Prebiotics and the Health Benefits of Fiber: Current Regulatory Status, Future Research, and Goals[1,2]

Amy M. Brownawell,[3] Wim Caers,[4] Glenn R. Gibson,[5] Cyril W. C. Kendall,[6] Kara D. Lewis,[3]* Yehuda Ringel,[7] and Joanne L. Slavin[8]

[3]Life Sciences Research Organization, Inc., Bethesda, MD; [4]Regulatory Affairs, Beneo Group, Tienen, Belgium; [5]Food Microbial Sciences Unit, Department of Food Biosciences, The University of Reading, Reading, UK; [6]Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada; [7]Department of Medicine, School of Medicine, University of North Carolina, Chapel Hill, NC; and [8]Department of Food Sciences and Nutrition, University of Minnesota, St. Paul, MN

Abstract

First defined in the mid-1990s, prebiotics, which alter the composition and activity of gastrointestinal (GI) microbiota to improve health and well-being, have generated scientific and consumer interest and regulatory debate. The Life Sciences Research Organization, Inc. (LSRO) held a workshop, Prebiotics and the Health Benefits of Fiber: Future Research and Goals, in February 2011 to assess the current state of the science and the international regulatory environment for prebiotics, identify research gaps, and create a strategy for future research. A developing body of evidence supports a role for prebiotics in reducing the risk and severity of GI infection and inflammation, including diarrhea, inflammatory bowel disease, and ulcerative colitis as well as bowel function disorders, including irritable bowel syndrome. Prebiotics also increase the bioavailability and uptake of minerals and data suggest that they reduce the risk of obesity by promoting satiety and weight loss. Additional research is needed to define the relationship between the consumption of different prebiotics and improvement of human health. New information derived from the characterization of the composition and function of different prebiotics as well as the interactions among and between gut microbiota and the human host would improve our understanding of the effects of prebiotics on health and disease and could assist in surmounting regulatory issues related to prebiotic use.   J. Nutr. 142: 962–974, 2012.

Introduction

Adequate intake of dietary fiber is increasingly being recommended by governmental public health agencies as a means to maintain and increase health and well-being. Some epidemiological studies have shown support for an inverse relationship between dietary fiber consumption and risk of some chronic diseases (1). Developing evidence suggests that dietary fiber protects against cardiovascular disease (CVD)[9] (2–16), obesity (17–22), and type 2 diabetes (23–28). Dietary fiber is considered essential for optimal digestive health (29,30). Dietary fiber is listed on the Nutrition Facts panel. The recommended DRI of total fiber is 25 g/d for young women and 38 g/d for young men; however, a usual intake of dietary fiber in the US is only ~15 g/d (31). Manufacturers are allowed to call a food a "good source of fiber" if it contains 10% of the recommended DRI (2.5 g/serving) and an "excellent source of fiber" if the food contains 20% of the DRI (5 g/serving).

In 2002, new definitions for fiber were published by the Institute of Medicine (31). Dietary fiber is nondigestible carbohydrate and lignin that are intrinsic and intact in plants. Functional fibers are isolated nondigestible carbohydrates that have beneficial physiological effects in humans. Total fiber is the sum of dietary fibers and functional fibers. Dietary fiber includes

[9] Abbreviations used: CVD, cardiovascular disease; DGAC, Dietary Guidelines for Americans 2010 Committee; DP, degree of polymerization; EFSA, European Food Safety Authority; FOS, oligofructose; FOSHU, foods for specific health uses; GALT, gut-associated lymphoid tissue; GI, gastrointestinal; GOS, galactooligosaccharide; hBD, human β-defensin; HDL-C, HDL cholesterol; IBS, irritable bowel syndrome; LDL-C, LDL cholesterol; PYY, peptide YY; scFOS, short-chain fructoologosaccharide; UC, ulcerative colitis.

plant nonstarch polysaccharides (e.g., cellulose, pectin, gums, hemicelluloses, β-glucans, and fiber contained in oat and wheat bran), lignin, and some resistant starches. Potential functional fibers include isolated, nondigestible plant (e.g., resistant starch, pectin, and gums), animal (e.g., chitin and chitosan), or commercially produced carbohydrates (e.g., resistant starch, polydextrose, inulin, and indigestible dextrins) (31). Fibers are not degraded and absorbed in the small intestine. They are usually fermented by the microbiota of the large intestine.

Colonic Microbiota and Fermentation

The human large intestine is one of the most diversely colonized and metabolically active organs in the human body (32). More than 1000 different species of bacteria reside in the colon, with microbial populations comprising $\sim 10^{11}$–10^{12} CFU/g of contents. The colonic environment is appropriate for bacterial growth due to its slow transit time, readily available nutrients, and favorable pH (33). Generally, bacteria having an almost exclusive saccharolytic metabolism can be considered beneficial because of their metabolic function and end products. Such a metabolic function is typical for lactobacilli and bifidobacteria. Mapping the diversity of and interactions among the human intestinal microbiota is of increasing interest (34,35). The Human Gut Microbiome Initiative was initiated to obtain a more comprehensive view of the distal human gut microbial ecosystem and to serve as a model for characterizing other human microbial ecosystems such as the skin and oral cavity (36).

The composition and activity of the intestinal microbiota can influence health and disease through its involvement in nutrition, host physiological functions, and pathogenesis of certain disease conditions (37,38). The mechanisms by which the intestinal microbiota induce these effects are not completely understood, but some hypotheses and mechanisms have been suggested: 1) increasing the colonization of favorable bacteria in the colon to compete with pathogenic microorganisms for ecological niches and metabolic substrates; 2) synthesizing energy for cells of the gut wall through the fermentation of carbohydrates to SCFA, mainly butyrate, acetate, and propionate; 3) increasing stool bulking and intestinal transit; 4) modulating the immune system, especially the gut-associated lymphoid tissue (GALT); and 5) modulating gene expression and cell differentiation in the gut wall, including endocrine L-cells in the colon.

Together with the gut immune system, the colonic and mucosal microbiota significantly contribute to the intestinal mucosal barrier that prevents pathogens from invading the gastrointestinal (GI) tract. The intestinal microbiota salvages energy through fermentation of substrates not digested in the upper gut. The main substrates are dietary carbohydrates that escape digestion or absorption in the upper GI tract. These include resistant starch, nonstarch polysaccharides (e.g., celluloses, hemicelluloses, pectins, and gums), nondigestible oligosaccharides, and sugar alcohols. The main fermentation pathway generates pyruvate from hexose sugars in the undigested carbohydrate. Colonic bacteria use a range of enzymes to produce organic acids and gases. These fermentation products provide energy fuel for colonocytes and other bacteria. Dietary components that stimulate fermentation lead to an increase in bacterial mass and, consequently, fecal mass and thus have a stool-bulking effect.

Fermentation and especially SCFA production play a multifaceted role at both the colonic and systemic levels (39). Colonic epithelial cells preferentially use butyrate as an energy source, even when competing substrates, such as glucose and glutamine,

are available. Butyrate is considered to be a key nutrient determining the metabolic activity and growth of colonocytes and may function as a primary protective factor against colon cancer and ulcerative colitis (UC) (40,41). SCFA are water soluble and are absorbed into the blood stream. Acetate is found in portal blood and passes through the liver. Acetate metabolism occurs in organs, including the brain (42), heart, and skeletal muscles (43,44). In contrast, propionate is mainly utilized as a gluconeogenic substrate in the liver (45) and may lower the hepatic production of cholesterol by interfering with its synthesis (46). Transport to and further metabolism of SCFA in the liver, muscle, or other peripheral tissues is thought to contribute ~ 7–8% of host daily energy requirements (33). Fermentation and SCFA production are also thought to inhibit the growth of pathogenic organisms by reducing luminal pH (47). A low pH reduces formation of toxic compounds such as ammonia, amines, and phenolic compounds from peptide degradation (48) and decreases the activity of undesirable bacterial enzymes (49).

GALT represents the largest mass of lymphoid tissue in the body. Approximately 25% of the intestinal mucosa consists of lymphoid tissue (50). About 60% of the total Ig produced daily is secreted into the GI tract (32). Immune responses that start in the gut have the potential to affect immune responses at other mucosal surfaces. The GI microflora provide the primary antigenic stimulus responsible for the migratory pathway and maturation of precursor lymphoid cells in the Peyer's patches of the GALT. Some prebiotics decrease the numbers of lymphocytes and/or leukocytes in GALT (51–53). Prebiotics may have direct or indirect effects on the immune system and may induce changes in the number of microbial genus or species. The colonic microbiota is the major stimulus for specific immune responses at local and systemic levels. Changes in the colon toward SCFA-producing bacteria may alter the pathogen-associated molecular patterns in the intestinal lumen (54), resulting in activation of NF-κB and secretion of proinflammatory cytokines (55). Abnormal intestinal response to a foreign antigen as well as local inflammatory reactions might, as a secondary event, induce impairment of colonic barrier and function.

Overall, a number of factors influence the composition of the microbiota. These include changes in physiological conditions of the host (e.g., age, stress, and health status) and environmental circumstances (e.g., diet and antibiotic therapy). Recognition of the health-promoting properties of certain gut microorganisms has encouraged dietary-based interventions to provide an optimal environment for beneficial microbiota composition and metabolism.

Prebiotic Fibers

Prebiotics were first 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" (56). This definition was later refined to include other areas that may benefit from selective targeting of particular microorganisms (57): "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits." Currently, the target genera are lactobacilli and bifidobacteria; however, prebiotic success has primarily been achieved with bifidobacteria. This may be due to the fact that more bifidobacteria usually reside in the human colon than lactobacilli and they exhibit a preference for oligosaccharides.

In 2010, the International Scientific Association for Probiotics and Prebiotics working group defined dietary prebiotics as "selectively fermented ingredients that result in specific changes,

TABLE 1 Criteria for characterization of an ingredient as a prebiotic[1]

Characterization of prebiotics
Resists gastric acidity, hydrolysis by mammalian enzymes, and absorption in the upper GI tract
Fermented by the intestinal microbiota
Selectively stimulates the growth and/or activity of intestinal bacteria potentially associated with health and well-being

[1] Adapted with permission from (56,57). GI, gastrointestinal.

in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" (32). Although all prebiotics can be classed as fibers, not all fibers are prebiotic. **Table 1** lists the criteria that must be scientifically demonstrated in order for an ingredient to be classified as a prebiotic (56,57). The latter of these criteria is what separates prebiotics from more traditional fibers.

Some prebiotics (i.e., inulin) occur naturally in several foods such as leeks, asparagus, chicory, Jerusalem artichokes, garlic, onions, wheat, oats, and soybeans (58). Their consumption in the typical U. S. and European diets has been estimated to be several grams per day (58,59). Many frequently eaten foodstuffs have been fortified with prebiotic ingredients, such as inulin, oligofructose (FOS), galactooligosaccharides (GOS), and other potential prebiotic candidates. Inulin, FOS, and GOS have been evaluated by the FDA and confirmed as "safe" (60–64). Studies conducted to evaluate potential toxic effects in animals and humans have revealed no or only mild adverse effects, including infrequent bloating, flatulence, and soft stools following ingestion of large quantities (65). These effects have also been reported after consuming dietary fibers in general (66). In practice, user concentrations of prebiotics (typically 2–4 g/serving) are far below the amounts at which intestinal discomfort occurs. Moreover, it is hypothesized that the prebiotic-induced increase in bifidobacteria may reduce these adverse effects, because this genus does not produce gas as part of its metabolism (67). The energy value of nondigestible oligosaccharides has been estimated between 1 and 2 kcal/g (68–70).

