides help to B cells, and promotes production of IgM, IgE, and IgG isotypes. IL-4 is essential for the development of a TH-2 response, and IFN-γ, IL-12, and IL-18 are important in the physiological development of TH-1 cells. At the beginning of an immune response, IFN-γ is generated by stimulation of T cells from activation of natural killer (NK) cells. IFN-γ induces the upregulation of IL-12 receptors on activated T cells. The generation of TH-2 cells depends critically on IL-4. Exposing naive helper cells to IL-4 at the beginning of an immune response causes them to differentiate into TH-2 cells. TH-2 cells secrete a profile of cytokines like IL-10 and IL-13 that promotes eosinophil activation and the synthesis of IgE. Probiotics (stabilizers of digestive mucosal barrier) exert immune-enhancing effects by augmenting both nonspecific and specific host immune responses.[22] Probiotics stimulate lymphocytes to produce cytokine INF-γ and prompt nonspecific phagocytic and lymphocytic activity. Introduction of antigen via oral route induces production of IgA and IgM.[22,95] Probiotics have also been found to improve the defective immune function via stimulating the cytokines IFN-γ, IL-12, and IL-10, all of which play a putative suppressive effect on antigen-specific immune responses.[96] *Lactobacillus johnsonii* and *L. casei* have been reported to stimulate the production IFN-γ and IL-10 secretion.[97] It thus may be concluded that probiotics have the capacity to stimulate a cytokine response, by local mononuclear cells or lymphocytes, and that it depends in part on their capacity to cross the gut epithelium before interacting with the cells of local immune system.[98] Oral administration of *L. casei* is reported to improve the innate immune response in BALB/C mice [99] and reduce skin inflammation due to contact sensitivity in animals sensitized to dinitrofluorobenzene.[99,100]

It has been reported very recently that dietary synbiotic supplementation (*L. casei* + dextran) elicited an enhanced murine and human NK cell activity.[101]

Shida et al.[102] showed that intraperitoneal injection of *L. casei* strain Shirota induced an IL-12 response in the serum of ovalbumin-specific T-cell receptor transgenic mice. On the other hand, anti-IL-12 antibody treatment blocked the ability of *Lactobacillus* Shirota to modulate cytokine production. Dieleman et al.[103] have

recently investigated the ability of *LGG* in the prevention of colitis by decreasing IL-1β and tumor necrosis factor-alpha (TNF-α), and increasing the cecal IL-10. These probiotics downregulate Th1 cytokine while maintaining transforming growth factor-beta (TGF-β). Both oral and subcutaneous administration of probiotics promotes this effect. This activity of lactobacilli via the subcutaneous route protects not only against colitis but also against collagen arthritis, a Th1 mediated model of autoimmunity.[99]

Lactobacillus acidophilus and *L. paracasei* are potential enhancers of systemic immunity and are nonpathogenic, as suggested by their bacterial translocation profiles in healthy mice.[104] Ulisse et al.[105] reported that VSL#3 is able to induce a significant increase in the expression of the antiinflammatory cytokine IL-10 in the mucosal pouch compared to inflamed and antibiotic-treated patients. Suzuki et al.[106] investigated the ability of 46 different *L. lactis* strains to induce production of the cytokines IL-6, IL-12, and TNF- α. The ability of these strains to induce TNF-α, but not IL-6 and IL-12, was lost after heat treatment, suggesting that the stimulus required for TNF-α induction is heat sensitive and is different from those required for IL-6 and IL-12 induction.

18.3.2.3 Urinary Tract Infections (UTI)

Infections of the urethra, bladder, ureter, and kidney affect nearly 3,000 million women per year worldwide. They are due to microbial invasion or an imbalance of the urinary tract microflora. Nearly 50 bacterial strains are found to cause UTIs. Bacterial and fungal infections of the urinary tract are the most promising field of application for probiotics other than the intestine. *Lactobacillus* organisms that predominate in the vagina of healthy women spread from their rectum and perineum and form a barrier to the entry of uropathogens from vagina into the bladder. They are believed to protect the host against infections by means of several mechanisms including (1) occupation of specific adhesion sites at the epithelial surface of the urinary tract; (2) maintenance of a low pH and production of antimicrobial substances like acids, hydrogen peroxide, and bacteriocins; (3) degradation of polyamines; and (4) the production of surfactants with antiadhesive properties.[107]

