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PROBIOTICS, PREBIOTICS AND SYNBIOTICS

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ABSTRACT

The benefits of Probiotics have been recognized and explored for over a century. Probiotics consist of bacteria or yeasts and can be considered functional foods that can re-colonize and restore the microflora symbiosis of the intestinal tract. Several health benefits associated with the Probiotics in various diseases includes Inflammatory Bowel Disease (IBD), Colon Cancer, Rotavirus-associated diarhoea, H.Pylori infection and Liver disease etc. Prebiotics are “non-digestible 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”. Dietary modulation of the gut microflora by Prebiotics is designed to improve health by stimulating numbers and/or activities of Probiotics like Bifidobacteria and Lactobacill.This review also discuss the results of randomized controlled clinical trials that used Prebiotics to treat IBD, Hypertension, Colon Cancer, Diabetes and Hepatic Encephalopathy as well as their potential applications. The combination of Probiotics and Prebiotics is known as Synbiotics. Recently Synbiotics have been proposed as a new therapeutic option in Pediatric surgery, Digestive organ surgery, Liver disease and Systemic Inflammatory Response Syndrome (SIRS). This article provides the overview of how Pro-, Pre- and Synbiotics contribute towards various health benefits.

INTRODUCTION:

Probiotics: Probiotics were first described by Metchnikoff in 1908 based on his observations on the longevity of individuals who lived in a certain part of Bulgaria and which he attributed to their ingestion, on a regular basis, of a fermented milk product. Probiotics, derived from the Greek and meaning “for life”, are defined as live organisms that, when ingested in adequate amounts, exert a health benefit to the host [1]. There are several commercially available supplements containing viable micro-organisms with probiotic properties.

There are several criteria for organisms as Probiotics include: It should be isolated from the same species as its intended host, Have a demonstrable beneficial effect on the host, non-pathogenic, and be able to survive transit through the gastrointestinal tract [2], large number of viable bacteria must be able to survive prolonged periods of storage [3, 4].

Examples of Probiotics are shown in **Table 1**.

TABLE 1: ORGANISMS AS PROBIOTICS [5, 6, 7]

L. casei CHCC3137	*L. plantarum Q47*	*B. bifidum DSM20239*
L. casei D12	*L. plantarum/pentosus I12*	*B. bifidum Z9*
L. casei F19	*L. reuteri DSM 12246*	*B. breve DSM20091*
L. casei Nikka	*L. reuteri E14*	*B. breve DSM20213*
L. casei 61R3	*L. reuteri M.7.1*	*B. longum subsp. infantis 20088*
L. casei 8R2	*L. rhamnosus 19070-2*	*B. longum Q45*
L. acidophilus NCFM	*L. rhamnosus E5*	*B. longum Q46*
L. acidophilus X37	*L. rhamnosus G26*	*B. longum Q50*
L. gasseri I23	*L. rhamnosus GG*	*B. longum Z8*
L. paracasei A14	*L. ruminus Q95*	*B. animalis subsp. lactis Bb 12*
L. paracasei B32	*L. delbrueckii subp Bulgaricus*	*B. longum M.16.2*
L. paracasei CRL431	*L. brevis*	*B. breve M.97.2*
L. paracasei Q85	*L. cellobiosus*	*B. longum ssp. infantis P30.5*
L. paracasei Z11	*L. curvatus*	*B. bifidum S.13.1*
L. salivarius	*L. johnsonii*	*B. infantis*
L. helviticus	*L. farciminis*	*B. thermophilum*
L. paraplantarum D14	*L. fermentum*	*B. adolescents*
L. plantarum 299v	*B. bifidum Bb-14*	*B. lactis*
L. plantarum M.1.1	*B. bifidum DSM20082*	*B. animalis*
B. breve	*Streptococcus thermophilus*	*Enterococcus faecium*
Lactococcus lactis	*Escherichia coli Nissle 1917*	*Propionibacterium freudenreichii*
Bacillus clausii	*Bacillus oligonitrophilis*	*Saccharomyces cerevisiae*
Bacillus coagulans	*Pediococcus acidilactici*	*Saccharomyces boulardii*

Criteria for the use of Probiotics in humans [8]: Identified at the genus, species, and strain level, safe for food and clinical use (nonpathogenic, susceptible to antibiotics), able to survive intestinal transit, able to adhere to mucosal surfaces, able to colonize the human intestine or vagina (at least temporarily), producing antimicrobial substances, able to antagonize pathogenic bacteria, clinically documented and validated health effects.