Prebiotics as Functional Foods

If added to many foods, including yogurts, cereals, breads, cookies, ice cream, spreads, drinks, and supplements, prebiotics can be considered a subcategory of functional food ingredients. According to The European Commission Concerted Action on Functional Food Science in Europe, functional foods are characterized by the following (71): *1*) conventional/everyday food or food ingredient; *2*) naturally occurring in foods; *3*) proven beneficial effect(s) on target functions beyond nutritive value/basic nutrition; and *4*) convincing human nutrition intervention studies demonstrating enhanced well-being and health and/or reduced risk of a disease and/or improved quality of life including physical, psychological, and behavioral performances.

Furthermore, a functional food must remain food and it should demonstrate its effects in amounts that can normally be expected to be consumed in the diet. It is not a pharmaceutical but part of the normal food pattern (71).

Disease Risk Reduction and Prebiotic Intake

After considering the relationship between dietary fiber and selected health outcomes, the Dietary Guidelines for Americans

2010 Committee (DGAC) reached the conclusion that "A moderate body of evidence suggests that dietary fiber from whole foods protects against cardiovascular disease, obesity, and type 2 diabetes and is essential for optimal digestive health" (30).

Thus far, health outcome data for prebiotic intake are substantially more limited than for dietary fiber in general. The suggested benefits of prebiotic intake are listed in **Table 2**.

Prospective and observational studies are being conducted to test these hypotheses and other typical fiber-related health outcomes.

GI infection and inflammation
Infectious diarrhea. In a study of 244 healthy study participants traveling to high- or medium-risk destinations for traveler's diarrhea, 10 g/d inulin ingested 2 wk prior to travel and 2 wk during travel reduced the prevalence and resulted in less severe attacks of diarrhea (72). A recent study assessed the effectiveness of a prebiotic GOS (B-GOS, Bimuno, Clasado) on the incidence and severity of travelers' diarrhea in 159 healthy participants (73). Either 5.5 g/d GOS or placebo (maltodextrin) was consumed 1 wk prior to travel and for the duration of travel to a country with a low or high risk for travelers' diarrhea. Significant differences (all $P < 0.05$) were observed between the GOS and placebo groups in the incidence of diarrhea (mean = 19 study participants vs. 30 study participants) and the duration of travelers' diarrhea (mean $\pm$ SD = 2.37 $\pm$ 2.06 d vs. 4.57 $\pm$ 3.03 d); there were similar findings for the duration of abdominal pain (mean $\pm$ SD = 2.00 $\pm$ 1.99 d vs. 3.53 $\pm$ 2.58 d) and in an overall quality of life assessment (mean score/d $\pm$ SD = 62.37 $\pm$ 5.51 vs. 53.12 $\pm$ 3.96).

Clostridium difficile-induced diarrhea. In a randomized, controlled study of 142 study participants, daily ingestion of 12 g/d FOS for 30 d resulted in fewer relapses with *C. difficile*-induced diarrhea compared with placebo (8.3% FOS vs. 34.3% placebo; $P < 0.01$; $\chi^2 = 14.35$) (74). Participants were concurrently treated with an antibiotic for 30 d and followed for an additional 30 d. Stool culture confirmed the prebiotic effect of FOS with an increase in fecal bifidobacteria [at baseline: 8.68 $\log_{10}$ cfu/g to discharge 9.37 $\log_{10}$ cfu/g ($P < 0.0001$; 95% CI: 0.45, 0.94), at 30 d: 9.64 $\log_{10}$ cfu/g ($P < 0.0001$; 95%CI: 0.74, 1.18), and at 60 d: 9.42 $\log_{10}$ cfu/g ($P < 0.0001$; 95%CI: 0.56, 0.93)]. For study participants given the placebo, bifidobacteria increased from baseline to discharge (8.65 $\log_{10}$ vs. 8.84 $\log_{10}$ cfu/g; $P = 0.027$; 95%CI: 0.024, 0.35) (74).

Inflammatory bowel disease
One randomized, double-blind, crossover, placebo-controlled study of 20 patients with pouchitis demonstrated that, compared with placebo, 24 g/d inulin for 3 wk reduced both endoscopic scores (mean $\pm$ SEM = 0.95 $\pm$ 0.22 inulin vs. 1.47 $\pm$ 0.32

TABLE 2 Previously suggested health benefits of prebiotic intake[1]

Health benefits of prebiotic intake
Reduced prevalence and duration of infectious agents and antibiotic-associated diarrhea
Reduced inflammation and symptoms associated with inflammatory bowel disease
Protective effects against colon cancer
Enhanced bioavailability and uptake of minerals, including calcium, magnesium, and possibly iron
Lowered risk factors for CVD
Promotion of satiety and weight loss and prevention of obesity

[1] CVD, cardiovascular disease.

placebo; $P = 0.04$) and histological pouchitis inflammation scores (mean $\pm$ SEM = 2.11 $\pm$ 0.14 inulin vs. 2.61 $\pm$ 0.26 placebo; $P = 0.04$), lowered fecal pH (mean $\pm$ SEM = 5.33 $\pm$ 0.12 inulin vs. 5.62 $\pm$ 0.10 placebo; $P = 0.02$), and increased fecal butyrate concentration (mean $\pm$ SEM = 18.9 $\pm$ 3.76 inulin vs. 11.7 $\pm$ 2.08 mmol/g wet feces placebo; $P = 0.01$). Concentrations of the secondary bile acids deoxycholic acid (mean $\pm$ SEM = 0.11 $\pm$ 0.06 inulin vs. 0.40 $\pm$ 0.13 μmol/g wet feces, placebo; $P = 0.01$) and ursodeoxycholic acid (mean $\pm$ SEM = 0.07 $\pm$ 0.02 inulin vs. 0.18 $\pm$ 0.05 μmol/g wet feces, placebo; $P = 0.04$) were reduced as were concentrations of *Bacteroides fragilis* (mean $\pm$ SEM = 6.77 $\pm$ 0.47 inulin vs. 7.68 $\pm$ 0.28 $\log_{10}$ cfu/g wet feces, placebo; $P = 0.02$) in fecal samples (75). However, these effects were not associated with significant differences in clinical symptoms between the inulin and placebo periods. A double-blind, randomized controlled study of 18 patients with active UC treated with a mixture of the probiotic *Bifidobacterium longum* and a prebiotic (Synergy 1, containing 6 g of fructo-oligosaccharide/inulin mix), showed a trend toward improved UC symptomology and a greater concentration of gut bifidobacteria (76). The authors also reported reduced sigmoidoscopy scores with the prebiotic and probiotic mixture [start = 4.5 $\pm$ 1.4 and end = 3.1 $\pm$ 2.5] compared to placebo [start = 2.6 $\pm$ 2.1 and end = 3.2 $\pm$ 2.2]. Differences between groups were of borderline significance ($P = 0.06$). The authors reported reductions in TNF ($P = 0.0177$), IL-1α ($P = 0.0051$), and reduced inflammation and regeneration of the epithelial tissue after treatment with the mixture compared with placebo [mean reduction in histological score for the mixture [start = 1.7 (1.4), end = 1.1 (1.2)] and the mean increase in histological score for the placebo [start = 0.9 (0.9), end = 1.9 (1.1)]. Significant differences in mRNA concentrations for human β-defensins (hBD) 2, 3, and 4, which are actively unregulated in UC (hBD2, $P = 0.0156$; hBD3, $P = 0.0379$; and hBD4, $P = 0.0078$), were reported after treatment with the mixture.

A randomized, double-blind, placebo-controlled study showed that 15 g/d FOS administered for 4 wk to individuals with Crohn's disease was ineffective at inducing clinical remission compared with administration of a placebo to individuals with Crohn's disease [FOS group ($n = 6$, 11%) and placebo group ($n = 10$, 20%; $P = 0.19$] by intention-to-treat analysis or by per-protocol treatment analysis [FOS group ($n = 6$, 15%) vs. placebo group ($n = 10$, 22%; $P = 0.40$)] (77). Treatment with FOS did not enhance bifidobacteria 9.4 (0.6) $\log_{10}$ cells/g dry feces compared with placebo [9.3 (0.8) $\log_{10}$ cells/g dry feces; $P = 0.20$]. The study also showed that FOS decreased the proportion of IL-6–positive dendritic cells in the lamina propria [FOS group (mean $\pm$ SD) = 29 $\pm$ 12 – -32 ± 15, $P = 0.036$; and placebo group (mean $\pm$ SD) = 32 $\pm$ 18 – -28 ± 15; $P = 0.46$] and enhanced the dendritic cell staining of IL-10 from baseline to wk 4 [FOS group: median (IQR) intensity ratio increased 1.3 (0.6) – 2.0 (1.6); $P = 0.035$)]; however, no difference was observed in patients who were given the placebo [median (IQR) intensity ratio 1.4 (1.4) – 1.6 (1.0); $P = 0.66$]. The production of IL-12p40 did not change.

In a small, randomized, double blind, placebo-controlled pilot study, participants with active UC were administered either 12 g/d of a FOS and inulin mixture (Beneo Synergy 1, a combination of long-inulin chains together with shorter FOS chains from chicory root) or a placebo for 2 wk (78). Both the prebiotic and the placebo produced significant reductions in disease activity from baseline to d 14 (mean $\pm$ SE for the prebiotic group = 8.9 $\pm$ 0.52 vs. 4.1 $\pm$ 0.4 and the placebo group = 8.3 $\pm$ 0.37 vs. 6.4 $\pm$ 0.63) ($P < 0.05$); however, disease activity did not

significantly differ between groups. All participants in the test group achieved clinical remission (score <6); however, 2 study participants in the placebo group had clinical activity. Consumption of the prebiotic also significantly decreased the concentration of calprotectin, an abundant neutrophil protein that is markedly elevated in patients with inflammatory bowel disease (d 0: 4377 $\pm$ 659 μg/g, d 7: 1033 $\pm$ 39 μg/g, d 14: 1211 $\pm$ 449 μg/g; $P < 0.05$ vs. d 0); however, consumption of the placebo did not elicit a significant effect (d 0: 5834 $\pm$ 1563 μg/g, d 7: 4084 $\pm$ 1395 μg/g, d 14: 3740 $\pm$ 2198 μg/g; P value not reported). Data reported for d 0 included 9 prebiotic and 9 placebo patients; for d 7, 8 prebiotic and 8 placebo patients; and at d 14, 7 prebiotic and 8 placebo patients.

