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REVIEW ARTICLE

Probiotics, Prebiotics and Synbiotics: A Health Benefit Supplement

Mehul R Chorawala*, Pratik M Oza, Gaurang B Shah

Department of Pharmacology, K. B. Institute of Pharmaceutical Education and Research, Gh-6, Sector-23, Gandhinagar-23.

ABSTRACT

A probiotic is a viable microbial dietary supplement that beneficial for the host through its effects in the intestinal tract. Probiotics are widely used to prepare fermented dairy products such as yogurt or freeze-dried cultures. In the future, they may also be found in fermented vegetables and meats. Several health-related effects associated with the probiotics, including alleviation of lactose intolerance and immune enhancement, have been reported in human studies. Some evidence suggests a role for probiotics in reducing the risk of rotavirus induced diarrhea and colon cancer. Prebiotics are non-digestible food ingredients that benefit the host by selectively stimulating the growth or activity of limited number of bacteria in the colon. Work with prebiotics has been limited, and only studies involving the inulin-type fructans have generated sufficient data for evaluation regarding their possible use as functional food ingredients. At present, claims about reduction of disease risk are only tentative and further research is needed. Among the claims are constipation relief, suppression of diarrhea and reduction of the risks of osteoporosis, atherosclerotic cardiovascular disease associated with dyslipidemia and insulin resistance, obesity and possibly type II diabetes. The combination of probiotics and prebiotics in a synbiotic has not been studied. This combination might improve the survival of the bacteria crossing the upper part of the gastrointestinal tract, thereby enhancing their effects in the large bowel. In addition, their effects might be additive or even synergistic.

Keywords: Probiotic, prebiotic, synbiotic, *lactobacilli*, *bifidobacteria*, inulin

*Corresponding author



INTRODUCTION

A probiotic is defined as a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract. This definition, however, was initially intended for use with animal feed products. For human nutrition, the following definition has been proposed: “a live microbial food ingredient that is beneficial to health” [1]. A prebiotics defined as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon” [2]. Modification by prebiotics of the composition of the colonic microflora leads to the predominance of a few of the potentially health-promoting bacteria, especially, but not exclusively, *lactobacilli* and *bifidobacteria* [2]. As discussed elsewhere in this volume [3] and as a working definition, a food can be said to be functional if it contains a component [which may or may not be a nutrient] that affects one or a limited number of functions in the body in a targeted way so as to have positive effects on health [4], or if it has a physiologic or psychologic effect beyond the traditional nutritional effect [5].

Among the most promising targets for functional foods are the gastrointestinal functions, including those that control transit time, bowel habits and mucosal motility as well as those that modulate epithelial cell proliferation. Promising targets are also gastrointestinal functions that are associated with a balanced colonic microflora, that are associated with control of nutrient bioavailability (ions in particular), that modify gastrointestinal immune activity, or that are mediated by the endocrine activity of the gastrointestinal system. Finally, some systemic functions such as lipid homeostasis that are indirectly influenced by nutrient digestion or fermentation represent promising targets [5,6].

This article reviews the scientific data showing that probiotics and prebiotics may positively affect various physiologic functions in ways that will permit them now or in the future to be classified as functional foods for which health claims (of enhanced function or of reduction in disease risk) will be authorized [3].

PROBIOTICS

The bacterial genera most often used as probiotics are *lactobacilli* and *bifidobacteria*. 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. After passage through the stomach and the small intestine, some probiotics survive and become established transiently in the large bowel. Indeed, the colon's fermentation capacity may be modified after probiotic intake, and oral intake of certain lactic acid bacteria will increase the number of *lactobacilli* or *bifidobacteria* in human feces. Several health-related effects associated with the intake of probiotics have been reported in ≥ 2 human nutrition studies by different research groups. These effects may be used to justify either functional claims or claims of reduced disease risk [7–10].