The concept of artificially boosting the lactobacilli numbers through probiotic instillation has long been conceived, but only in recent years has it been shown to be possible. It is to be noted that not all lactobacilli are effective, and to date only *L. rhamnosus* GR-1 and *L. reuteri* B-54 and RC-14 have been found to be clinical effective.[108] *Lactobacillus rhamnosus* GR-1, *L. fermentum* RC-14, and *L. crispatus* CTV-05 have been proved successful against urogenital infections, such as UTIs and bacterial vaginosis. Clinical trials increasingly provide a profound scientific basis for the use of probiotics in UTIs.[109]

Tomoda et al.[110] reported that oral administration of *B. longum*, in an open study, reduced *Candida* infections in urethra by up to 70 percent. *In vitro* adhesion of *C. albicans* and *Staphylococcus aureus* have been reported to be reduced on epithelial cell lines of the urinary tract by *L. acidophilus*, *L. rhamnosus*.[108] An open, randomized, clinical study showed that local application of *L. rhamnosus* was effective

in controlling UTIs in up to 73 percent of the cases.[111] Asahara et al.[112] reported that mice previously infected with *E. coli* showed a decrease in *E. coli* growth and inflammation after local application of heat-killed *L. casei* Shirota.

18.3.2.4 Bacterial Vaginosis (BV)

The vagina and its microflora form a finely balanced ecosystem. Disruption of this ecosystem can lead to a microbiological imbalance and symptoms of vaginosis.[113,114] Earlier considered to be a mere annoyance, vaginosis is now being examined for a role in serious conditions including pelvic inflammatory disease, pregnancy-related complications (low-birth-weight babies), and increased susceptibility to AIDS infection. The organisms associated with BV include a variety of anaerobic Gram-negative rod-shaped bacteria, namely, *Gardnerella*, *Mobiluncus*, *Bacteroides*, and possibly *Fusobacterium*, *Prevotella*, Pepo*Streptococcus*, *Porphyromonas,* and *Mycoplasma* species.[115] Elucidation of pathogenic mechanisms of BV indicates a role of inflammatory cytokines and antibodies to cytolysins. These factors are not easily resolved by antibiotic treatment, thus traditional approaches to patient management like probiotic therapy are being reevaluated.[116–118] The failure of antibiotic therapy to control bacterial vaginosis reflects organisms already having ascended the uterus or the antibodies being unable to eradicate pathogen biofilms and negate their sialidase activity. Reid and Bocking,[119] along with others,[120] have drawn attention to reduced presence of *Lactobacilli* (especially those producing hydrogen peroxide) in patients with BA. Standard antibiotic therapy for BV with metronidazole is quite ineffective in that more than 30 percent of women have yeast vaginitis after therapy and more than 50 percent get a recurrent BV infection within 3 to 6 months.[121]

A study constituting 13 women showed that consumption of yogurt containing *L. acidophilus* decreased the incidence of *C. albicans* yeast infections. Hydrogen peroxide (H_2O_2) production is a key factor in resisting BV disease.[122] H_2O_2-producing strains of lactobacilli have been found in 61 percent of pregnant women with normal flora, and in only 5 percent of women with BV. The H_2O_2 has been shown to be toxic to BV-causing organisms, namely, *Gardnerella vaginalis* and *Prevotella bivia*.[115] Comparable results were obtained in open and placebo-controlled studies, in which lyophilized *L. acidophilus* was applied locally or *L. acidophilus* yogurt was given orally.[123,124] In these studies, success rates for control of bacterial vaginosis or *Candida* vaginitis ranged from 57 to 87 percent in the probiotic group and from 0 to 22 percent in the control group.[124]

Various molecular methods have shown *L. crispatus* and *L. johnsonii* to be the most common vaginal isolates from "normal" women of childbearing age.[125] The administration of *L. rhamnosus* GR-1 in combination with *L. fermentum* B-54 and RC-14 by mouth and intravaginally have been shown to be safe and to reduce the risk of UTIs, BV, and yeast vaginitis.[126] As with urogential pathogens, *Lactobacilli* ascend from the rectum into the vagina and, subsequently, alter the microenvironment and potentially modulate the immunologic status of the host such that a normal vaginal flora is more often restored and retained.[126,128]