Functional bowel disorders

Recently, the efficacy of *trans*-GOS in changing the colonic microbiota and improving symptoms was investigated in a randomized, controlled patient-blinded crossover trial in 44 patients with Rome II positive irritable bowel syndrome (IBS) (79). The study included a 2-wk baseline period and two 4-wk treatment periods separated by 2-wk washout phases. *Trans*-GOS administration for 4 wk enhanced fecal bifidobacteria at 3.5 g/d [mean $\pm$ SD bacterial proportion at the beginning of treatment (b) vs. at the end of treatment (e) = 3.25 $\pm$ 0.51 (b) vs. 5.51 $\pm$ 0.43 (e); $P < 0.005$] and at 7 g/d [mean $\pm$ SD = 3.01 $\pm$ 0.38 (b) vs. 7.48 $\pm$ 0.59 (e); $P < 0.001$]. There were differences in bifidobacteria concentrations at the end of treatment between placebo and 3.5-g/d prebiotic treatment ($P < 0.05$) and between placebo and 7.0-g/d prebiotic treatment ($P < 0.001$). Placebo treatment had no effect on the group who were also given 3.5 g/d prebiotic [mean $\pm$ SD = 2.99 $\pm$ 0.45 (b) vs. 2.95 $\pm$ 0.27 (e)] or the group who also received 7.0 g/d prebiotic. *Trans*-GOS at 3.5 g/d given for 4 wk also changed (all $P < 0.05$) stool consistency [mean $\pm$ SD = 4.4 $\pm$ 1.4 (b) vs. 3.8 $\pm$ 1.0 (e)], improved flatulence [mean $\pm$ SD = 2.0 $\pm$ 0.6 (b) vs. 1.3 $\pm$ 0.6 (e)], decreased bloating [mean $\pm$ SD = 4.1 $\pm$ 1.3 (b) vs. 2.8 $\pm$ 0.9 (e)], improved the rating on the composite Likert scale [mean $\pm$ SD = 9.9 $\pm$ 6.2 (b) vs. 6.2 $\pm$ 4.3 (e)], and improved subjective global assessment scores [mean $\pm$ SD = 4.2 $\pm$ 0.5 (b) vs. 3.1 $\pm$ 0.8 (e)]. *Trans*-GOS, at 7 g/d, significantly improved subjective global assessment scores [mean $\pm$ SD = 4.1 $\pm$ 0.8 (b) vs. 3.6 $\pm$ 0.9 (e)] and anxiety scores [mean $\pm$ SD = 9.7 $\pm$ 4.3 (b) vs. 7.8 $\pm$ 4.6 (e) (both $P < 0.05$)]. The effects were apparent in diarrhea and constipated and alternating forms of IBS.

Colon cancer

Human studies have yielded inconsistent effects of prebiotics on biomarkers of colon cancer (80–82). The largest well-designed U.S. study of 89,000 nurses examining the association between dietary fiber consumption and colon cancer found no correlation (83). More research is needed to evaluate the role of prebiotics in protecting against colon cancer in humans.

Bioavailability and uptake of calcium

Calcium absorption is enhanced with prebiotic intake, mainly fructans (84–86). These effects may be due to lowered luminal pH resulting from SCFA production by bifidobacteria after prebiotic fermentation. This reduced pH shifts calcium speciation and solubility such that bioavailability increases. Inulin-type fructans and other nutrients may also synergize to improve calcium absorption (87); crude fractions of chicory improved bone characteristics such as total bone mineral content and diaphysal bone mineral density of femurs relative to native inulin, reformulated inulin, and control for total bone mineral

content (means ± SEM = 0.706 ± 0.047 g vs. 0.688 ± 0.084 g, 0.671 ± 0.057 g, and 0.646 ± 0.056 g, respectively; P = 0.041) and for diaphyseal bone mineral density (g/cm^2) (means ± SEM = 0.273 ± 0.005 vs. 0.274 ± 0.006, 0.269 ± 0.006, and 0.264 ± 0.004, respectively; P = 0.026) in rats. A 12-mo study of 100 adolescents ingesting 8 g/d short- and long-chain inulin fructans showed a significant increase in calcium absorption that led to greater bone mineral density (88). Oral ^{42}Ca and i.v. ^{46}Ca absorption have also been quantified in young adults following 8 wk of supplementation with 8 g of inulin/FOS (89). Calcium absorption increased at least 3% in young adults with a mean calcium intake of 900 mg/d. Absorption increased 22.7 ± 11.3 to 31.0 ± 15.3%, with colonic absorption representing 69.6 ± 18.6% of the increase or a total of 49 ± 28 mg/d.

CVD

Despite consistent evidence from prospective epidemiologic studies showing that dietary fiber exerts a protective effect against CVD, the components of dietary fiber that exert this effect have not been well defined. The Institute of Medicine (31) concluded that cereal fibers and viscous functional fibers, such as gums and pectins, are most effective. Soluble and viscous fibers appear to favorably alter biomarkers of CVD, including LDL cholesterol (LDL-C) and C-reactive protein. Evidence suggests that high-fiber, vegetable-based diets can dramatically reduce LDL-C concentrations in a manner comparable to statin drugs (90–92). Whether all isolated, functional fibers protect against CVD is unclear. The U.S. FDA allows health claims for soluble fiber from oats, barley, and psyllium (1).

A prospective study examined the effect of consuming 18 g/d of inulin on the serum lipid profiles of hypercholesterolemic men and women (n = 21) (93). Although differences in responses between inulin and control periods were significant (P < 0.05) for LDL-C (−14.4%) and total cholesterol (−8.7%), the variable lipid responses in the crossover treatment groups suggest a need for additional research. A double-blind, randomized, placebo-controlled study examined the lipid-modifying ability of 10 g/d inulin/FOS administered for 6 mo to 17 normolipidemic participants who consumed their normal diet and did not modify their habits (94). Compared with placebo, inulin/FOS had no effect on plasma TG concentrations and hepatic lipogenesis and induced only a nonsignificant trend for reduced plasma total and LDL-C concentrations and a higher HDL cholesterol (HDL-C) concentration.

A recent randomized, controlled, crossover study of 23 hyperlipidemic adults investigated whether prebiotics could potentiate the cholesterol-lowering effect of soy (95). Participants completed three 4-wk diet intervention phases: a low-fat dairy diet plus 10 g/d FOS-enriched inulin, a 30-g/d soy food diet with 61 mg/d isoflavones from soy foods plus 10 g/d placebo (maltodextrin), and a soy food-containing diet plus 10 g/d prebiotic. Intake of soy with the prebiotic resulted in a greater decrease in LDL-C (−0.18 ± 0.07 mmol/L; P = 0.042) and in the LDL-C:HDL-C ratio (−0.28 ± 0.11; P = 0.041) compared with prebiotic. HDL-C increased with soy plus prebiotic consumption compared with prebiotic alone (0.06 ± 0.02 mmol/L; P = 0.029). The authors suggested that co-ingestion of a prebiotic may increase the effectiveness of soy foods as part of the dietary strategy to lower serum cholesterol.

Obesity, satiety, and weight loss

Some human studies have reported that obesity is associated with elevated concentrations of Firmicute bacteria and reduced concentrations of the Bacteroidetes phylum (96–98). Other studies have not supported these findings or have indicated that other groups of bacteria are associated (99–101). Armougam et al. (99) determined that compared with lean participants, obese participants had reduced fecal concentrations of Bacteroidetes [mean = 1.35×10^{10} bacteria/(archaea copies · g of feces) (lean) vs. 3.76×10^9 bacteria/(archaea copies · g of feces) (obese); P < 0.01] and higher concentrations of Lactobacillus [mean = 2.77×10^6 bacteria/(archaea copies · g of feces) (lean) vs. 4.38×10^7 bacteria/(archaea copies · g of feces) (obese); P < 0.0197]. Another study reported that obese individuals had a reduced relative proportion of Bacteroidetes (P < 0.001) and an elevated relative proportion of Firmicutes (P = 0.002) compared with lean controls prior to being placed on either a fat- or carbohydrate-restricted diet. Following either a carbohydrate- or fat-restricted diet increased the relative abundance of fecal Bacteroidetes (P < 0.001) and decreased the abundance of Firmicutes (P = 0.002) (98). A study conducted on adult female monozygotic and dizygotic twin pairs concordant for leanness or obesity and their mothers showed that obesity was associated with phylum-level changes in microbiota, decreased bacterial diversity, and changed representation of genes and metabolic pathways (97). Obese participants had lower proportions of Bacteroidetes (P = 0.003), an elevated proportion of Actinobacteria (P = 0.002), no difference in Firmicutes (P = 0.09), and reduced species diversity compared with lean twins, suggesting that deviations from a core microbiome may be associated with different physiologic states (obese vs. lean). Nadal et al. (96) showed that weight loss was associated with a change in gut microbial composition of obese adolescents. Weight loss resulted in reduced concentrations of Clostridium histolyticum (Firmicutes division) and E. rectale-C. coccoides. Weight loss >4 g resulted in increased concentrations of the Bacteroides-Prevotella group [median proportions of bacterial cells hybridizing with specific group probes to total bacteria hybridizing with EUB probe 338 and ranges before intervention = 2.51 (6.92–1.13) and after intervention = 3.09 (16.14–0.93); P = 0.047] and decreased proportions of C. histolyticum [before intervention = 5.38 (13.04–2.02) and after intervention = 2.95 (13.12–0.47); P = 0.011], C. lituseburense [before intervention = 2.53 (17.3–0.33) and after intervention = 1.45 (17–0.16); P = 0.049], and E. rectale/C. coccoides [before intervention = 7.51 (19.4–1.53) and after intervention = 4.55 (20.57–0.51); P = 0.033]. Santacruz et al. (102) reported that a reduction in body weight of >4 kg for overweight adolescents was related to increased counts of the B. fragilis [median and IQR of cell number/g of fecal samples: before intervention = 7.6 (6.7–8.2) log cells/g fecal sample vs. after intervention = 8.6 (8.1–9.3) log cells/g fecal sample; P = 0.001] and Lactobacillus groups [median and IQR of cell number/g of fecal samples: before intervention = 6.4 (5.9–6.9) and after intervention = 7.0 (6.3–7.1); P = 0.007) and decreased counts of the C. coccoides group [median and IQR of cell number/g of fecal samples: before intervention = 8.6 (8.3–9.0) vs. after intervention = 7.7 (7.4–8.5); P = 0.001]. In another study, 16 overweight pregnant women at 24 wk of pregnancy compared with 34 normal-weight pregnant women had lower numbers of Bifidobacterium [median and IQR of bacterial numbers (log genome equivalents/g feces): overweight pregnant women = 8.36 (7.74–8.57) vs. normal-weight pregnant women = 9.10 (8.53–9.52); P > 0.001] and Bacteroides [median and IQR of bacterial numbers (log genome equivalents/g feces): overweight pregnant women = 6.20 (6.00–6.66) vs. normal-weight pregnant women = 6.88 (6.21–7.23); P = 0.035, respectively] and elevated numbers of Staphylococcus [median and IQR of bacterial numbers (log genome equivalents/g feces): overweight

pregnant women = 5.78 (4.83–6.37) vs. normal-weight pregnant women = 4.40 (3.94–4.74); $P = 0.006$], *Enterobacteriaceae* [median and IQR of bacterial numbers (log genome equivalents/g feces): overweight pregnant women = 7.23 (6.65–7.90) vs. normal-weight pregnant women = 6.37 (6.10–6.76); $P = 0.001$] and *E. coli* [median and IQR of bacterial numbers (log genome equivalents/g feces): overweight pregnant women = 6.20 (5.50–7.14) vs. normal-weight pregnant women = 5.17 (4.68–5.70); $P = 0.005$] (103). Women who experienced excessive weight gain during pregnancy had higher *E. coli* concentrations than women with normal weight gain [median and IQR of bacterial numbers (log genome equivalents/g feces): excessive weight gain = 6.25 (5.06–8.08) vs. normal weight gain = 5.26 (4.70–5.94); $P = 0.045$] and lower concentrations of *Akkermansia muciniphila* (median and IQR of bacterial numbers (log genome equivalents/g feces): excessive weight gain = 8.12 (6.52–8.50) vs. normal weight gain = 8.54 (7.90–9.50); $P = 0.020$]. Duncan et al. (101) reported no difference between the proportion of fecal Bacteroides in lean and obese study participants (21 vs. 27.2%; SED = 2.96; $P = 0.08$) when obese participants consumed weight maintenance diets and no significant relationship between weight loss and the proportion of Bacteroides in total fecal bacteria for obese participants compared with lean participants ($R^2 = 0.08$, $P = 0.11$). They reported that weight loss resulted in decreases in the Roseburia + *E. rectale* bacteria of the *C. coccoides* group of Firmicutes for obese individuals ($P < 0.001$) and bifidobacteria when participants consumed weight loss diets [low carbohydrate, ketogenic and high protein, moderate-carbohydrate, nonketogenic] compared with the maintenance diet: (1.87 and 2.09%, SED = 0.88, respectively, $P < 0.037$).