Prebiotics: Prebiotics are "non-digestible 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" [9]. For a dietary substrate to be classed as prebiotic, criteria required are [10]:

The substrate must not be hydrolyzed or absorbed in the stomach or small intestine; it must be selective for beneficial commensal bacteria in the large intestine such as the bifidobacteria, Fermentation of the substrate should induce beneficial luminal/systemic effects within the host.

The rationale behind prebiotic use is to elevate the endogenous numbers of beneficial bacterial strains including lactobacillus and bifidobacterium [11]. This increase will impart the beneficial effects seen by probiotic administration, including an increase in SCFA production, particularly butyrate, which can provide fuel for enterocytes, prevention of pathogenic adherence, production of anti-bacterial substances, and decreased luminal pH [12].Examples of Prebiotics are shown in **Table 2**.

TABLE 2: SUBSTRATES AS PREBIOTICS [13, 14, 15]

Inulin (legumes, vegetables and cereals)	Fructo- oligosaccharides – FOS (legumes, vegetables, cereals)	Xylo-oligosaccharides (plant sources)
Resistant starches (legumes, vegetables and cereals)	Soybean oligosaccharides (soy bean)	Trans Galacto-oligosaccharides (lactose synthetic)
Lactulose (lactose synthetic)	Lactitol (lactose synthetic)	Goat's milk oligosaccharides

Germinated Barley Foodstuff (High glutamine rich protein & hemi-cellulose-rich dietary fiber)	Human breast milk oligosaccharides	Gluco-oligosaccharides
Glyco-oligosaccharides	Isomalto-oligosaccharides	Malto-oligosaccharides
Stachyose	Raffinose	Sucrose thermal oligosaccharides
Gentio-oligosaccharides		

Synbiotics: Synbiotics refer to nutritional supplements combining Probiotics and Prebiotics that are thought to act together; i.e. synergism. It has been suggested that a combination of a probiotic and a prebiotic, i.e. Synbiotics, might be more active than either a probiotic or prebiotic alone in preventing GI Disorders [16, 17]. The potential benefits of synbiotic therapy are obvious, however, the great challenge, as is the case with Probiotics and Prebiotics alone, is to determine the best combination for each disease setting and each individual.

The first attempts should be to combine Probiotics and Prebiotics which have demonstrated individual benefits to determine if there are additive effects, alternatively, a more structured approach would be to determine the specific properties that a prebiotic requires to be beneficial to the probiotic and select the prebiotic accordingly [18].

Example of Synbiotics includes [19]: Bifidobacteria + Fructo-oligosaccharides, Lactobacilli + Lactitol, Bifidobacteria + Galacto-oligosaccharides

Probiotics & Health Benefits:

Inflammatory Bowel Disease: Inflammatory bowel disease (IBD) comprises a spectrum of disorders characterized by inflammation, ulceration and abnormal narrowing of the gastrointestinal tract resulting in abdominal pain, diarrhoea and gastrointestinal bleeding [20]. The role of intestinal microorganisms as the mediators of this intestinal inflammation is supported by a number of clinical findings.

A number of investigators have used Probiotics in patients with IBD. In 1997, Kruis *et al*., randomized 103 patients with inactive ulcerative colitis to receive a non-pathogenic *E. coli* or Mesalazine for 12 weeks [21]. Malin and colleagues administered *Lactobacillus GG* to 14 children with Crohn's disease and demonstrated enhanced serum IgA levels, suggesting immunostimulation.