HEALTH BENEFICIAL EFFECTS OF PROBIOTICS

Alleviation of lactose intolerance

Lactose intolerance is a problem for $\leq 70\%$ of the world's populations who have a low amount of intestinal β -galactosidase activity and for whom lactose behaves like an osmotic, non-digestible carbohydrate. Probiotics have been shown to improve lactose digestion by reducing the intolerance symptoms as well as by slowing orocecal transit [7].

Immune enhancement

Infants supplemented with a strain of *Lactobacillus casei* have enhanced concentrations of circulating immunoglobulin A (IgA), which correlates with shortened duration of rotavirus induced diarrhea [11]. In addition, consumption of *L. acidophilus* and *Bifidobacterium bifidum* significantly enhances the non-specific immune phagocytic activity of circulating blood granulocytes [12]. This latter effect may explain, in part, the stimulation of intestinal IgA antibody responses in infants. Indeed, it is known that phagocytic activity is involved with natural immunity and that phagocytes are implicated in antibody immune responses acting as antigen-presenting cells. Finally, ingestion of yogurt has been shown to stimulate the production of cytokines by blood mononuclear cells.

Decrease in fecal enzymes and mutagenicity

Probiotic *lactobacilli* and *bifidobacteria* strains decrease the quantity of such fecal microbial enzymes as β -glucuronidase, β -glucosidase, nitroreductase and urease, which are involved in the metabolic activation of miscellaneous mutagens and carcinogens. In addition, these or similar strains have been reported to decrease fecal and urinary mutagenicity in healthy volunteers consuming fried ground beef.

Hypocholesterolemic effect

The hypocholesterolemic effects of probiotics are the subject of controversy. Studies published in the 1970s and 1980s consistently reported 5–17% reductions in serum cholesterol concentrations after 2–4 wk of daily consumption of fermented milk products, but these data have been challenged by the results of more recent studies, almost all of which did not report any significant effect. As discussed recently by Jackson et al [13], the major limitations of these earlier studies were as follows: the excessive volumes (0.5–8.4 L) of yogurt consumed daily in most of the positive studies, failure to assess or control for the background diet and exercise patterns of the subjects studied, failure to randomize groups for confounding factors, lack of run-in periods during which the volunteers adapted to the diet, lack of multiple baseline measurements and changes in control groups. Jackson et al [13] concluded that experimental evidence does not support a hypocholesterolemic effect for probiotics when consumed in easily achievable quantities.

Reduction of risk of disease

Rotavirus-induced diarrhea and possibly colon cancer are the only disorders for which there is evidence of disease reduction from probiotic consumption. For *rotavirus* diarrhea, different groups of investigators working under a variety of conditions have consistently reported that humans consuming probiotic fermented dairy products had a shortening of episodes or a reduction in risk of the disease [9]. For colon cancer, the evidence comes exclusively from experimental animals, in which *lactobacilli* and *bifidobacteria* were shown to decrease the number of aberrant crypt foci, a marker for risk of cancer development after treatment with a chemical carcinogen [14].