Schweirtz et al. (100) examined microbial composition and SCFA production in lean and overweight study participants. They reported that obese participants had a higher mean total amount of SCFA in their fecal samples (103.9 ± 34.3 vs. 84.6 ± 22.9 mmol/L; $P = 0.024$) than lean participants, increased propionate (41%; 19.3 ± 8.7 vs. 13.6 ± 5.2 mmol/L; $P = 0.002$), and a nonsignificant increase in butyrate (28%; 18.1 ± 10.0 vs. 14.1 ± 7.6 mmol/L; $P = 0.10$). In addition, they reported a lower Firmicutes:Bacteroidetes ratio in overweight (1.1 vs. 3.3; $P = 0.001$) and obese (1.2 vs. 3.3; $P = 0.005$) study participants compared with lean participants. Obese study participants had a lower proportion of Methanobrevibacter compared with lean participants [6.2 ± 3.24 vs. 8.0 ± 3.92 median $\log_{10}$ cells/g of feces (dry weight ± SD); $P = 0.018$].

A limited number of studies have investigated the effect of prebiotics on obesity, satiety, and weight gain in humans. One 2-d study reported no added effect of FOS, β-glucan, or a combination on appetite or energy intake (104). Study participants consumed a meal replacement bar at breakfast containing 0.3 g β-glucan (control) or bars containing an additional 0.9 g β-glucan (barley group), 8 g FOS (FOS group), or 0.9 g β-glucan + 8 g FOS (barley + FOS group), an ad libitum lunch 4 h later, and the same type of bar for a snack 2 h after lunch on d 1. On d 2, participants consumed the bar only at breakfast. On d 1, energy intakes for the control, barley, FOS, and FOS + barley groups were 629, 624, 655, and 651 g (SE = 49), respectively. On d 2, energy intake values were 595, 608, 612, and 590 g (SE = 44), respectively. The authors reported no significant effect of on appetite ratings or food intake.

In another study, 33 study participants were given 3 different meal challenges 7 d apart after an overnight fast (105). The meal challenges were a conventional, full-fat sausage patty, a patty in which one-half the fat in the conventional patty was replaced with inulin, and a patty with one-half the fat replaced by lupin-kernel

fiber, an insoluble nondigestible carbohydrate. Participants had reduced 24-h energy intake after partially replacing fat in a sausage patty with 24 g inulin [control, inulin-fiber (df 32; $P = 0.039$]. There was a strong trend toward lower energy intake on the day of consumption of lupin kernel fiber patties (df 32; $P = 0.053$) compared with the day of consumption of the full-flavored patty breakfast.

A randomized, double-blind, placebo-controlled trial was conducted to examine the effects of FOS supplementation on body weight and satiety hormone concentrations in overweight and obese adults (106). Forty-eight otherwise healthy adults with a BMI (in kg/m^2) >25 received 21 g FOS/d or placebo (maltodextrin) for 12 wk. The FOS-supplemented group had a reduction in body weight of 1.03 ± 0.43 kg, whereas the control group had an increase in body weight of 0.45 ± 0.31 kg over 12 wk ($P = 0.01$). A lower AUC for ghrelin (23% decrease; $P = 0.004$) and a higher AUC for peptide YY (PYY) were reported with FOS (13% increase; $P = 0.03$) for initial values compared to values at the end of the study. Suppressed ghrelin and enhanced PYY may contribute to the reduction in energy intake. Similar results were obtained in a randomized, double-blind, parallel, placebo-controlled trial of 10 healthy adults who received either 16 g/d fructan prebiotics or 16 g/d dextrin maltose for 2 wk (107). Prebiotic treatment increased breath hydrogen excretion, a marker of gut microbiota fermentation (increased fermentation at 30 min and 120 min; $P < 0.05$), by ~3-fold and compared with the placebo, there was a time × treatment effect ($P = 0.0156$). The prebiotic also reduced hunger visual analog scale scores at 180 min compared with placebo (time × treatment, $P = 0.0147$). Prebiotics increased plasma glucagon-like peptide 1 (treatment × time interaction, $P = 0.038$) and PYY concentrations (treatment × time interaction, $P = 0.0498$); however, postprandial glucose AUC decreased after the standardized meal prebiotic treatment (treatment × time interaction, $P = 0.05$).

A single-blind, crossover, placebo-controlled study investigated the effect of FOS supplementation on satiety in men and women with BMI ranging from 18.5 to 27.4 kg/m^2 (108). Study participants were given 16 g/d FOS or placebo for 2 wk followed by a 2-wk washout phase and the other treatment for 2 wk. Consumption of FOS enhanced satiety during breakfast and dinner ($P = 0.04$) and decreased hunger ($P = 0.04$) and prospective food consumption after dinner ($P = 0.05$). Energy intakes at breakfast and lunch were lower after FOS supplementation than after supplementation with the placebo [breakfast: percentage from placebo vs. percentage from FOS = 100 vs. 91 ± 3.3; $P < 0.01$; lunch: percentage from placebo vs. percentage from FOS (mean ± SEM) = 100 vs. 89.5 ± 3; $P < 0.05$]. In another study, children were given 8 g/d of a 1:1 mixture of FOS and long-chain inulin for 12 mo or a placebo (89). The prebiotic group had no increase in BMI Z-score (mean ± SEM = 0.03 ± 0.01; $P = 0.30$); however, the BMI Z-score for the control group increased (mean ± SEM = 0.13 ± 0.04; $P = 0.001$).

In a randomized, double-blind, crossover study, Hess et al. (109) investigated the effect of consumption of short-chain fructooligosaccharides (scFOS) on satiety. Healthy men and women with a BMI of 18–27 kg/m^2 adhered to a low-fiber lead-in diet 24 h prior to testing on 3 different occasions. Men were tested 1 wk apart and women were tested 2–3 wk apart. Participants were given a hot cocoa beverage containing 0, 5, or 8 g of scFOS with breakfast and 3 solid chocolate-flavored candies containing the same dose as a pre-dinner snack. Computerized visual analogue scales were used to report satiety, hunger, fullness, and prospective food consumption at baseline

and 15, 30, 45, 60, 90, 120, 180, and 240 min from baseline. Breath hydrogen samples were obtained at baseline and 240 min postintervention. Food consumption was recorded for 24 h after the start of each visit. scFOS elicited a dose-dependent increase in breath hydrogen, a marker of fermentation (0, 10, and 16 g scFOS = -0.4 ± 1.6, 5.8 ± 1.6, and 14.9 ± 1.6 ppm, respectively; $P < 0.05$), but these doses had no effect on the AUC for hunger (207 ± 15, 219 ± 15, and 222 ± 15; P-trend = 0.12), satisfaction (167 ± 15, 155 ± 15, and 148 ± 15; P-trend = 0.06), or fullness (166 ± 17, 151 ± 17, and 149 ± 17; $P = 0.10$). There was a significant difference in prospective food intake between the control (232 ± 17) and 16-g FOS (251 ± 17) groups (P-trend = 0.026). There was no effect on food intake at 0, 10, and 16 g scFOS (1034 ± 58, 1040 ± 58, and 1024 ± 58 kcal; P-trend = 0.86) at lunch. During the rest of the day, high-dose scFOS was associated with decreased food intake in women (1261 ± 161 vs. 891 ± 160 kcal; $P = 0.037$) and increased food intake in men compared with placebo (1613 ± 159 vs. 1259 ± 160 kcal; $P = 0.05$).

Regulatory Agency and Public Health Positions on Prebiotics

Many countries have no requirement for premarket approval of prebiotics, because there is no established or implemented system for health claims, although scientific substantiation should be available on request by authorities. The following countries have specific positions and/or policies regarding the use of and claims that can be made for prebiotics.

United States
The DGAC completed a non-Nutrition Evidence Library review of systematic reviews published since 2004 on probiotics, prebiotics, and health (30). The DGAC thinks that the gut microbiota plays a role in health and recognizes that consumer interest in altering the microbiota is high. Additionally, the DGAC thinks that investigation of the gut microbiota is an important emerging area of research. However, insufficient evidence was available for DGAC to make dietary recommendations for Americans regarding either prebiotics or probiotics. The DGAC did note that although not all dietary fibers are prebiotics, all prebiotics are dietary fibers. Therefore, the recommended intakes of dietary fiber can provide prebiotics to the diet. In conclusion, the DGAC suggested that foods high in prebiotics (wheat, onions, garlic) should be consumed as well as food concentrated in probiotics (yogurt).

The most common claims in the marketing of prebiotics are structure/function claims. Health claims are not used, because the FDA has not approved such claims for prebiotics and nutrient content claims cannot be made because a daily value has not been established for prebiotics. However, because the DGAC notes that "all prebiotics are dietary fibers" and promotes consumption of recommended intakes of dietary fiber, perhaps a nutrient content claim for prebiotics could be argued based on dietary fiber daily values.

Canada
Health Canada is currently developing guidelines to clarify the acceptable use of the term prebiotic. A Guidance Document on the Use of Probiotic Microorganisms in Food was published in May 2009 (110).

Europe
France. The French Agency for Food, Environmental, and Occupational Health and Safety has approved both inulin and FOS as prebiotics at 5 g/d (111,112). Allowed claims include: *1)*

"a bifidogenic effect at a daily dose of 5 g oligofructose per day;" *2)* "Native inulin from chicory is bifidogenic (stimulation of the growth of intestinal bifidobacteria) at a daily dose of 5 g/d;" *3)* "Native inulin from chicory is prebiotic at a daily dose of 5 g/d;" and *4)* "Native inulin from chicory at a daily dose of 5 g/d helps to maintain a healthy intestinal flora in the colon."

The Netherlands. In The Netherlands, inulin was approved as a prebiotic component of Vitaalbrood at 5 g inulin/100 g (113). The package may carry the following statement: "Three slices per day supports a well-balanced gut flora composition and colonic function by selectively stimulating the growth of *Bifidobacterium.*"

European Food Safety Authority
Among recent rulings, the European Food Safety Authority (EFSA) rejected an Article 14 (1)(a) health claim referring to reduction of disease risk (travelers' diarrhea) for a *Trans*-GOS called BiMuno (114), produced by Clasado and marketed in the UK (114). Also rejected were two Article 13 (5) health claims (115) based on newly developed scientific evidence and/or including a request for the protection of proprietary data for BiMuno, one claiming the product "helps maintain a healthy gastrointestinal function" and one claiming it "supports your natural defenses." The reason for rejection was the same for all claims: a cause and effect relationship between the consumption of the food for which the claim is made and the claimed effect has not been established. This is despite several well-designed human studies of this product (116,117), including a recent double-blind, placebo-controlled study on traveler's diarrhea (73).