Probiotics have also been used in chronic relapsing pouchitis [22]. Gienchitto and colleagues prescribed a mixture of eight different probiotic strains to twenty patients with pouchitis [23]. Several in vitro studies on cell models of IBD have shown the ability of certain probiotic strains such as *L. rhamnosus* GG to modulate the immune system by down regulating TNF-a-induced IL-8 production [24]. *In vivo* animal studies have indicated the importance of commensal bacteria in the development of a functional immune system. *B. lactis* Bb12 initially elevated levels of IL-6 expression, but rats maintained normal gut histology [25]. Several studies have shown that Probiotics might have had beneficial effect on IBD patients.

Alleviation of Lactose Intolerance: Lactose upon ingestion is hydrolyzed by lactase in the brush border membrane of the mucosa of the small intestine into constitutive monosaccharides, glucose and galactose, which are readily absorbed in the blood stream. However, the activity of intestinal lactase in lactose intolerant individuals is usually less than 10% of childhood levels [26]. This decline, termed hypolactasia, causes insufficient lactose digestion in the small intestine, characterized by an increase in blood glucose concentration or hydrogen concentration in breath upon ingestion of 50 g lactose, conditions designated as lactose maldigestion [27].

Probiotics have been shown to improve lactose digestion by reducing the intolerance symptoms as well as by slowing orocecal transit [28].

Pediatric Intestinal Disease: Breastfed babies have a predominant colonization with *E. coli* and *Streptococci bifidobacteria*, while those fed with formula milk have microbiotas with predominance of bifidobacteria, bacteroidi, clostridia and other enterobacteria. The gradual establishment of the flora from the early hours of life allows modulating the immune response in favor of the acquisition of oral tolerance [29, 30], defined as "specific immunological hyporesponse to a previous exposure to mucosal antigen".

Infants who are fed with formula milk supplemented with probiotic bacteria may experience promotion of the natural production of this immunoglobulin. Supplementation with Probiotics is generally considered safe because they are identical to the microorganisms present in vaginal flora and in the human gastrointestinal tract [31].

Necrotizing Enterocolitis: Necrotizing enterocolitis (NEC) is a major cause of morbidity and mortality in premature infants; the etiology of this disease has not yet been fully clarified. Based on observations in animal models, some studies evaluated the effects of Probiotics supplementation on the incidence of NEC in newborns. A recent review examined the results of 11 studies, showing that the risk of NEC and death in the population treated with Probiotics is lower and confirming significant benefits of a supplementation with Probiotics in premature and very-low birth weight infants [32].

Infantile Diarrhoea: Rotavirus is the most common cause of acute childhood diarrhea in the world and is an important cause of infant mortality. This condition is generally self-limiting and treatment is supportive, with careful replacement of fluid and electrolyte losses. In a study of 100 children, admitted to hospital with acute diarrhea, Guarino demonstrated a reduced duration of diarrhea in children receiving *Lactobacillus GG* (3 days) compared with controls (6 days) [33]. Probiotic therapy has been aimed at the treatment of established rotaviral infections in children and also in prevention of the disease.

The potential benefit of probiotics in the treatment of rotavirus infection appears, however, to be a strain related phenomenon. In summary, there is good evidence to support the use of Probiotics in patients with infantile diarrhea [2].

Traveller's Diarrhoea: Diarrhoeal illness is common in travellers. Bacterial infections with enterotoxigenic *Escherichia coli*, *Shigella* species, *Campylobacter jejuni* and *Salmonella* cause at least 80% of traveller's diarrhoea [34]. A wide variety of Probiotics have been used to treat or as prophylaxis against diarrhoea with varying results. Trevis capsules, containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were administered to 195 Danish tourists in Egypt and conferred a protection rate of 39% against diarrhoea [35]. Given the contrary nature of these results, it is difficult to advocate the use of Probiotics in travelers at the present time.