PREBIOTICS

The only prebiotics for which sufficient data have been generated to allow an evaluation of their possible classification as functional food ingredients are the inulin-type fructans, which include native inulin, enzymatically hydrolyzed inulin or oligofructose, and synthetic fructooligosaccharides [15,16]. Inulin has been defined as a polydisperse carbohydrate material consisting mainly, if not exclusively, of β -[2-1] fructosyl-fructose links. Inulin-producing plant species are found in several monocotyledonous and dicotyledonous families, including Liliaceae, Amaryllidaceae, Gramineae and Compositae. However, only one inulin-containing plant species (Chicory, *Cichorium intybus*) is used to produce inulin industrially. In chicory inulin, both Gpy-Fn [α -D-glucopyranosyl- [β -D-fructofuranosyl] n-1 β -D-fructofuranoside] compounds are considered to be included under the same nomenclature, and the number of fructose units varies from 2 to >70. Native inulin is processed by the food industry to produce either short-chain fructans, specifically oligofructose (degree of polymerization: 2–10; average: 5) as a result of partial enzymatic hydrolysis (inulinase, EC 3.2.1.7), or long-chain fructans by applying an industrial physical separation technique. An analytic method has been developed to quantify inulin and oligofructose in plants and food products. After a multicenter validation ring test, this method was adopted as Association of Official Analytical Chemists method 997.08: “Fructans in food products, ion exchange chromatography method” [17]. Inulin and oligofructose are present in significant amounts in miscellaneous edible fruit and vegetables; average daily consumption has been estimated at 1–4 g in the United States and 3–11 g in Europe. The most common sources are wheat, onion, banana, garlic and leeks [18]. Chicory inulin and oligofructose are officially recognized as natural food ingredients in most European countries, and they have a self-affirmed generally recognized as safe status in the United States. The synthetic inulin-type fructans are obtained by enzymatically catalyzed fructosyl transfer (using fungal fructosyl transferase) from and between sucrose molecules. In Europe, these are classified as novel foods. Inulin-type fructans are used as sugar substitutes, as fat replacers (inulin only), and as a means of providing texture, stabilizing foam, or improving mouth feel in miscellaneous products such as fermented dairy products; desserts such as jellies and ice creams; bakery products such as cookies, breads and pastries; spreads; and infant formulas. In a recent consensus paper inulin-type fructans were classified as non-digestible oligosaccharides [19].

HEALTH BENEFICIAL EFFECTS OF PREBIOTICS

Fate in the gastrointestinal tract

Because of the β -configuration of the anomeric C-2 in their fructose monomers, inulin-type fructans resist digestion in the upper part of the gastrointestinal tract. Moreover, there is evidence that they are not absorbed to any significant extent. Thus, it has been proposed that they be called a colonic food (i.e. a food entering the colon and serving as a substrate for the endogenous bacteria, thereby directly providing the host with energy and metabolic substrates). The idea that inulin-type fructans are fermented by bacteria colonizing the large bowel is supported by many in-vitro (both analytic and microbiological) and in-vivo studies, which, in addition, confirm the production of lactic and short-chain carboxylic acids as end products of the fermentation. Furthermore, it was shown in human in vivo studies that this fermentation leads to the selective stimulation of growth of the *bifidobacteria* population, making inulin-type fructans the prototypes of prebiotics [2].

Effect on mineral absorption

The non-digestible carbohydrates (dietary fiber) have been reported to impair the small-intestinal absorption of minerals because of their binding or sequestering action. However, the minerals that are bound or sequestered and, consequently, not absorbed in the small intestine, do reach the colon, where they may be released from the carbohydrate matrix and absorbed. Moreover, a high concentration of short-chain carboxylic acids resulting from the colonic fermentation of the non-digestible carbohydrates facilitates the colonic absorption of minerals, particularly Ca^{2+} and Mg^{2+} . In addition, independent of any binding or sequestering of minerals, some non-digestible carbohydrates [e.g., inulin-type fructans] may improve mineral absorption and balance because of an osmotic effect that transfers water into the large bowel, thus increasing the volume of fluid in which these minerals can dissolve. Furthermore, these carbohydrates, being extensively fermented, acidify the colonic content and consequently raise the concentration of ionized minerals, in particular Ca^{2+} and Mg^{2+} , a condition that favors passive diffusion. Finally, studies of ileostomy patients showed that inulin-type fructans do not impair mineral absorption in the small intestine [20]. Indeed, this study showed that the amount of calcium, magnesium and iron ions recovered in the ileostomate over 3 days is not significantly modified after supplementing the diet with 17 g of these fructans daily. It was reported that inulin-type fructans enhance Ca^{2+} and Mg^{2+} absorption in growing rats and increase ionic iron and Zn^{2+} balance without having a significant effect on Cu^{2+} bioavailability [21]. More recently, in-vivo human studies confirmed the positive effect of inulin and oligofructose on the absorption and balance of dietary calcium but not of iron, magnesium or zinc. In the first published report, 9 men (mean age: 21.5 ± 2.5 yrs) who ingested 850 mg Ca^{2+} /day and a dietary supplement of 40 g/day of inulin had a significant increase in the apparent absorption [$\pm 12\%$] and balance [100 mg/day] of calcium without any significant change in urinary excretion [21]. In the second study, the calcium balance of 12 males between the ages of 15 and 18 yrs who consumed 16.8 g oligofructose/day, as measured by the double

stable isotope technique, increased 11% [P =0.09] with no significant effect on urinary excretion [22].