EFSA has recently published a guidance document focused on 2 key issues regarding the substantiation of health claims related to the GI tract and immune system (i.e., which claimed effects considered to be beneficial physiological effects and which studies/outcome measures are considered to be appropriate for the substantiation of function claims and disease risk reduction claims) (118). Currently, it is the opinion of the EFSA Panel on Dietetic Products, Nutrition and Allergies that "it is not possible to define the exact numbers of the different bacterial groups which constitute a 'normal' microbiota" and that the available data and "evidence available to the Panel does not establish that increasing the number of any groups of microorganisms, including lactobacilli and/or bifidobacteria, is in itself a beneficial physiological effect" (118).

Japan
Numerous prebiotics have been commercialized in Japan during the last decade following their classification as foods for specific health uses (FOSHU) by the Japanese Ministry of Health, Labor, and Welfare. FOSHU status is allowed for "foods containing an ingredient with functions for health and officially approved to claim its physiological effects on the human body" (119). Oligosaccharides and various dietary fibers are among the principal ingredients of foods approved "to modify gastrointestinal conditions." Prebiotic fiber functional ingredients include indigestible dextrin, psyllium seed husk, polydextrose, partially hydrolyzed guar gum, wheat bran, raffinose, beer yeast fiber, low-molecular weight sodium alginate, and agar-derived fiber. The oligosaccharide list is almost as long, with fructo-, galacto-, and isomalto- oligosaccharides as well as lactosucrose and lactulose and, most recently, a coffee bean mannanoligosaccharide in instant coffee and coffee premixes. All products containing dietary fiber and/or oligosaccharides are allowed on-label health

claims such as "suitable for improving the regulation and control of the gastrointestinal tract" or "improves bowel movement," or the equivalent and may carry the FOSHU seal of approval.

Korea
The Korean FDA allows the following health claims for inulin and chicory extracts: "Helps to maintain a healthy blood cholesterol level" (9–10 g inulin/d); "helps to maintain healthy postprandial glucose levels" (9–10 g inulin/d); and "helps to maintain healthy bowel function" (8–20 g inulin/d) (120).

Southeast Asia
In Singapore, inulin and FOS have been approved as prebiotics (121). Inulin and FOS can be added to all foods (1.25 g/portion) in Malaysia, except infant formula (122). In Malaysia, nutrient function claims describe the physiological role of the nutrient in growth, development, and normal functions in the body (123,124). They should not imply that the nutrient cures, treats, or protects the consumer from diseases. Permitted Malaysian nutrient function claims for prebiotics include: "Inulin/FOS is prebiotic," "Inulin/FOS helps increase intestinal bifidobacteria and helps maintain a good intestinal environment," and "Inulin/FOS is bifidogenic." In Thailand, the Ministry of Health within the Thai FDA has developed guidelines and criteria to support the safety, quality, and efficacy evaluation of probiotics (published in 2009) and prebiotics (draft) in food products (125). Thailand allows nutrient content claims, nutrient comparative claims, and nutrient function claims, where 29 nutrient function claims have been approved (126). A ministerial notification is currently being drafted for health claims based on relevant Codex guidelines.

South America
Brazil. Inulin and FOS have been approved as prebiotics in Brazil at 2.5 g/portion with a recommendation for consumption of 5 g/d (127). The permitted claim is "Inulin/FOS as prebiotics contribute to a balance/equilibrium of the intestinal flora. Their consumption should be associated with a balanced diet and a healthy life-style."

Chile. Inulin and FOS have been approved as prebiotics in Chile at 1.5 g/portion with a recommendation of a minimum of 3 g/d (128). The permitted claim is "Contributes to maintain the balance of the intestinal flora."

Columbia. Prebiotics have been accepted by el Ministerio de la Protección Social of Columbia (129). The allowed claim is "a moderate nutrition and a regular consumption of foods with prebiotics, stimulates the growth of beneficial intestinal bacteria and helps to improve the intestinal function and the natural resistance."

South Africa
"Novel fibers" in South Africa means an "edible carbohydrate," of which a physiological effect of benefit to health was demonstrated by generally accepted scientific eidence and approved and registered by the South African Health Products Regulatory Authority (130). Novel fibers are classified as having ≥10 monomeric units, not hydrolyzed by the endogenous enzymes in the small intestine of humans; have been produced synthetically; or are obtained from natural sources that are not ordinarily consumed as fruits, vegetables, or cereals in the diet, or any oligomers (FOS), polymers (inulin), or mixtures thereof in which the degree of polymerization (DP) varies from 2 to 60 monomeric units for which a prebiotic claim could be made.

Prebiotic activity should be demonstrated by the following criteria (130): resistance to gastric acidity, hydrolysis by mammalian enzymes, and GI absorption; fermentation by intestinal microbiota; stimulation of the growth of the whole indigenous population of bifidobacteria; and the selective stimulation of growth and/or activity of other indigenous GI microbiota that contribute to health and well-being.

Codex alimentarius, fiber, and fructooligosaccharide
An unresolved regulatory question is whether DP 3–9 should be classified as dietary fiber based on the current Codex Alimentarius Definition of Dietary Fiber (footnote 2). At the Joint ILSI North America/ILSI Europe session at the 9th Vahouny Fiber Symposium, June 2010 (131), ~90% of respondents agreed with the inclusion of carbohydrate polymers of DP 3 and in the definition of dietary fiber (132). The percentage of respondents agreeing that in order to qualify as dietary fiber, carbohydrates falling into groups 2 and 3 of the Codex Alimentarius definition (as adopted in June 2009) should demonstrate scientific evidence of at least one of, but not limited to, the following physiological effects was: 99% for reduction in blood total and/or LDL-C; 96% for reduction in postprandial blood glucose and/or insulin; 99% for increased stool bulk and/or decreased gut transit time; 83% for fermentability by colonic microbiota; and 31% for "other proposed effects" (132).

WHO/FAO
Recognizing the possible beneficial effect of prebiotics in food, the FAO convened a technical meeting to start work on the evaluation of the functional and health properties of prebiotics (133). At the technical meeting, a group of international experts agreed on guidelines and recommended criteria and methodology for systematically approaching the evaluation of prebiotics for safe use in food. It was recommended that a full expert consultation be convened under the auspices of FAO. The work on these guidelines is ongoing (133).

Current Status

Prebiotics decrease GI infection, including the incidence and severity of traveler's and *C. difficile*-induced diarrhea and have shown preliminary signals of beneficial effects in inflammatory bowel disease (pouchitis and UC) and IBS. Prebiotics also increase the bioavailability and uptake of calcium. Hyperlipidemic men and women administered 10 g/d FOS-enriched inulin in combination with a diet containing soy foods had an enhanced reduction in LDL-C (-0.18 ± 0.07 mmol/L; $P = 0.042$) and in the LDL-C:HDL-C ratio (-28 ± 0.11; $P = 0.041$) compared with the prebiotic (95). However, additional research is needed to determine if prebiotics decrease the risk of CVD, because administration of inulin-type fructans for 6 mo had no lipid-lowering effect on normolipidemic individuals ($P < 0.30$ for total cholesterol, LDL-C, and HDL-C) (94). Because results are mixed for the effects of prebiotics on colon cancer and colon cancer biomarkers, further studies are needed in this area. Consumption of scFOS for 3 mo resulted in no difference in crypt cell proliferation from the beginning to the end of the study for participants with and without adenomas (mean overall difference in proportion of proliferating cells = 0.9 ± 0.91, $P = 0.62$, and -0.2 ± 9.8, $P = 0.87$, respectively) (80). Supplementation with a synbiotic containing the prebiotic Beneo Synergy1 (ORAFTI) and the probiotics LGG and BB12 changed transepithelial resistance ($128.4 \pm 4.5\%$ vs. $124.2 \pm 4.6\%$; $P = 0.025$) in polypectomized study participants but did not affect

study participants with cancer (104.9 ± 6.2% vs. 101.9 ± 6.6%; $P = 0.65$) when compared with a placebo (81). Limburg et al. (82) reported that the change in percentage of aberrant crypt foci did not significantly differ after study participants ingested ORAFTI Synergy1 compared with placebo consumption (−88 to 83 vs. −100 to 117; $P = 0.92$). Prebiotics may potentially have favorable effects on reducing obesity, decreasing risk of weight gain, and increasing satiety. FOS supplementation decreased body weight over 12 wk (FOS group: −1.03 ± 0.43 kg vs. control group: +0.45 ± 0.31 kg; $P = 0.01$) and resulted in a lower AUC for ghrelin (23% decrease; $P = 0.004$) and higher AUC for PYY (13% increase; $P = 0.03$) for initial values compared with values at the end of the study (106). Prebiotics have also been shown to decrease hunger VAS scores at 180 min compared with placebo (time × treatment; $P = 0.0147$); increase plasma glucagon-like peptide 1 (treatment × time interaction; $P = 0.038$) and PYY (treatment × time interaction; $P = 0.0498$); and decrease AUC for glucose after a standard meal (treatment × time interaction; $P < 0.05$) (107). Although developing evidence supports a role for prebiotics in reducing the risks of some chronic diseases, positive and negative results as well as no effect have been obtained for the effects of prebiotics on other diseases (134).

The United States, France, The Netherlands, Japan, Korea, Singapore, Malaysia, Thailand, Brazil, Chile, Columbia, and South Africa have established guidelines for prebiotics and Canada is developing guidelines. EFSA has rejected article 14 (1) (a) health claims and two article 13 (5) health claims and issued guidelines on substantiation of health claims related to the GI tract and the immune system (118). Inclusion of scFOS as a dietary fiber in Codex Alimentus is an unresolved issue. WHO/FAO is developing guidelines for evaluation of the functional and health properties of prebiotics.

Research Needs and Future Directions

At the February 10, 2011 Workshop on Prebiotics and the Health Benefits of Fiber: Future Research and Goals (135), the following research needs and future directions of the prebiotic field, including research on fiber and specifically prebiotic fiber, gut microbiota, and human studies, were discussed. **Table 3** identifies research needs in each of these areas.

Fiber/prebiotic fiber
The general consensus of prebiotic researchers is that not all fiber is the same; separation into functional categories is necessary. Prebiotics differ in source, structure, and functional characteristics from traditional fibers like pectin, cellulose, and others. Further research is needed to better characterize the effects of different fibers and prebiotics on the composition and function of the intestinal microbiota. Intervention studies are needed to identify potential benefits of fiber and prebiotics on human health and specific disease conditions. Future research studies should include various types of fiber and prebiotics and provide clear descriptions of the fiber/prebiotics used (i.e., monomeric composition, chain length, type of binding, branching, and side chains).

Gut microbiota
To further strengthen prebiotic research, attention must focus not only on prebiotics but also on understanding the composition, function, and complex interactions among and between the gut microbiota and the human host.