Antibiotic Associated Diarrhoea: The use of antibiotics is widespread and is often associated with gastrointestinal side effects such as diarrhoea. *Pseudomembranous colitis* is a serious, occasionally fatal, complication of antibiotic therapy which is characterized clinically by profuse diarrhoea, toxemia and 'pseudomembrane' formation at sigmoidoscopy [36]. Many Probiotics have been employed in the treatment of antibiotic related and clostridial diarrhoea, with variable results. A recent study in 188 children who received oral antibiotics for acute infectious disorders, also demonstrated decreased stool frequency when *Lactobacillus GG* was given concomitantly [37]. Notwithstanding the encouraging results demonstrated with the use of *Lactobacillus GG* the routine clinical use of Probiotics for all cases of antibiotic related diarrhoea cannot be justified at present.

Food Allergy Reaction: Human beings are exposed to numerous environmental antigens through food. The intestinal mucosa is efficient in assimilating antigens encountered by the enteric route, but high-level antigen exposure during the first few months of life may pre dispose individuals to allergic sensitization [38]. Oral introduction of Probiotics can help in treatment of such food allergies by alleviating intestinal inflammation. It has also been suggested that intestinal microorganisms could down-regulate the allergic inflammation by counter-balancing T-helper cell Type 2 responses and by enhancing antigen exclusion through induction of an IgA response [39]. Majamaa and Isolauri suggest that probiotic bacteria may promote endogenous barrier mechanisms in the patients with atopic dermatitis and food allergy [40].

Immunomodulatory activity: Immunomodulation by Probiotics is a subject of growing interest. *Lactobacillus rhamnosus* GG and *Propionibacterium freudenreichii* ssp. *shermanii* JS have shown a specific dose- and duration-dependent Immunomodulatory effects on the proliferative activity of murine B and T lymphocytes [41].

In another study the effects of heat-killed cells, cell walls, and cytoplasmic extracts of *Bifidobacterium*, *Lactobacillus acidophilus*, *L. bulgaricus*, *L. casei*, *L. gasseri*, *L. helviticus*, *L. reuteri*, and *Streptococcus thermophilus*, on cell proliferation and cytokine and nitric oxide (NO) production, were examined using the RAW 264.7 macrophage cell line and in murine cultures composed of peritoneal, spleen, and Peyer's patch cells.

Oral administration of *Bifidobacterium breve* YIT4064 has been reported to activate the humoral immune system by augmenting anti-rotavirus IgA production or anti-influenza virus, IgG production, and thus protect against rotavirus infection or influenza infection, respectively [42].

Hypocholesterolemic effects: 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 [28].

The administration of *Lactobacillus reuteri* CRL 1098 (10 cells /day) to hypercholesterolemic mice for 7 days decreased total cholesterol by 38%, producing serum cholesterol concentrations similar to that of the control group. This dose of *Lactobacillus reuteri* caused a 40% reduction in triglycerides and a 20% increase in the ratio of high density lipoprotein to low density lipoprotein without bacterial translocation of the native microflora into the spleen and liver [43].

Anti-mutagenic, Anti-genotoxic & Anti-carcinogenic activity: Antigenotoxicity, antimutagenicity and anticarcinogenicity are important potential functional properties of Probiotics, which received much attention recently. Mutagens are frequently formed during stress or due to viral or bacterial infections and phagocytosis but also commonly obtained via foods. Endogenous DNA damage is one of the contributors to ageing and age-related degenerative diseases. Some epidemiological researchers have emphasized that probiotic intake may be related to a reduced colon cancer incidence [44] and experimental studies showed the ability of lactobacilli and bifidobacteria to decrease the genotoxic activity of certain chemical compounds [45] and increase in antimutagenic activity during the growth in selected media [46].

Antimutagenic effect of fermented milks has also been detected against a range of mutagens and promutagens including 4-nitroquinoline-N0-oxide, 2-nitrofluorene, and benzopyrene in various test systems based on microbial and mammalian cells. Experiments carried out in animal models showed certain strains of *L. acidophilus* and *Bifidobacterium* spp. was capable of decreasing the levels of enzymes such as b-glucuronidase, azo reductase, and nitroreductase responsible for activation of procarcinogens.