Effect on the metabolism of lipids

The effects of inulin-type fructans on triglyceridemia have been studied in both animals and humans. In rats, a decrease in serum triglyceridemia (in both the fed and the fasted state) was consistently reported in several studies; in healthy humans, only fasting triacylglycerol was measured, and it was modified in only one study. No data have been published for hypertriglyceridemic patients. Data concerning the effects of inulin-type fructans on cholesterolemia or lipoproteinemia are scarce [14]. Feeding rats a diet supplemented with oligofructose [10% in the diet] significantly lowers serum triacylglycerol and phospholipid concentrations but does not modify free fatty acid concentrations in serum. The hypotriglyceridemia is due mostly to a decrease in the concentration of plasma VLDL. This effect likely results from a decrease in the hepatic synthesis of triacylglycerol rather than from a higher catabolism of triacylglycerol-rich lipoproteins. Hepatocytes isolated from oligofructose-fed rats have a slightly lower capacity to esterify [¹⁴C] palmitate into triacylglycerol but a 40% decrease in capacity to synthesize triacylglycerol from [¹⁴C] acetate [23]. These data support the hypothesis that decreased de-novo lipogenesis in the liver through a coordinate reduction of the activities of all lipogenic enzymes is a key event in reducing VLDL triacylglycerol secretion in fructan-fed rats. That de-novo lipogenesis is the basis for the hypotriglyceridemic effect of fructans in the rat liver might explain the lack of effect observed in healthy humans, who ordinarily eat much less carbohydrate than do rodents. Some experiments should be performed in either obese patients or insulin-resistant persons consuming high-carbohydrate, high-energy diets [14]. To explain a possible effect of inulin-type fructans on the modulation of triacylglycerol metabolism, 2 effects are hypothesized. The first effect is the modification of glucose or insulin concentrations, because dietary modulation of lipogenesis is often linked to such physiologic changes. Indeed, the induction of lipogenic enzymes by glucose, occurring via an increased gene transcription, is potentiated by insulin. This association has also been shown for resistant starch, which in rats' decreases serum triacylglycerol concentrations, reduces fatty acid synthase activity by 20%, and concomitantly lowers postprandial insulinemia [24]. The second effect is the production in the large bowel of short-chain carboxylic acids, which results in a more than 2-fold increase in the portal concentration of both acetate and propionate in oligofructose-fed rats. Moreover, propionate was reported to inhibit fatty acid synthesis, whereas acetate is a lipogenic substrate.

Long-term (16 wk) administration of oligofructose also decreases total serum cholesterol concentrations in rats, but does not influence either the absorption of dietary cholesterol or the excretion of cholesterol or bile acids in ileostomy subjects. Preliminary data for slightly hypercholesterolemic human volunteers indicate that inulin [18 g/day for 3 wk] may lower both total and LDL serum cholesterol [25].

Reduction of risk of disease

For inulin-type fructans, claims that they reduce the risk of disease are only tentative and still need to be supported and validated by further research. These claims include the following: Constipation relief resulting from fecal bulking and possible effects on intestinal motility and suppression of diarrhea, especially when associated with intestinal infections [26].

Reduction of risk of osteoporosis if inulin-type fructans improve the bioavailability of calcium and if this functional effect is followed by a more physiologic change in peak bone density and mineral bone mass [27].