18.3.2.5 Carcinogenesis

Colorectal cancer is the fourth most common cause of cancer morbidity and mortality worldwide (8.9 percent of all new cancers, with about 400,000 deaths/year). High incidence rates are found in Western Europe, North America, and Australia.[128] Colon cancer occurs due to somatic mutations in colon cells occurring during the lifetime of an individual. Genotoxic carcinogens including heterocyclic aromatic amines, which are formed during cooking of meat, are a potential risk factor of colon cancer in high meat consumers. These enzymes include glycosidase, β-glucuronidase, azoreductase, and nitroreductase. The protective role of probiotics and prebiotics in colon cancer has been reviewed in the past decade. At present, direct experimental evidence is lacking for suppression of cancer in humans by probiotic bacteria, but a good deal of indirect evidence has been described. Some suggested mechanisms (Figure 18.5) are (1) inhibition of carcinogens and/or procarcinogens, (2) inhibition of bacteria that convert procarcinogens to carcinogens, (3) activation of host's immune system, (4) reduction of intestinal pH to reduce microbial activity, and (5) alteration of colonic motility and transit time. *In vitro* studies have demonstrated that the cell wall of lactic acid bacteria can bind with heterocyclic amines.[27] There has been evidence that some probiotics produce butyric acid and this molecule can influence the rate of apoptosis in enterocytes. Probiotics also neutralize the activity of mutagens, such as 4-nitroquinoline-*N*-oxide, 2-nitrofluorene, and benzopyrene.[128] Some probiotics may decrease the fecal concentration of enzymes, mutagens, and secondary bile salts that may be involved in colon carcinogenesis. Kim et al.[129] assessed the anticancer activity and bacterial enzyme inhibition of *B. adolescentis* SPM0212. *Bifidobacterium adolescentis* SPM0212 inhibited the proliferation of three human colon cancer cell lines: HT-29, SW 480, and Caco-2. SPM0212 also dose-dependently inhibited TNF-α production and changes in cellular morphology. *B. adolescentis* SPM0212 inhibited harmful fecal enzymes, including α-glucuronidase, α-glucosidase, tryptophanase, and urease. Thus, *B. adolescentis* SPM0212 exerts an anticancer effect and inhibits harmful fecal enzymes.

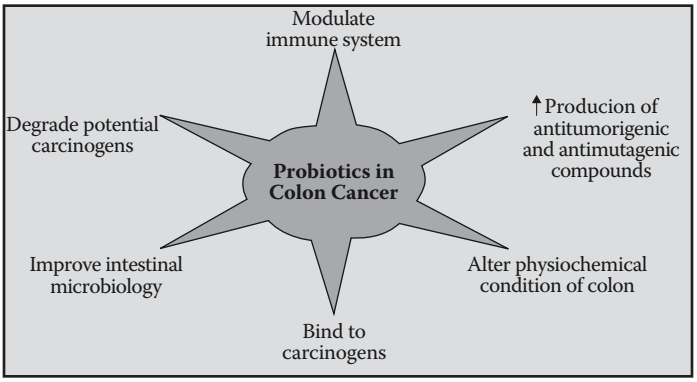

Figure 18.5 Modulatory effect of probiotics on colon cancer.

Intravesical instillation of epirubicin plus oral administration of *L. casei* preparation is a novel, promising treatment for preventing recurrence after transurethral resection for superficial bladder cancer.[130] Consumption of *L. acidophilus* 74-2 could be beneficial for the expression of cytoprotective COX-1.[131]