This inactivation consequently led to a substantial decline of the risk associated with tumor development. Several studies have shown that preparations containing LAB inhibit the growth of tumor cells in experimental animals or indirectly lower carcinogenicity by decreasing bacterial enzymes that activate carcinogenesis [47]. Short-chain fatty acids produced by L. acidophilus and bifidobacteria were also reported to inhibit the generation of carcinogenic products by reducing enzyme activities.

Probiotics and Sepsis in surgical and critically ill patients: Septic complications in surgical and intensive care patients are common. The majority of Nosocomial infections are caused by intestinally derived organisms such as *E coli* [48]. There is increasing evidence to suggest that passage of these organisms across the intestinal barrier to normally sterile extra intestinal sites may be the cause of this septic morbidity. In the elective group, 64 patients were randomized to receive the *Lactobacillus plantarum* 299v in a fruit drink (Proviva™) for one week pre-operatively with 65 controls.

At the beginning of the trial, no prophylactic antibiotics were given to the probiotic group at the induction of anesthesia. The rate of bacterial translocation to mesenteric lymph nodes and serosa was documented as was the incidence of septic complications. In the intensive care unit, 45 critically ill patients were randomized to receive *Lactobacillus plantarum* 299v and compared with 45 controls. The rate of infective complications and clinical outcome were unchanged, however CRP and IL-6 levels and endotoxin exposure

did tend to be lower in the probiotic group, just failing to reach statistical significance. These findings suggest that Probiotics may modulate barrier function when it is deficient, but not when it is undisrupted, as in the elective situation above [2].

Inhibition of Helicobacter pylori and other intestinal pathogens: Probiotic cultures produce a wide range of antibacterial compounds including organic acids (e.g., lactic acid and acetic acid), hydrogen peroxide, bacteriocins, various low-molecular mass peptides, and antifungal peptides/proteins, fatty acids, phenyllactic acid, and OH-phenyllactic acid. Lactic and acetic acids are the main organic acids produced during the growth of Probiotics and their pH lowering effect in the gastrointestinal tract has a bactericidal or bacteriostatic effect.

Moreover, a heat-stable, low- molecular- weight antibacterial substance different from lactic acid was present in the cell-free culture supernatant resulting in the inactivation of a wide range of Gram-negative bacteria and inhibition of the adhesion to and invasion of Caco-2 cells by *Salmonella enteric* ser. typhimurium [49, 50]. *Helicobacter pylori* are an intestinal pathogen, long-term infection by which leads to chronic gastritis, peptic ulcer and increases the risk of gastric malignancies [51]. Probiotic organisms do not appear to eradicate *H. pylori*, but they are able to reduce the bacterial load and inflammation in animal and human studies. It has been suggested that the suppression effect is strain dependent [52]. *L. casei* Shirota strain showed a significant reduction in the levels of H. pylori colonization in the antrum and body mucosa in vivo mouse model [53].

Recent study concluded that regular intake of yogurt containing *B. animalis* Bb12 and *L. acidophilus* La5 may effectively suppress *H. pylori* infection in humans [54.] In the other studies in humans treated either with lyophilized culture of *L. brevis* [55] or yogurts containing L. acidophilus and *B. lactis* [54] *or L. johnsonii* La1 [56,] a decrease in the *H. pylori* bacteria load was observed indirectly via the urea breath test.

Respiratory Infections: All Probiotics induce an immune response, whose characteristics are related to the strain or the combination of bacteria that have been used. Recent studies have shown positive effects of Probiotics on the respiratory system, especially in preventing and reducing the severity of respiratory infections, due to an increase in IgA-secreting cells in the bronchial mucosa [57.] Hereafter, we present the results of studies performed on different target populations aimed at investigating the effects of Probiotics on infectious diseases of the respiratory system.

Irritable Bowel Syndrome: The pathogenesis of irritable bowel syndrome remains unknown, and abnormal neural activation, neural innervations, or abnormal innate immune responses have all been implicated. The therapy of irritable bowel syndrome has remained largely unsatisfactory. Against this background, there have been attempts to evaluate a role for Probiotics in the therapy of patients with irritable bowel syndrome.