TABLE 3 Future research needs: fiber/prebiotic fiber, gut microbiota, and human studies[1]

Future research needs
Fiber/prebiotic fiber
Better characterization (structure and function) of different fibers and prebiotics
Intervention studies to identify the potential benefits of fiber and prebiotics
Gut microbiota
Complete the human gut microbiome project to identify microbiota beyond bifidobacteria and to differentiate ``normal/healthy'' and disease-state microbiota
Define a panel of target microbes for prebiotics and demonstrate functional efficacy
Clarify the functional importance of changes in flora
Determine the long-term effects of prebiotic intakes and develop structure/function models
Develop biomarkers for beneficial bacteria and their health outcomes
Improve methods to study fermentation in vivo and in vitro
Promote the development and use of molecular techniques to identify/quantify gut microbiota (e.g., qPCR) and detect/localize (e.g., FISH of 16S ribosomal RNA, high throughout sequencing, microarrays) for different gut microbial types/genera
Assess the metabolic and health-related impact of gut microbiome modulation and the metabonomic impact of gut microbiota modulation by diet
Human studies
Identify biomarkers of exposure for prebiotic intake
Identify biomarkers of effect for prebiotic intake in the general population and in diseased/at risk subpopulations
Define relevant clinical endpoints [i.e., blood glucose/lipids, bowel function, mineral metabolism (calcium/magnesium), immune function and gut inflammation, gut symptomology, daily functioning, and quality of life]
Define dose-response relationships for various categories of prebiotic
Define how much the observed benefits of various fibers are related to prebiotic effects
Determine the duration of effect of prebiotics
Define relationships among prebiotics and baseline diet
Investigate variability in response to prebiotics observed among individuals
Promote studies investigating relationship between GI health and prebiotic intake in children and the elderly

[1] The composition of the prebiotic GOS (B-GOS) for DP and saccharide linkages, as percentage of the GOS content: DP 2 ($n = 52$), DP 3 ($n = 26$), DP 4 ($n = 14$), DP 5 ($n = 8$), and linkages $\beta 1 \rightarrow 3$ ($n = 26$), $\beta 1 \rightarrow 4$ ($n = 23$), and $\beta 1 \rightarrow 6$ ($n = 51$), average molecular weight (kDa) = 496. The GOS content of B-GOS was 48% (wt:wt). DP, degree of polymerization; GI, gastrointestinal; GOS, galactooligosaccharide.

Human studies
Generally, there is a lack of prebiotic population-based studies, although it must be taken into account that this is a relatively new area of nutritional sciences. It has been suggested that ongoing large-scale studies could be tapped into as a short-term way to study the effects of prebiotics in subpopulations of interest.

Regulatory and funding issues
Population-based studies of prebiotic intake are expensive. A potential way to fund these initiatives is to forge industry-government collaborations. Increasing the visibility of the prebiotics field to federal agencies may also help meet funding needs. Currently, one of the most important regulatory issues is to identify a strategy most likely to succeed with regulatory agencies like EFSA. Perhaps an interim strategy could be adopted to gain approval of prebiotics as a class of fiber instead of an independent category while research is being conducted to address the concerns of the regulators. However, if prolonged, such a strategy would incur a long-term cost, undervaluing the unique properties and benefits of prebiotics.

Disseminating the health benefits of prebiotics in general and of specific prebiotic fibers in particular is currently restricted by

government regulations on advertising and label claims. The recognition that advertising and label claims are a powerful force to influence public behavior motivates government regulatory agencies to carefully evaluate the scientific evidence that serves as the basis of the claim prior to approval. Advocates of prebiotics face a considerable challenge to gather sufficient evidence to convince regulatory bodies in the US and EU that the beneficial effects of prebiotics are sufficiently well defined and accepted by the scientific community that such claims are acceptable.

The LSRO-organized Workshop on Prebiotics and the Health Benefits of Fiber: Future Research and Goals determined that to increase acceptance of prebiotics by the scientific community and the likelihood of regulatory acceptance of claims for the beneficial effect of prebiotics on human health, additional research is needed on fiber, gut microbiota, and human studies. Developing these data maybe more easily achievable for some diseases than for others. Although this research will be costly, it has the potential to reduce the risk of some diseases and improve human health.

Literature Cited

1. Slavin JL. Position of the American Dietetic Association: health implications of dietary fiber. J Am Diet Assoc. 2008;108:1716–31.

2. Eshak ES, Iso H, Date C, Kikuchi S, Watanabe Y, Wada Y, Wakai K, Tamakoshi A. Dietary fiber intake is associated with reduced risk of mortality from cardiovascular disease among Japanese men and women. J Nutr. 2010;140:1445–53.

3. Mozaffarian D, Kumanyika SK, Lemaitre RN, Olson JL, Burke GL, Siscovick DS. Cereal, fruit, and vegetable fiber intake and the risk of cardiovascular disease in elderly individuals. JAMA. 2003;289:1659–66.

4. Pietinen P, Rimm EB, Korhonen P, Hartman AM, Willett WC, Albanes D, Virtamo J. Intake of dietary fiber and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. Circulation. 1996;94:2720–7.

5. Rimm EB, Ascherio A, Giovannucci E, Spiegelman D, Stampfer MJ, Willett WC. Vegetable, fruit, and cereal fiber intake and risk of coronary heart disease among men. JAMA. 1996;275:447–51.

6. Wolk A, Manson JE, Stampfer MJ, Colditz GA, Hu FB, Speizer FE, Hennekens CH, Willett WC. Long-term intake of dietary fiber and decreased risk of coronary heart disease among women. JAMA. 1999;281:1998–2004.

7. Fraser GE, Sabate J, Beeson WL, Strahan TM. A possible protective effect of nut consumption on risk of coronary heart disease. The Adventist Health Study. Arch Intern Med. 1992;152:1416–24.

8. Jacobs DR Jr, Meyer KA, Kushi LH, Folsom AR. Whole-grain intake may reduce the risk of ischemic heart disease death in postmenopausal women: the Iowa Women's Health Study. Am J Clin Nutr. 1998; 68:248–57.

9. Morris JN, Marr JW, Clayton DG. Diet and heart: a postscript. BMJ. 1977;2:1307–14.

10. Bolton-Smith C, Woodward M, Tunstall-Pedoe H. The Scottish Heart Health Study. Dietary intake by food frequency questionnaire and odds ratios for coronary heart disease risk. II. The antioxidant vitamins and fibre. Eur J Clin Nutr. 1992;46:85–93.

11. Todd S, Woodward M, Tunstall-Pedoe H, Bolton-Smith C. Dietary antioxidant vitamins and fiber in the etiology of cardiovascular disease and all-causes mortality: results from the Scottish Heart Health Study. Am J Epidemiol. 1999;150:1073–80.

12. Bazzano LA, He J, Ogden LG, Loria CM, Whelton PK. Dietary fiber intake and reduced risk of coronary heart disease in US men and women: the National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study. Arch Intern Med. 2003;163: 1897–904.

13. Jensen MK, Koh-Banerjee P, Hu FB, Franz M, Sampson L, Gronbaek M, Rimm EB. Intakes of whole grains, bran, and germ and the risk of coronary heart disease in men. Am J Clin Nutr. 2004;80:1492–9.

14. Pereira MA, O'Reilly E, Augustsson K, Fraser GE, Goldbourt U, Heitmann BL, Hallmans G, Knekt P, Liu S, Pietinen P, et al. Dietary fiber and risk of coronary heart disease: a pooled analysis of cohort studies. Arch Intern Med. 2004;164:370–6.

15. Khaw KT, Barrett-Connor E. Dietary fiber and reduced ischemic heart disease mortality rates in men and women: a 12-year prospective study. Am J Epidemiol. 1987;126:1093–102.

16. Liu S, Buring JE, Sesso HD, Rimm EB, Willett WC, Manson JE. A prospective study of dietary fiber intake and risk of cardiovascular disease among women. J Am Coll Cardiol. 2002;39:49–56.

17. Davis JN, Hodges VA, Gillham MB. Normal-weight adults consume more fiber and fruit than their age- and height-matched overweight/obese counterparts. J Am Diet Assoc. 2006;106:833–40.

18. Howarth NC, Huang TT, Roberts SB, McCrory MA. Dietary fiber and fat are associated with excess weight in young and middle-aged US adults. J Am Diet Assoc. 2005;105:1365–72.

19. Liu S, Willett WC, Manson JE, Hu FB, Rosner B, Colditz G. Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. Am J Clin Nutr. 2003;78:920–7.

20. Maskarinec G, Takata Y, Pagano I, Carlin L, Goodman MT, Le Marchand L, Nomura AM, Wilkens LR, Kolonel LN. Trends and dietary determinants of overweight and obesity in a multiethnic population. Obesity (Silver Spring). 2006;14:717–26.

21. Tucker LA, Thomas KS. Increasing total fiber intake reduces risk of weight and fat gains in women. J Nutr. 2009;139:576–81.

22. Ludwig DS, Pereira MA, Kroenke CH, Hilner JE, Van Horn L, Slattery ML, Jacobs DR Jr. Dietary fiber, weight gain, and cardiovascular disease risk factors in young adults. JAMA. 1999;282: 1539–46.

23. de Munter JS, Hu FB, Spiegelman D, Franz M, van Dam RM. Whole grain, bran, and germ intake and risk of type 2 diabetes: a prospective cohort study and systematic review. PLoS Med. 2007;4:e261.

24. Schulze MB, Schulz M, Heidemann C, Schienkiewitz A, Hoffmann K, Boeing H. Fiber and magnesium intake and incidence of type 2 diabetes: a prospective study and meta-analysis. Arch Intern Med. 2007;167:956–65.

25. Giacco R, Parillo M, Rivellese AA, Lasorella G, Giacco A, D'Episcopo L, Riccardi G. Long-term dietary treatment with increased amounts of fiber-rich low-glycemic index natural foods improves blood glucose control and reduces the number of hypoglycemic events in type 1 diabetic patients. Diabetes Care. 2000;23: 1461–6.

26. Kaline K, Bornstein SR, Bergmann A, Hauner H, Schwarz PE. The importance and effect of dietary fiber in diabetes prevention with particular consideration of whole grain products. Horm Metab Res. 2007;39:687–93.

27. Simpson HC, Simpson RW, Lousley S, Carter RD, Geekie M, Hockaday TD, Mann JI. A high carbohydrate leguminous fibre diet improves all aspects of diabetic control. Lancet. 1981;1:1–5.

28. Hopping BN, Erber E, Grandinetti A, Verheus M, Kolonel LN, Maskarinec G. Dietary fiber, magnesium, and glycemic load alter risk of type 2 diabetes in a multiethnic cohort in Hawaii. J Nutr. 2010;140:68–74.

29. Aldoori W, Ryan-Harshman M. Preventing diverticular disease. Review of recent evidence on high-fibre diets. Can Fam Physician. 2002;48:1632–7.

30. USDA. Carbohydrates. 2010 [cited 2010 Sep 22]. Available from: http://www.cnpp.usda.gov/Publications/DietaryGuidelines/2010/DGAC/Report/D-5-Carbohydrates.pdf.

31. Institute of Medicine. Dietary Reference Intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington, DC: National Academies Press; 2002.

32. Gibson GR, Scott KP, Rastall RA, Tuohy KM, Hotchkiss A, Dubert-Ferrandon A, Gareau M, Murphy EF, Saulnier D, Loh G, et al.

Dietary prebiotics: current status and new definition. Food Science and Technology Bulletin: Functional Foods. 2010;7:1–19.

33. Cummings JH, Macfarlane GT. The control and consequences of bacterial fermentation in the human colon. J Appl Bacteriol. 1991;70:443–59.

34. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell. 2006;124:837–48.

35. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. Science. 2005;308:1635–8.

36. Gordon JI, Ley RE, Wilson R, Mardis E, Xu J, Fraser CM, Relman DA. Extending our view of self: the Human Gut Microbiome Initiative (HGMI); 2011 [cited 2011 Feb 21]. Available from: http://www.genome.gov/Pages/Research/Sequencing/SeqProposals/HGMISeq.pdf.