18.3.2.6 Hypercholesterolemia

Hypercholesterolemia has been linked with increased risk for coronary heart disease, one of the leading causes of death today. Cholesterol is a precursor to certain hormones and vitamins and is a component of cell membranes and nerve cells. However, elevated levels of total blood cholesterol or other blood lipids are considered to be a high risk factor for coronary heart disease. Cholesterol-lowering effect of lactic acid bacteria (*Streptococcus*, *Lactobacillus,* and *Bifidobacterium*) is well established.[132] To date, 11 strains of *Lactobacillus* have been found to remove cholesterol via various mechanisms and can be used as a dietary adjunct to lower serum cholesterol *in vivo*.[133] *Lactobacillus acidophilus* deconjugates bile acids into free acids that are rapidly excreted from the intestinal tract. Because free bile salts are excreted from the body, the synthesis of new bile acids from cholesterol lowers its concentration in the body. Further, it has been suggested by Andersson et al.[134] that the bile flow is stimulated by regular milk consumption (1 L/day). Isolates of *L. acidophilus* from human intestine are better able to assimilate cholesterol and actively deconjugate bile salts than commercially used cultures of *L. acidophilus*. *Lactobacillus plantarum* PH04 and *L. reuteri*. showed cholesterol-lowering activities.[132] A few human studies have suggested a decrease in serum cholesterol concentrations during consumption of very large amounts (8 L/day) of yogurt or fermented milk per day.[135] Very recently, Greany et al.[136] reported that *L. acidophilus* strain DDS-1 and *B. longum* strain UABL-14 did not produce beneficial effect on plasma lipids in 55 normocholesterolemic subjects aged 18 to 36 (33 premenopausal women and 22 men).

18.3.2.7 Dental Caries

The microflora of the oral cavity comprises more than 100 bacterial species. There are anecdotal reports of attempts to make the plaque flora on the teeth surface less cariogenic by adding harmless bacteria; however, no positive results of controlled studies have been published.[137,138] *Lactobacillus* from bio-yogurt was reported to colonize on the surface of the teeth, increasing the cariogenicity of plaque flora. Ingestion of heat-killed lactobacilli for 6 months has, however, been reported to reduce the incidence of caries by 42 percent during a 2-year follow-up.[138]

18.3.2.8 Respiratory Tract Infection

The mucosa of the respiratory tract is an appropriate area for probiotics to induce immune stimulation. In clinical studies, ingestion of *B. longum* or yogurt bacteria increased the number of macrophages in the lungs, and intranasal administration

of a *Bifidobacterium/Lactobacillus* preparation to mice stimulated immunological parameters.[139] In a double-blind, placebo-controlled, randomized trial including 371 healthy children receiving *L. rhamnosus* for 7 months, relative reduction in the number of children suffering from respiratory infections with complications and a lowering of respiratory tract infections were observed.[140]

18.3.2.9 Hypertension

In the United States, nearly 50 to 60 percent of the population is hypertensive. There is evidence to support that consumption of certain lactobacilli or milks fermented with lactobacilli may result in decreased blood pressure.[141] Studies done with hypertensive rats have shown a positive effect; studies with human subjects are limited. It is reported that fermented milk may decrease systolic pressure by 10 to 22 mm Hg. Antihypertensive effect has been demonstrated by two tripeptides formed during the growth of *L. helveticus* upon fermentation of milk. These tripeptides were shown to inhibit angiotensin-converting enzyme, a key enzyme catalyzing the conversion of angiotensin I to angiotensin II, which elevates blood pressure due to its potent vasoconstrictor effects.[142]

18.3.2.10 Kidney Stones

A high level of oxalate in the urine is a risk factor for development of kidney stones. A probiotic preparation able to degrade oxalate *in vitro* was shown to reduce oxalate fecal excretion in six patients. This result, although intriguing, is still preliminary.[143,144]

18.3.2.11 Surgical Wound Infections

Certain strains of probiotic lactobacilli and their by-products have been reported to treat or prevent (prophylactically) surgical infections.[145] Howard et al.[145] indicate that some strains of probiotics also ameliorate *Staphylococcus*-related infections of surgical implants. The wound-bed tissue of gunshot-wounded animals indicated better results with the probiotic treatment group over the antibiotic-treated group. The protective effect was indicated to be based on the natural defense mechanism activated after injury—the bacterial translocation of saprophytic bacteria from the gut to the wound.[146] *Lactobacillus plantarum* and/or its by-products showed therapeutic effects against *Pseudomonas aeruginosa* burn infections both *in vitro* and *in vivo.* There was also an improvement in tissue repair, enhanced phagocytosis of *P. aeruginosa,* and a decrease in apoptosis.[146]