Several recent meta-analyses have examined the evidence for benefit from Probiotics in irritable bowel syndrome [58] in all analyses, it appeared that Probiotics were associated with a modest benefit (approximately 20-24%) compared to placebo. These were short term studies, and since irritable bowel syndrome requires long term therapy whether Probiotics will provide sustained clinical benefit over longer periods of time, and whether they are useful in specific sub-groups, needs to be evaluated.

Liver disease: In recent years, it has been recognized that several of the manifestations of chronic liver disease including encephalopathy, endotoxemia and bacterial peritonitis have primary origins in the gut through translocation of molecules or bacteria past the intestinal barrier. Probiotics enhance intestinal barrier function; they also influence innate immune activity and adaptive immunity.

Probiotics have therefore been tried in the therapy of chronic liver disease. Small pilot studies or open label studies suggest that Probiotics may benefit patients with non-alcoholic steato-hepatitis, alcoholic liver disease, and minimal hepatic encephalopathy and lowers endotoxemia in liver cirrhosis [59, 60]. These findings need to be confirmed in large randomized trials before a place can be established for the use of Probiotics in chronic liver disease.

Urogenital Infections: Urogenital infections not caused by sexual transmission in women are still one of the most important medical issues. Recurrent urinary tract infection (UTI) is, in most cases caused by the uropathogens *E. coli*; recurrent bacterial vaginosis (BV) is usually caused by *Gardnerella vaginalis*; recurrent yeast vaginosis is usually caused by *Candida albicans*. The predominant bacteria in the urinary tract of healthy women are lactobacilli. Zuccotti *et al*., reported some studies highlighting that Probiotics could be a good alternative to antibiotic therapy due to their quality to adhere to uroepithelial cells and produce inhibitors of pathogenic growth and biosurfactant secretion [61].

Reid *et al*., reported that a daily intake of probiotic strains *L. rhamnosus* GR-1 and Lactobacillus fermentum RC-14 resulted in some asymptomatic BV patients reverting to a normal lactobacilli dominated vaginal microflora [62]. They reported a crossover study involving 33 patients with recurrent vaginitis treated with eight ounces of *L. Acidophilus* supplemented yogurt daily for six months and then switched to a yogurt free diet. Czaja *et al.*, performed a phase I trial to assess the safety and tolerance of a Lactobacillus vaginal suppository for prevention of recurrent UTI involving premenopausal women with a history of recurrent UTI [63].

Pancreatitis: Pancreatic necrosis and associated pancreatic infection are determinates of poor outcome in patients with severe acute pancreatitis, and the nature of microbial species inhabiting the intestine can influence subsequent infection rates. Two small randomized double-blind trials have been published by the same research group examining the effect of naso-jejunal treatment with *Lactobacillus plantarum* in patients with acute pancreatitis [64, 65]. Both trials compared live *L. plantarum* with killed bacteria as a control, and both showed significantly lower rates of infection in the groups treated with the live probiotic. Replication of these results, ideally in larger studies, would provide excellent evidence for probiotic use in this setting.

Clinical studies on Probiotics: A randomized, double-blind, placebo-controlled trial was performed to determine whether Probiotics may reduce the risk of infections in infants. The children involved in the research were younger than 2months of age and were daily provided with milk containing *L. rhamnosus* GG and *Bifidobacterium lactis* Bb-12, or placebo milk, administered until 12 months of age. The results suggest that Probiotics may represent a mean to reduce the risk of early acute otitis media and the use of antibiotics for recurrent respiratory infections during the first year of life [66].

Similar results have emerged in a study performed on a target population of 326 children aged between 3 and 5 years, showing more than 65% decrease in the incidence of antibiotic use and 25% reduction in school missed days among children treated with Probiotics [67].