37. Roberfroid MB. General introduction: prebiotics in nutrition. In: Gibson GR, Roberfroid MB, editors. Handbook of prebiotics. Boca Raton (FL): CRC Press; 2008. p. 1–11.

38. Ringel Y, Carroll IM. Alterations in the intestinal microbiota and functional bowel symptoms. Gastrointest Endosc Clin N Am. 2009;19:141–50.

39. Flint HJ, Duncan SH, Scott KP, Louis P. Interactions and competition within the microbial community of the human colon: links between diet and health. Environ Microbiol. 2007;9:1101–11.

40. Hague A, Singh B, Paraskeva C. Butyrate acts as a survival factor for colonic epithelial cells: further fuel for the *in vivo* versus *in vitro* debate. Gastroenterology. 1997;112:1036–40.

41. Barcenilla A, Pryde SE, Martin JC, Duncan SH, Stewart CS, Henderson C, Flint HJ. Phylogenetic relationships of butyrate-producing bacteria from the human gut. Appl Environ Microbiol. 2000;66:1654–61.

42. Juhlin-Dannfelt A. Ethanol effects of substrate utilization by the human brain. Scand J Clin Lab Invest. 1977;37:443–9.

43. Lindeneg O, Mellemgaard K, Fabricius J, Lundquist F. Myocardial utilization of acetate, lactate and free fatty acids after ingestion of ethanol. Clin Sci. 1964;27:427–35.

44. Lundquist F, Sestoft L, Damgaard SE, Clausen JP, Trap-Jensen J. Utilization of acetate in the human forearm during exercise after ethanol ingestion. J Clin Invest. 1973;52:3231–5.

45. Landau BR, Schumann WC, Chandramouli V, Magnusson I, Kumaran K, Wahren J. 14C-labeled propionate metabolism in vivo and estimates of hepatic gluconeogenesis relative to Krebs cycle flux. Am J Physiol. 1993;265:E636–47.

46. Salminen S, Bouley C, Boutron-Ruault MC, Cummings JH, Franck A, Gibson GR, Isolauri E, Moreau MC, Roberfroid M, Rowland I. Functional food science and gastrointestinal physiology and function. Br J Nutr. 1998;80 Suppl 1:S147–71.

47. Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol Rev. 2001;81:1031–64.

48. Smith EA, Macfarlane GT. Enumeration of human colonic bacteria producing phenolic and indolic compounds: effects of pH, carbohydrate availability and retention time on dissimilatory aromatic amino acid metabolism. J Appl Bacteriol. 1996;81:288–302.

49. Thornton JR. High colonic pH promotes colorectal cancer. Lancet. 1981;1:1081–3.

50. Kagnoff MF. Immunology of the digestive system. In: Johnson LR, editor. Physiology of the gastrointestinal tract. 2nd ed. New York: Raven Press; 1987. p. 1699–728.

51. Field CJ, McBurney MI, Massimino S, Hayek MG, Sunvold GD. The fermentable fiber content of the diet alters the function and composition of canine gut associated lymphoid tissue. Vet Immunol Immunopathol. 1999;72:325–41.

52. Pierre F, Perrin P, Champ M, Bornet F, Meflah K, Menanteau J. Short-chain fructo-oligosaccharides reduce the occurrence of colon tumors and develop gut-associated lymphoid tissue in Min mice. Cancer Res. 1997;57:225–8.

53. Gaskins HR, Mackie RI, May T, Garleb KA. Dietary fructo-oligosaccharide modulates large intestinal inflammatory responses to *Clostridium difficile* in antibiotic-compromised mice. Microb Ecol Health Dis. 1996;9:157–66.

54. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol. 2001;2:675–80.

55. Abreu MT. Immunologic regulation of toll-like receptors in gut epithelium. Curr Opin Gastroenterol. 2003;19:559–64.

56. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr. 1995;125:1401–12.

57. Gibson GR, Probert HM, Van Loo J, Rastall RA, Roberfroid MB. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev. 2004;17:259–75.

58. van Loo J, Coussement P, de Leenheer L, Hoebregs H, Smits G. On the presence of inulin and oligofructose as natural ingredients in the western diet. Crit Rev Food Sci Nutr. 1995;35:525–52.

59. Moshfegh AJ, Friday JE, Goldman JP, Ahuja JK. Presence of inulin and oligofructose in the diets of Americans. J Nutr. 1999;129:S1407–11.

60. Imperial-Sensus LLC. Generally recognized as safe (gras) notification for Frutafit. GRN 118 inulin. Sugarland, TX: Imperial-Sensus LLC; 2002 Nov 14. Report No.: GRN No. 118.

61. GTC Nutrition Company. Generally recognized as safe notification for short-chain fructooligosaccharide. GRN 44. Golden (CO): GTC Nutrition Company; 2000 Apr 17. Report No.: GRN No. 44.

62. GTC Nutrition Company. Generally recognized as safe notification for galacto-oligosaccharide. GRN 285. Golden (CO): GTC Nutrition Company; 2009 Mar 12. Report No.: GRAS GRN 285.

63. Coussement PA. Inulin and oligofructose: safe intakes and legal status. J Nutr. 1999;129:S1412–7.

64. Speigel JE. Safety and benefits of fructooligosaccharides as food ingredients. Food Technol. 1994;48:85–9.

65. Briet F, Achour L, Flourie B, Beaugerie L, Pellier P, Franchisseur C, Bornet F, Rambaud JC. Symptomatic response to varying levels of fructo-oligosaccharides consumed occasionally or regularly. Eur J Clin Nutr. 1995;49:501–7.

66. Livesey G. Tolerance of low-digestible carbohydrates: a general view. Br J Nutr. 2001;85 Suppl 1:S7–16.

67. Rogosa M. Genus III. Bifidobacterium Orla-Fensen 1924, 472. In: Buchanan RE, Gibbons NE, editors. Bergey's manual of determinative bacteriology. 8th ed. Baltimore (MD): Waverly Press; 1974. p. 669–74.

68. Livesey G. The energy values of dietary fibre and sugar alcohols for man. Nutr Res Rev. 1992;5:61–84.

69. Roberfroid M, Gibson GR, Delzenne N. The biochemistry of oligofructose, a nondigestible fiber: an approach to calculate its caloric value. Nutr Rev. 1993;51:137–46.

70. Roberfroid MB. Caloric value of inulin and oligofructose. J Nutr. 1999;129:S1436–7.

71. FUFOSE. Scientific concepts of functional foods in Europe. Consensus document. Br J Nutr. 1999;81 Suppl 1:S1–27.

72. Cummings JH, Christie S, Cole TJ. A study of fructo oligosaccharides in the prevention of travellers' diarrhoea. Aliment Pharmacol Ther. 2001;15:1139–45.

73. Drakoularakou A, Tzortzis G, Rastall RA, Gibson GR. A double-blind, placebo-controlled, randomized human study assessing the capacity of a novel galacto-oligosaccharide mixture in reducing travellers' diarrhoea. Eur J Clin Nutr. 2010;64:146–52.

74. Lewis S, Burmeister S, Brazier J. Effect of the prebiotic oligofructose on relapse of *Clostridium difficile*-associated diarrhea: a randomized, controlled study. Clin Gastroenterol Hepatol. 2005;3:442–8.

75. Welters CF, Heineman E, Thunnissen FB, van den Bogaard AE, Soeters PB, Baeten CG. Effect of dietary inulin supplementation on inflammation of pouch mucosa in patients with an ileal pouch-anal anastomosis. Dis Colon Rectum. 2002;45:621–7.

76. Furrie E, Macfarlane S, Kennedy A, Cummings JH, Walsh SV, O'Neil DA, Macfarlane GT. Synbiotic therapy (*Bifidobacterium longum*/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. Gut. 2005;54:242–9.

77. Benjamin JL, Hedin CR, Koutsoumpas A, Ng SC, McCarthy NE, Hart AL, Kamm MA, Sanderson JD, Knight SC, Forbes A, et al. Randomised, double-blind, placebo-controlled trial of fructo-oligo-saccharides in active Crohn's disease. Gut. 2011;60:923–9.

78. Casellas F, Borruel N, Torrejon A, Varela E, Antolin M, Guarner F, Malagelada JR. Oral oligofructose-enriched inulin supplementation in acute ulcerative colitis is well tolerated and associated with lowered faecal calprotectin. Aliment Pharmacol Ther. 2007;25:1061–7.

79. Silk DB, Davis A, Vulevic J, Tzortzis G, Gibson GR. Clinical trial: the effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. Aliment Pharmacol Ther. 2009;29:508–18.

80. Boutron-Ruault MC, Marteau P, Lavergne-Slove A, Myara A, Gerhardt MF, Franchisseur C, Bornet F. Effects of a 3-mo consumption of short-chain fructo-oligosaccharides on parameters of colorectal carcinogenesis in patients with or without small or large colorectal adenomas. Nutr Cancer. 2005;53:160–8.

81. Rafter J, Bennett M, Caderni G, Clune Y, Hughes R, Karlsson PC, Klinder A, O'Riordan M, O'Sullivan GC, Pool-Zobel B, et al. Dietary synbiotics reduce cancer risk factors in polypectomized and colon cancer patients. Am J Clin Nutr. 2007;85:488–96.

82. Limburg PJ, Mahoney MR, Ziegler KL, Sontag SJ, Schoen RE, Benya R, Lawson MJ, Weinberg DS, Stoffel E, Chiorean M, et al. Randomized phase II trial of sulindac, atorvastatin, and prebiotic dietary fiber for colorectal cancer chemoprevention. Cancer Prev Res (Phila). 2011;4:259–69.

83. Fuchs CS, Giovannucci EL, Colditz GA, Hunter DJ, Stampfer MJ, Rosner B, Speizer FE, Willett WC. Dietary fiber and the risk of colorectal cancer and adenoma in women. N Engl J Med. 1999; 340:169–76.

84. Coudray C, Fairweather-Tait SJ. Do oligosaccharides affect the intestinal absorption of calcium in humans? Am J Clin Nutr. 1998;68:921–3.

85. van den Heuvel EG, Schoterman MH, Muijs T. Transgalactooligosaccharides stimulate calcium absorption in postmenopausal women. J Nutr. 2000;130:2938–42.

86. Tahiri M, Tressol JC, Arnaud J, Bornet FR, Bouteloup-Demange C, Feillet-Coudray C, Brandolini M, Ducros V, Pepin D, Brouns F, et al. Effect of short-chain fructooligosaccharides on intestinal calcium absorption and calcium status in postmenopausal women: a stable-isotope study. Am J Clin Nutr. 2003;77:449–57.

87. Demigné C, Jacobs H, Moundras C, Davicco MJ, Horcajada MN, Bernalier A, Coxam V. Comparison of native or reformulated chicory fructans, or non-purified chicory, on rat cecal fermentation and mineral metabolism. Eur J Nutr. 2008;47:366–74.

88. Abrams SA, Griffin IJ, Hawthorne KM, Liang L, Gunn SK, Darlington G, Ellis KJ. A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. Am J Clin Nutr. 2005;82:471–6.

89. Abrams SA, Griffin IJ, Hawthorne KM, Ellis KJ. Effect of prebiotic supplementation and calcium intake on body mass index. J Pediatr. 2007;151:293–8.