18.3.2.12 Chronic Fatigue Syndrome (CFS)

CFS is a medically unexplained illness, characterized by persistent and relapsing fatigue, in addition to cognitive dysfunction, headaches, joint pains, and central nervous system disturbances.[147] Recent research indicates that there are marked

alterations in the intestinal microflora of patients with CFS, including a lowered level of bifidobacteria and small intestinal bacterial overgrowth (SIBO).[148] States of stress associated with anger and fear have been shown to be related to an increase in bacteroides, normally comprising only 2 to 4 percent of the gut flora. These bacteroides increase to 20 to 30 percent under conditions of anger and fear.[149] Although it is uncertain whether oxidative stress is a cause or effect of illness, it is becoming clear that patients with CFS have increased markers of oxidative stress and an impaired antioxidant capacity.[150,151] Some studies show that antioxidant support can allay the symptoms of CFS along with improvements in erythrocyte fragility, a marker of oxidative stress.[152] Essential fatty acid (EFA) deficiency, both viral and immune induced and/or through abnormalities of metabolism, is also reported to play a role in pathogenesis of CFS.[147] Administration of specific strains of lactic acid bacteria has been shown to help regulate the composition of the intestinal flora. Furthermore, lactic acid bacteria are reported to have the potential to act as strong antioxidants in a patient population that has been shown to be under increased oxidative stress or have compromised endogenous antioxidant capacity. They also have the potential to improve the EFA status in serum phospholipids and have been used therapeutically in the treatment of SIBO,[153] a condition common in CFS and the other so-called functional somatic disorders.

18.4 PREBIOTICS

Prebiotics are food ingredients that are largely undegraded in the small bowel and can beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria.[9] Prebiotics positively modulate mineral absorption, energy expenditure, lipid metabolism, and glucose level.[154] Prebiotics are believed to be beneficial in alleviation of osteoporosis, obesity, and diabetes.

18.4.1 Osteoporosis

Osteoporosis is a growing global problem especially so with postmenopausal women. Lifestyle changes to build peak bone mass during growth, to prevent osteoporosis as well as to treat the disease in later life, is the key area of focus.[154] Functional foods have enjoyed a niche in enhancing bone health. Enhancers of calcium absorption, such as inulin or whey proteins, that is, prebiotics, are the likely agents to be developed in the future. These agents are reported to play an important role in calcium bioavailability both in absorption and retention.[155] Consumption of 15 g oligofructose per day increased stable isotopic tracer calcium absorption from 47.8 percent in a placebo group to 60 percent. Feeding of 40 g inulin per day increased apparent calcium absorption in adults in treated group from 21.3 to 33.7 percent.[156] In a study, a total of 59 subjects were studied using a balanced, randomized, crossover design. They received oligofructose or the mixture of inulin + oligofructose for 3 weeks, and a total of approximately 1,500 mg/day dietary calcium throughout the study. Calcium absorption was significantly higher in the group receiving the inulin

+ oligofructose mixture than in the placebo group, but no significant difference was seen between the oligofructose group and the placebo group. The authors concluded that modest intakes of an inulin + oligofructose mixture increases calcium absorption in girls at or near menarche.[154]

Scholz-Ahrens et al.[5] reported that prebiotics are the most promising, but also best investigated substances with respect to a bone health-promoting potential, compared with probiotics and synbiotics.

18.4.2 Obesity and Diabetes

Although the etiology of obesity and diabetes is complex, diet clearly plays an important role both in development and management of these diseases.[11] This mainly involves food products that help in management of hunger, increase satiety, and stimulate energy expenditure. Recently, Yadav et al.[157] reported that the probiotic dahi (yogurt)-supplemented diet significantly delayed the onset of glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative stress in high fructose-induced diabetic rats, indicating a lower risk of diabetes and its complications. Ludwig et al.[158] found that high dietary fiber (prebiotics) has a protective effect against weight gain. Functional foods have also been reported to ameliorate diabetes by improving insulin sensitivity and blood glucose levels.