A randomized double-blind, placebo-controlled trial assessed whether the consumption for 3 months of Lactobacillus gasser PA 16/8, *Bifidobacterium longum* SP 07/3, B. bifidum MF 20/5, had impacts on symptoms severity, incidence and duration of common cold. Similar conclusions were obtained in a study that assessed the effect of long-term intake of Probiotics on the same pathology [68].

Two multicentre, randomized, controlled, double-blind studies were conducted in two successive vaccine seasons (pilot study and control). 86 and 222 elderly volunteers consumed, respectively, a fermented milk drink containing *L. casei* DN-114 001, a fermented yogurt or a control unfermented dairy product, twice a day for a period of 7 or 13 weeks. Vaccination took place after 4 weeks. The study showed that Probiotics improve antibody responses to influenza vaccination in individuals over 70 years [69]. *L. casei* DN-114001 was also evaluated in a multicentre, double-blind, controlled study on 1.072 elderly, to assess the resistance to respiratory infections. The product containing Probiotics, well tolerated, induced a reduction in the duration of respiratory infections, especially URTI and nasopharyngitis [70].

Recombinant Probiotic:

Subalin: A new probiotic class based on recombinant strains of bacteria has been designed to produce predetermined therapeutic proteins. The biological properties of *Bacillus subtilis* 2335 strain were transformed by the plasmid encoding the synthesis of human interferon alpha-2. The recombinant strain was demonstrated to preserve the high antagonistic

activity of the parent culture (biosporin) and to acquire marked antiviral properties due to interferon synthesis. Using this strain, Sorokulova *et al*., designed the new probiotic possessing a combination of antibacterial and antiviral properties [71]. Clinical and bacteriological investigations during administration and after termination of the course of oral administration of subalin have suggested innocuousness as well as to the absence of any side-effects of subalin [72]. Both biosporin and subalin have been proven to be safe when injected intravenously and intraperitoneally into animals at a dose of 5310 cells per 0.5 ml of physiological saline [73].

Subalin has been tested for its ability of increasing the effectiveness of anti-tumor therapy with cyclophosphamide. Furthermore, the application of gene engineering methods may aid in designing a new generation of Probiotics with predict able biological properties.

Prebiotics & Health Benefits:

Inflammatory Bowel Diseases: In Experimental colitis, Studies using Prebiotics have been performed mostly in animal models. Lactulose and Inulin have been shown to attenuate inflammation in IL-10 knockout mice and DSS-induced colitis respectively [74, 75]. The combination of Inulin and oligofructose (mixture 1:1) is also effective in preventing the development of colitis in HLA-B27 transgenic rats [76]. This beneficial effect is observed in conjunction with an increase of intestinal bifidobacteria and lactobacilli. DSS-induced colitis rats fed with goat's milk oligosaccharides maintain their body weight, have reduced colonic Myeloperoxidase activity and clinical symptoms and increased MUC-3 expression compared with control rats [77]. Lactulose has also demonstrated a dose-dependent beneficial effect on DSS induced colitis in rats, including improvements of colonic ulceration areas, body weight changes, diarrhea, bloody stools and a reduction of Myeloperoxidase activity and Microscopic colitis [78].

Several studies have investigated the use of an insoluble mixture of glutamine-rich protein and hemicellulose-rich dietary fiber termed germinated barley foodstuff (GBF). Fukuda *et al* found that feeding GBF to rats with DSS induced colitis results in significantly reduced colonic inflammation scores, and increased butyrate concentrations in cecal contents [79].

In Ulcerative colitis, although there is a paucity of human studies using Prebiotics, but the few emerging studies showed that there is potential for this treatment modality. A multi-centered open-label trial reported that oral administration of GBF to patients with mild to moderately active UC for 24 wk results in a significant decrease in clinical activity index, compared to controls [80]. An open-label study of 22 UC patients in remission showed that a daily oral intake of 20 g GBF results in a significantly improved clinical activity index and endoscopic score at 3, 6 and 12 mo, and a reduced relapse rate, compared with controls [81].