90. Jenkins DJ, Kendall CW, Popovich DG, Vidgen E, Mehling CC, Vuksan V, Ransom TP, Rao AV, Rosenberg-Zand R, Tariq N, et al. Effect of a very-high-fiber vegetable, fruit, and nut diet on serum lipids and colonic function. Metabolism. 2001;50:494–503.

91. Jenkins DJ, Kendall CW, Faulkner D, Vidgen E, Trautwein EA, Parker TL, Marchie A, Koumbridis G, Lapsley KG, Josse RG, et al. A dietary portfolio approach to cholesterol reduction: combined effects of plant sterols, vegetable proteins, and viscous fibers in hypercholesterolemia. Metabolism. 2002;51:1596–604.

92. Jenkins DJ, Kendall CW, Marchie A, Faulkner DA, Wong JM, de Souza R, Emam A, Parker TL, Vidgen E, Lapsley KG, et al. Effects of a dietary portfolio of cholesterol-lowering foods vs lovastatin on serum lipids and C-reactive protein. JAMA. 2003;290:502–10.

93. Davidson MH, Maki KC. Effects of dietary inulin on serum lipids. J Nutr. 1999;129:S1474–7.

94. Forcheron F, Beylot M. Long-term administration of inulin-type fructans has no significant lipid-lowering effect in normolipidemic humans. Metabolism. 2007;56:1093–8.

95. Wong JM, Kendall CW, de Souza R, Emam A, Marchie A, Vidgen E, Holmes C, Jenkins DJ. The effect on the blood lipid profile of soy foods combined with a prebiotic: a randomized controlled trial. Metabolism. 2010;59:1331–40.

96. Nadal I, Santacruz A, Marcos A, Warnberg J, Garagorri M, Moreno LA, Martin-Matillas M, Campoy C, Marti A, Moleres A, et al. Shifts in clostridia, bacteroides and immunoglobulin-coating fecal bacteria associated with weight loss in obese adolescents. Int J Obes (Lond). 2009;33:758–67.

97. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, et al. A core gut microbiome in obese and lean twins. Nature. 2009;457:480–4.

98. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006;444:1022–3.

99. Armougom F, Henry M, Vialettes B, Raccah D, Raoult D. Monitoring bacterial community of human gut microbiota reveals an increase in Lactobacillus in obese patients and Methanogens in anorexic patients. PLoS ONE. 2009;4:e7125.

100. Schwiertz A, Taras D, Schafer K, Beijer S, Bos NA, Donus C, Hardt PD. Microbiota and SCFA in lean and overweight healthy subjects. Obesity (Silver Spring). 2010;18:190–5.

101. Duncan SH, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P, Flint HJ. Human colonic microbiota associated with diet, obesity and weight loss. Int J Obes (Lond). 2008;32:1720–4.

102. Santacruz A, Marcos A, Warnberg J, Marti A, Martin-Matillas M, Campoy C, Moreno LA, Veiga O, Redondo-Figuero C, Garagorri JM, et al. Interplay between weight loss and gut microbiota composition in overweight adolescents. Obesity (Silver Spring). 2009;17:1906–15.

103. Santacruz A, Collado MC, Garcia-Valdes L, Segura MT, Martin-Lagos JA, Anjos T, Marti-Romero M, Lopez RM, Florido J, Campoy C, et al. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br J Nutr. 2010;104:83–92.

104. Peters HP, Boers HM, Haddeman E, Melnikov SM, Qvyjt F. No effect of added β-glucan or of fructooligosaccharide on appetite or energy intake. Am J Clin Nutr. 2009;89:58–63.

105. Archer BJ, Johnson SK, Devereux HM, Baxter AL. Effect of fat replacement by inulin or lupin-kernel fibre on sausage patty acceptability, post-meal perceptions of satiety and food intake in men. Br J Nutr. 2004;91:591–9.

106. Parnell JA, Reimer RA. Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. Am J Clin Nutr. 2009;89:1751–9.

107. Cani PD, Lecourt E, Dewulf EM, Sohet FM, Pachikian BD, Naslain D, De Backer F, Neyrinck AM, Delzenne NM. Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. Am J Clin Nutr. 2009;90:1236–43.

108. Cani PD, Joly E, Horsmans Y, Delzenne NM. Oligofructose promotes satiety in healthy human: a pilot study. Eur J Clin Nutr. 2006;60:567–72.

109. Hess JR, Birkett AM, Thomas W, Slavin JL. Effects of short-chain fructooligosaccharides on satiety responses in healthy men and women. Appetite. 2011;56:128–34.

110. Health Canada. Guidance document :the use of probiotic microorganisms in food. Ottawa: Health Canada; 2009.

111. AFSSA. French approval for inulin as prebiotic; 2004 [cited 2011 Mar 9]. Available from: http://www.afssa.fr/Documents/NUT2004-sa0365.pdf.

112. AFSSA. AFSSA report of the effects of probiotics and prebiotics on flora and immunity. AFFSA; 2005 [cited 2011 Mar 9]. Available from: http://www.isapp.net/docs/AFFSAprobioticprebioticfloraimmunity05.pdf.

113. Netherlands Nutrition Centre. Assessment report of Netherlands Nutrition Centre on inulin in Vitaalbrood. Netherlands Nutrition Centre; 2008 [cited 2011 Mar 9]. Available from: http://www.voedingscentrum.nl/resources2008/beoordelingsrapport_vitaalbrood_florapdf.pdf.

114. Bimuno. Improve digestive health with Bimuno; 2010 [cited 2010 Oct 12]. Available from: http://www.bimuno.com/.

115. EUROPA. European Union Register of nutrition and health claims made on food: rejected health claims; 2010 [cited 2010 Sep 23]. Available from: http://ec.europa.eu/food/food/labellingnutrition/claims/community_register/rejected_health_claims_en.htm.

116. Depeint F, Tzortzis G, Vulevic J, I'anson K, Gibson GR. Prebiotic evaluation of a novel galactooligosaccharide mixture produced by the enzymatic activity of Bifidobacterium bifidum NCIMB 41171, in healthy humans: a randomized, double-blind, crossover, placebo-controlled intervention study. Am J Clin Nutr. 2008;87:785–91.

117. Vulevic J, Drakoularakou A, Yaqoob P, Tzortzis G, Gibson GR. Modulation of the fecal microflora profile and immune function by a novel trans-galactooligosaccharide mixture (B-GOS) in healthy elderly volunteers. Am J Clin Nutr. 2008;88:1438–46.

118. EFSA Panel on Dietetic Products Nutrition and Allergies. Guidance on the scientific requirements for health claims related to gut and immune function. EFSA J. 2011;9:1984.

119. Japanese Ministry of Health Labour and Welfare. Food for Specified Health Uses (FOSHU). Japanese Ministry of Health, Labour, and Welfare; 2010 [cited 2010 Oct 12]. Available from: http://www.mhlw.go.jp/english/topics/foodsafety/fhc/02.html.

120. Korea FDA. Health functional food code. Chungcheongbuk-do, Korea: Korea Food and Drug Administration; 2010.

121. Agri-Food and Veterinary Authority of Singapore. Food labelling and advertisements. Agri-Food and Veterinary Authority of Singapore; 2011 [cited 2011 Mar 8]. Available from: http://www.ava.gov.sg/FoodSector/FoodLabelingAdvertisement/.

122. Tee E-S, Sulong F. Make effective use of nutrition information on food labels. Ministry of Health Malaysia; 2010 [cited 2011 Mar 8]. Available from: http://www.nutrition.moh.gov.my/pdf/mdg2010/Key%20Message%2014.pdf.

123. Nik Daud NI. Malaysian legal requirements for innovative foods. World of Food Science; 2011 [cited 2011 Mar 9]. Available from: http://www.worldfoodscience.org/cms/?pid=1004358.

124. Food Safety and Quality Division. Guide to nutrition labelling and claims. Malaysia: Ministry of Health Malaysia; 2010.

125. Food and Drug Administration Thailand. Prebiotics [in Thai]. Medical and health product bulletin; 2011 [cited 2011 Mar 8]. 5:1–31. Available from: http://www.fda.moph.go.th/fda-net/html/product/apr/about/bulletin%205-4.pdf.

126. Southeast Asia Region ILSI. Seminar and Workshop on Nutrition Labeling, Claims and Communication Strategies for the Consumer, Sept 20–21; 2010, Kuala Lumpur, Malaysia, Meeting Summary. ILSI Southeast Asia Region 2010 [cited 2011 Mar 9]. Available from: http://www.ilsi.org/SEA_Region/Documents/Nutrition%20Labeling%20Claim%20Meeting%20Summary%20Sept2010.pdf.

127. Agencia Nacional de Vigláncia Sanitária. Alimentos com Alegações de Propriedades Funcionais e ou de Saúde, Novos Alimentos/Ingredientes, Substâncias Bioativas e Probióticos. ANVISA; 2011 [cited 2011 Mar 11]. Available from: http://www.anvisa.gov.br/alimentos/comissoes/tecno_lista_alega.htm.

128. de Chile Ministerio de Salud R. Normas Técnicas Sobre Directrices Nutricionales Que Indica, Para La Declaración De Propiedades Saludables De Los Alimentos. Resolucion Exenta No. DE 2005. Republica de Chile; 2005 [cited 2011 Mar 11]. Available from: http://juridico1.minsal.cl/resolucion_556_2005.doc.

129. Ministerio de la Protección Social Columbia South America. Resolucion 288 De 2008 (enero 31). Diario Oficial No. 46.894 de 6 de febrero de 2008. Por la cual se establece el reglamento técnico sobre requisitos de rotulado o etiquetado nutricional que deben cumplir los alimentos envasados para consumo humano. FAO; 2008 [cited 2011 Mar 11]. Available from: http://faolex.fao.org/docs/texts/col78822.doc.

130. South African Health Products Regulatory Authority. Foodstuffs, cosmetics and disinfectants Act, 1972 (ACT 54 of 1972). Regulations relating to the labelling and advertising of food stuffs. Department of Health South Africa; 2011 [cited 2011 Mar 11]. Available from: http://www.hahnlaw.co.za/Food%20Labelling%20Regs%20R%20146%20of%201March%202010.pdf.

131. ILSI North America, ILSI Europe. 9th Vahouny Fibre Symposium: Session 10: implementation Issues of the Codex definition of dietary fibre. Part 2. Building scientific agreement on resolution of the issues Workshop format facilitated by Julie Miller-Jones and Martine Champ (Session sponsored by ILSI North America and ILSI Europe); Washington, DC: ILSI North America; 2010.

132. Howlett JF, Betteridge VA, Champ M, Craig SA, Meheust A, Jones JM. The definition of dietary fiber: discussions at the Ninth Vahouny Fiber Symposium: building scientific agreement. Food Nutr Res. 2010;54:5750.

133. Pineiro M, Asp NG, Reid G, Macfarlane S, Morelli L, Brunser O, Tuohy K. FAO Technical meeting on prebiotics. J Clin Gastroenterol. 2008;42 Suppl 3:S156–9.

134. Geier MS, Butler RN, Giffard PM, Howarth GS. Prebiotic and synbiotic fructooligosaccharide administration fails to reduce the severity of experimental colitis in rats. Dis Colon Rectum. 2007;50:1061–9.

135. Life Sciences Research Organization. Workshop on Prebiotics and the Health Benefits of Fiber: future research and goals. Life Sciences Research Organization; 2011 [cited 2011 Feb 18]. Available from: http://www.lsro.org/prebiotics/prebiotics_summary4wkshp.html.