18.5 PROBIOTICS AS VACCINE CARRIERS

Delivery of vaccine antigens by live bacterial carriers has resulted in the elimination of humoral and cellular responses at the level of both systemic and mucosal compartments. Commensal lactic acid bacteria is being exploited to deliver vaccines and other biologically active material to the GIT.[159] Their use for vaccine delivery is of special value because they provide protection at the site of pathogen entry, and their immunomodulative effect makes them an attractive model antigen delivery vehicle or *de novo* vaccines. The advantages of lactic acid bacteria delivery includes ease of administration, survival in the gastric acid, inherent safety, particulate nature, economic technology in that the bacteria manufacture the vaccine or therapeutic agents. *Lactococcus lactis* and *L. plantarum* are being used as a means of antigen delivery for mucosal immunization. Novel high-efficacy *Lactobacillus* expression vectors have been designed to allow antigen expression intracellularly, extracellularly, or secreted and anchored to the surface.[160] These expression vectors have been used successfully to construct different *Lactobacillus*-expressing antigens, such as tetanus toxin fragment C (TTFC), several rotavirus proteins, or urease A and B subunits from *H. pylori*.[161]

Several recent publications report potential of probiotic vaccine vectors, for example, *Lactococcus lactis* prototype vaccine against *H. pylori,*[161] *Brucella abortis,*[162] *Streptococcus gordonii* prototype vaccines against HIV[163] and measles.[164] *Lactobacillus* prototype vaccines against anthrax[165] and rotavirus[166] are under development. Intrinsic immunogenicity as well as resistance to bile acid and persistence

in the GIT are several features that make *Lactobacillus* a potentially better vehicle for oral vaccination than *S. gordonii*.

Westendorf et al.[167] demonstrated the potential of *E. coli* Nissle 1917 to serve as a safe carrier for targeted delivery of recombinant proteins to the intestinal mucosa. The excellent colonization properties of the strain rendered it an ideal carrier for gut-focused *in situ* synthesis of therapeutic molecules.

Moreover, the successful phase I clinical trial with IL-10-producing *Lactococcus lactis* for Crohn's disease has opened new avenues for the use of transgenic bacteria as delivery vehicles. The major advantage of this novel strategy is the avoidance of systemic side effects associated with conventional therapies. This methodology opens up an alternative method for local delivery of therapeutic proteins to various mucosal tissues.[168]

18.6 FORMULATION OF PROBIOTICS

Probiotics are living organisms in food and dietary supplements that have beneficial health effects beyond their inherent nutritive value. A prerequisite for any effect of ingested bacteria is successful implantation in the GIT. So, bacteria must remain viable during gastric transit. Based on the acid stability, it is essential to consume these microbes with food or to protect them by encapsulation.[169] For particularly critical applications, microencapsulation technologies have been developed and successfully applied using various matrices to protect the bacterial cells from damage caused by the external environment, to improve their survival during gastroduodenal transit, and to enhance their stability profile. Microencapsulation is the process by which small particles or droplets are surrounded by a coating to produce capsules in micrometer to millimeter range known as microcapsules. The concept of microencapsulation allows the functional core ingredient (in this case the probiotic bacterium) to be separated from its environment by a protective coating. Separation of the functional core ingredient from the environment continues until the release of the functional ingredient is desired.[170]

In a broad sense, encapsulation can be used for many applications, such as stabilizing the core material, controlling the oxidative reaction, providing sustained or controlled release (both temporal and time-controlled release), masking flavors, colors, or odors, extending the shelf life, and protecting components against nutritional loss. Polymers, such as alginate, chitosan, carboxymethyl cellulose (CMC), carrageenan, gelatin, pectin, and so forth, are mainly applied, using various encapsulation technologies. Some of techniques and processes used for encapsulating probiotic microrganisms include spray drying, spray-congealing, fluidized bed coating/air suspension, extrusion-spheronization, coacervation/phase separation technique, and electrostatic method.[171] However, microencapsulation by spray drying is a well-established process that can produce large amounts of material. Nevertheless, this economical and effective technology for protecting materials is rarely considered for cell immobilization because of the high mortality resulting from simultaneous dehydration and thermal inactivation of microorganisms. Even though the viability of the