Reduction of the risk of atherosclerotic cardiovascular disease associated with dyslipidemia, especially hyper-triglyceridemia, and insulin resistance, which in particular is known to be associated with hyper-energetic, high-carbohydrate feeding regimens [28]. The reduction of risk via a hypocholesterolemic effect needs further investigation as does the proposal of a sound mechanistic hypothesis to be tested in humans [29]. Reduction of the risk of obesity and possibly of type 2 diabetes, both of which are known to be associated with insulin resistance [30].

Cancer is a last area for further research on the ability of inulin and oligofructose to reduce risk of disease [31]. In 2 studies, feeding rats with inulin significantly reduced the incidence of the so-called aberrant crypt foci induced by such colon carcinogens as azoxymethane and dimethylhydrazine [26,27]. In the strategy for functional food development [3], finding cancer inhibitory effects in experimental animals is the first step, i.e., identifying effects that, because of their potential implications for human health, will require careful evaluation, including relevant human studies.

SYNBIOTIC: COMBINATION OF PROBIOTICS AND PREBIOTICS AS FUNCTIONAL FOOD.

The term synbiotic is used when a product contains both probiotics and prebiotics. Because the word alludes to synergism, this term should be reserved for products in which the prebiotic compound selectively favors the probiotic compound. In this sense, a product containing oligofructose and probiotic *bifidobacteria* would fulfill the definition, whereas a product containing oligofructose and a probiotic *Lactobacillus casei* strain would not. However, one might argue that synergism is attained in vivo by ingestion of *lactobacilli* on the one hand and promotion of indigenous *bifidobacteria* on the other hand [31-33].

HEALTH BENEFICIAL EFFECTS OF SYNBIOTICS

The strength of experimental evidence supporting claims of a functional effect from probiotics and prebiotics is as strong, promising, or preliminary or as no effect or unknown. Some probiotics are known to alleviate lactose intolerance [34]. The selective stimulation of growth of bifidobacteria in the colonic microbiota has been shown for inulin-type fructans in both experimental and human studies [35]. The scientific evidence for a bifidogenic effect is strong and can be used to support an application for an enhanced function claim of modifying the composition of the colonic flora [36]. At least 3 important questions remain to be

answered, however: (1) what is the persistence of the “bifidogenic” effect while maintaining a fructan-rich diet and after this diet is terminated? (2) What is the possibility of functional effects owing to the so-called “synbiotic” approach, which combines fructans as prebiotics with a probiotic microbial strain? (3) What are the healths benefits of having a colonic flora in which *bifidobacteria* predominate? A second effect worth considering when discussing potential improvement of a functional effect is increased bioavailability of minerals [37]. Although data on the balance of magnesium, iron or zinc are too preliminary to be taken into consideration, scientific evidence exists to support effects of inulin-type fructans on calcium absorption in both experimental animals and humans and the evidence is assessed as promising [38]. Such a promising observation opens a new avenue for research and might have health implications if this effect is shown to contribute to reducing the risk of osteoporosis. Data for the effect of probiotics on the bioavailability of minerals are not available. The effects on lipid metabolism of both probiotics and prebiotics are also discussed in this review. Experimental data are convincing in support of the hypothesis that one prebiotic [oligofructose] inhibits hepatic lipogenesis in rats and consequently induces a significant hypotriglyceridemic effect [39]. Hypothesized mechanisms of this effect include metabolic effects of short-chain carboxylic acids and the lowering of glycemia or insulinemia. Except for 2 studies [13,37], however, this hypolipidemic effect has not yet been confirmed in human volunteers. When assessing these data, I conclude that preliminary evidence exists for a hypotriglyceridemic effect of oligofructose and possibly inulin but not for the synthetic fructans [40]. The effect of probiotics on triglyceridemia has not been evaluated [41].

With regard to cholesterolemia, even if a large daily intake of yogurt has a lowering effect, the present state of scientific knowledge does not support the conclusion that probiotics have any significant hypocholesterolemic effect [42]. Similarly, with present knowledge, it is not possible to conclude that prebiotics have a hypocholesterolemic effect, but preliminary evidence is suggestive.