bacteria after spray drying remained low, these microparticles showed good cell protection in gastric juice and controlled release of probiotic bacteria under simulated intestinal conditions. To improve the survival of probiotics, a few approaches used are the incorporation of thermoprotectants, such as trehlose, nonfat milk solids, adnitol, granular starch, and so forth. Spherical polymer beads with diameters ranging from 0.3 to 3.0 mm and immobilizing active biomass are produced using extrusion or emulsification technique, by thermal (κ-carrageenan, gellan, agrose, gelatin) or ionotropic (alginate, chitosan) gelation of the droplets. The conventional encapsulation method, with sodium alginate in calcium chloride ($CaCl_2$), has been used to encapsulate *Lactobacillus acidophilus* to protect this organism from the harsh acidic conditions in gastric fluid. Studies have shown that calcium-alginate-immobilized cell cultures are better protected, shown by an increase in the survival of bacteria under different conditions; than they are in the nonencapsulated state. The results from these studies indicate that the viability of encapsulated bacteria in simulated gastric fluid increases with an increase in capsular size.[171,172] However, although promising on a laboratory scale, the developed technologies for producing gel beads still present serious difficulties for large-scale production of microencapsulated organisms. The use of microencapsulated probiotics for controlled-release applications is a promising alternative to solving the major problems of these organisms that are faced by industry. Even so, the challenges are to select the appropriate microencapsulation technique and encapsulating materials. To date, the research on the encapsulation of probiotics has been focused mainly on maintaining the viability of probiotic bacterial cell at low pH and high bile concentration.[171] Very recently we have developed floating beads of proboitics (*L. acidophilus*) using alginate-HPMC and studied their survival and effectiveness against ethanol-induced ulcers in rats.[173] The formulated beads showed good viability and significantly better gastro protection compared to the unformulated probiotic.

The survival of probiotics in oral solid dosage forms, such as tablets, pellets, and capsules, have also been investigated in an attempt to formulate a stable oral dosage forms. A range of compaction forces (1 to 300 MPa) were employed to investigate the susceptibility of *L. acidophilus* incorporated into lactose and a microcrystalline formulation mixture, to forces produced during tablet compression. A strong negative correlation between bacterial survival and compaction pressure was observed, suggesting that survival decreased with the increase in tablet compaction forces. However, stability testing of *L. acidophilus* formulation showed that bacteria do not remain viable after 8 to 9 days in a mixture with lactose and microcrystalline cellulose, respectively. While for extrusion, survival of Gram-positive probiotic organisms after extrusion, spheronization, and drying were significantly higher than Gram-negative organisms. The level of mortality was not affected by extrusion speed or the ratio of die length to radius. However, survival was found to be inversely proportional to extrusion pressure over the range of 1 to 8,000 kPa. Capsule filling with the *L. acidophilus*/lactose mixture was proved to be the most successful approach, as the lethal effects of drying and pressure were kept to a minimum. Furthermore, these capsules were successfully coated with an ethylcelluose/amylase colon-specific coat, without loss of bacteria viability.[169] To stabilize the lyophilized cell and to target the

release of the probiotics to the terminal ileum and the beginning of the colon in the human GIT, Chan and Zang[174] carried out compression coating of the lyophillzed cell powder, using metha-acrylic acid copolymers and pectin. *In vitro* tests further revealed that the release could occur near the end of the ileum and the beginning of the colon. The cells showed a 10^4- to 10^5-fold increase in the cell survival compared with free cells under acidic conditions.

18.7 HURDLES AND ROAD AHEAD: THE FUTURE OF PROBIOTICS

Traditional probiotic strains have a long history of safe and effective use in a range of diseases, and with each passing day they are finding new therapeutic applications, but the fact that a complete absence of risk does not exist with the use of microbial systems cannot be overlooked.[175] The reported benefits can be better realized but for the following limitations with probiotic therapy:

1. Temporary adherence: For a reasonable impact of probiotics on the intestinal microflora, relatively longer duration of adherence/contact is required. Clinical trials with probiotic strains have shown that these probiotic strains usually disappear from the GIT within a couple of weeks after the ingestion is discontinued. To achieve desired long-term effects, repeated administration is required.[175]
2. Altered metabolic activity: Probiotics especially lactobacilli are involved in deconjugation of bile salts. Fortunately, the decrease in concentration of conjugated bile acids in the small bowel due to the production of bile salt hydrolase by lactobacilli does not have clinically relevant effects on metabolism.[175]
3. Gene transfer: The risk of gene transfer is a serious concern with the use of genetically modified probiotics, especially w.r.t. the transfer of antibiotic resistance.[176]
4. Altered immunomodulation and adjuvant effects: The immunomodulating/enhancing effects of probiotics may have negative implications in patients suffering from autoimmune disorders; however, only limited instances of any disease relapse (e.g., in autoimmune hepatitis) have been reported.[176]