Because a metabolic link was recently shown between insulin resistance and the associated risk factors for atherosclerotic cardiovascular disease, especially hyper-triglyceridemia and because of the growing awareness that hyper-triglyceridemia itself may be a risk factor in atherogenesis, the potential functional effects described, primarily of prebiotics, need to be studied carefully in humans, especially in conditions known to be associated with hyper-insulinemia and hyper-triglyceridemia. Other effects of probiotics on body function for which preliminary evidence is available are immuno-stimulation, decrease in fecal mutagenicity and change in fecal enzyme activities [43-45].

Reduction of disease risk

With regard to a possible role for probiotics or prebiotics in reducing the risk of diseases, the evidence is much more limited; in most cases it is either preliminary or there is no evidence at all. The only areas where evidence can be considered promising are diarrhea (probiotics) and constipation (prebiotics). For both types of food products, preliminary animal data support anti-carcinogenic effects. Even though prebiotics improve calcium absorption,

their positive role in reducing the risk of osteoporosis needs to be supported by more human studies. Neither the probiotics nor the prebiotics can be claimed to reduce the risk of cardiovascular disease, but the hypolipidemic effects of inulin-type fructans should be investigated further in at-risk human populations [46,47].

Similarly, to prevent/treat colorectal cancer, several methods are available. Almost all the strategies have some limitations. One of the promising new ways to prevent/treat colorectal cancer is to use synbiotics, which is a combination of pro- and prebiotics. It has been observed that the administration of synbiotics may be beneficial in the prevention of initiation/early stage of cancer, as well as in the treatment of existing tumors. This subject needs further investigation which seems to be a promising new strategy for prevention and treatment of colorectal cancer [48].

CONCLUSION

Probiotics are already widely used to prepare fermented dairy products that are becoming popular in Europe and Japan. These products favorably influence digestive functions and colonic flora. The most promising health benefits are the prevention of diarrhea and enhancement of the immune system. These 2 effects may be mechanistically related. As we discussed previously [19], dietary carbohydrates make up a large family of miscellaneous compounds with different physiologic effects and diverse nutritional properties that deserve the attention of nutritionists. In the carbohydrate family, the indigestible oligosaccharides are of particular interest and in the next decade, be one of the most fascinating functional food ingredients. Inulin-type fructans are indigestible oligosaccharides for which a wide range of scientific observations are already available and which demonstrate an array of potential health benefits. To justify claims of enhanced function or reduction in the risk of disease, most of the available information must be confirmed in humans in relevant nutrition studies focusing on well-validated endpoints. Such studies will be of much value if they are based on sound mechanistic hypotheses. Changes in the composition of the colonic microbiota, modulation of the metabolism of triacylglycerol, modulation of insulinemia, improved bioavailability of dietary calcium, and negative modulation of colon carcinogenesis are the most promising areas for further research. In addition to having nutritional properties, which may justify their classification as functional food ingredients, inulin-type fructans are low-energy carbohydrates [49], which have interesting technologic properties in food product development. These properties depend on the molecular structure of the various inulin-type fructans, especially their degree of polymerization, which determines their water solubility, viscosity, water retention capacity, and capacity to form a creamlike texture, a property of inulin used to process it as a fat replacer. As we suggested previously [2], combining probiotics and prebiotics in what has been called a synbiotic could beneficially affect the host by improving survival and implantation of live microbial dietary supplements in the gastrointestinal flora, by selectively stimulating the growth or activating the catabolism of one or a limited number of health-promoting bacteria in the intestinal tract, and by improving the gastrointestinal tract's microbial balance, but the creation of a synbiotic has not been investigated. Combining probiotics with prebiotics could improve the survival of the bacteria crossing the upper part of

the gastrointestinal tract, thus enhancing their effects in the large bowel. Moreover, probiotic and prebiotic effects might be additive or even synergistic. This has been the case when combining the anti-carcinogenic effects of inulin and bifidobacteria in experimental animals [27].

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