Industry-centered research focused on prolonging the shelf life and likelihood of survival through the intestinal tract, optimizing adhesion capacity, and developing proper production, handling,[171] and packaging procedures to ensure that desired benefits are delivered to the consumer needs to be undertaken. Gene technology will play a major role in this field for obtaining new strains with desired properties.[98]

Apart from the above aspects, the following considerations are also important for the development of efficient probiotic therapy:[175]

1. Addressing important issues like the consumer aspects, regulatory control,[27] and trade offers.
2. Interlinking the expertise and scientific knowledge on food, GIT functionality, and human health.
3. Studying the mechanism of action of probiotics in the GIT and to developing diagnostic tools and biomarkers for their assessment.

4. Developing newer production technologies to ensure the stability and viability of probiotics.[177]
5. Conducting larger controlled clinical studies to clarify optimal agents; doses; combinations of various probiotics, prebiotics, and antibiotics; and usefulness in other therapeutic conditions.[176]

Despite the problems associated with dosage, viability, lack of industrial standardization, and potential safety issues, there is a considerable potential for the development of probiotics for a wide range of clinical conditions. Some of the commercially available probiotic preparations include Allergy Research 73390 (containing 20 billion CFU of LGG), Allergy Research 72780 (a combination of *L. plantarum, L. salivarius, L. rhamnosus*), Pro-culture Gold™ (containing *L. rhamnosus*), and Pro-immune support™ (β 1,3/1, 6 glucan, *L. plantarum*).

18.8 CONCLUSIONS

With the increasing consumer awareness regarding linkage of diet and health, research in probiotics seems a highly fascinating challenge. Even though the idea that probiotics are a panacea for a multitude of diseases as yet seems farfetched, use of these agents as an adjunct to other established therapies has definitely shown potential benefits. It is a well-documented fact that probiotics can provide a stabilizing influence on the human ecosystem. Although the precise mechanism of action needs to be illustrated to provide a scientific rationale for their use, the information regarding therapeutic effectiveness can help in designing large, double-blind, controlled clinical trials. The importance of specifying the probiotic strain used during each clinical study must be emphasized within the scientific community as it may have important implications for assessing the studies and for planning future studies. Looking at the immense clinical/research data indicating the wide therapeutic applications of probiotics, it seems important to develop these agents as pharmaceuticals. A pharmaceutical scientist can help in proposing the systems, which can result in improved adhesion of probiotics in the GIT. The future of probiotics will rely on better elucidation of their mechanism of action and on maximization of their therapeutic potential, a burning challenge for zealous scientists.

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Food Science and Technology

Handbook of PREBIOTICS AND PROBIOTICS INGREDIENTS

Health Benefits and Food Applications

While there is little dispute that probiotics and prebiotics, alone and together, have been proven to promote gastrointestinal health and proper immune function, the challenge faced by researchers is finding not only the right combinations, but also finding those that are fully compatible with the formulation, processing, packaging, and distribution of functional foods. The **Handbook of Prebiotics and Probiotics Ingredients: Health Benefits and Food Applications** comprehensively explores these variables and highlights the most current biological research and food applications.

In this volume, a team of experts offers insight into the many facets of these products, describing the prebiotic, probiotic, and synbiotic applications in use today as well as those currently being studied. The book first examines the sources of prebiotics and probiotics and then describes the physiological functions of both products. The contributors discuss promising applications for a plethora of disorders, including inflammatory bowel disease, pediatric diarrhea, cancer, and various chronic diseases.

The **Handbook of Prebiotics and Probiotics Ingredients: Health Benefits and Food Applications** contains chapters contributed by experts from around the world. The book takes a global perspective, providing a thorough reference for product developers and regulatory agencies, as well as for nutritionists and forward-thinking professionals.

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