A small, uncontrolled study of 15 active Crohn's disease patients reported that 21 d of Fructo-oligosaccharide (15 g) intake results in a significant decrease of disease activity, an increase of intestinal bifidobacteria and modifications of Toll-like receptors and IL-10 expression in mucosal dendritic cells [82].

Antihypertensive effects: Hypertension is one of the major risk factors for cardiovascular disease. Dietary components including Prebiotics are seen as better alternatives than drug therapy to treat hypertension considering that Prebiotics has a long history of safe use and has been a natural component present in our foods. Plant fibers are also found to exert prebiotic properties, health enhancing effects and strongly influence the metabolism of carbohydrates and lipids, which are directly associated with the increased risks of hypertension. Direct association of Prebiotics and antihypertension has been established via various *in-vivo* trials which is shown in following **table 3**.

TABLE 3: VARIOUS *IN-VIVO* TRIALS SHOWING ASSOCIATION BETWEEN PREBIOTICS & THEIR ANTI-HYPERTENSIVE ACTIVITY

I Intervention	Experimental Design	Subjects	Dose	Effects	Ref
Soluble fiber extracted from oat bran	Randomized, double-blind, placebo-controlled	n=110; 30 to 65 years; not on hypertension treatment; SBP of 125-159 mmHg and DBP of < 95 mmHg	8 g/d of fiber for 12 weeks	A reduction in SBP of 2mmHg & DBP of 1.0 mmHg	83

Diet containing soy protein isolate & supplementation of fiber extracted from psyllium	Randomized, double-blind, parallel	n=36; nonsmoking men or women > 20 years old; on anti hypertensive drug therapy for > 6 months ; SBP of 130-160mmHg	12 g fiber/d for 8 weeks	A reduction in SBP of 5.9 mmHg	84
Dietary fiber in the form of pill supplementation	Randomized, double-blind, parallel, placebo controlled	n=63; 18-70 yrs old; hypertensive with a minimum DBP of > 90 mmHg	7 g/d of dietary fiber for 12 weeks	A reduction in DBP of 5 mmHg	85
Beta-glucan from whole oats cereals	Randomized, parallel, pilot trial	n=18; 27-59 years old; healthy, untreated hypertensive with SBP of 130-160 mmHg and DBP of 85-100 mmHg	5.52 g/d of beta glucan for 6 weeks	A reduction in SBP of 7.5 mmHg and DBP of 5.5 mmHg	86
Bread substituted with lupin kernel flour	Randomized, parallel	n=74; 20-70 years old; overweight and obese men and women with BMI of 25-35; SBP<150 mmHg and DBP<95 mmHg	4 x 40g of bread/d for 16 weeks; Bread contained 9.5% w/w of fiber	A reduction in SBP of 3.0 mmHg	87

SBP: systolic blood pressure; DBP: diastolic blood pressure

Diabetes: Hypertension is highly associated with diabetes, where a lowering in cholesterol levels and improvement of glucose levels has been found to lower blood pressure. Dietary intervention is one of the main therapies proposed to diabetics, thus Prebiotics have gained increasing attention because of their beneficial effects on lowering blood glucose. Therefore, Prebiotics could act as antihypertensive agents upon the exhibition of blood glucose lowering effects. In a study evaluating the improvement of glucose tolerance via the intake of Prebiotics, Cani *et al.* administered an oligofructose-supplemented diet [10% (w/w)] to male mice for 14 weeks. The study used 32 male mice and the results showed that mice in the group consuming diet supplemented with oligofructose had improved glucose tolerance as compared to those in the control group [88]. Mice on the oligofructose-supplemented diet showed normal fasting plasma insulin levels and restored glucose induced insulin secretion while the control did not. Various studies have highlighted the beneficial effects of Prebiotics on physiological conditions such as lipid and glucose profiles that are directly associated with hypertension. Hence, there is a strong basis for continuous evaluation on Prebiotics specifically aimed at utilizing longer and larger *in-vivo* trials.

TABLE 4: *IN-VIVO* TRIALS SHOWING ASSOCIATION BETWEEN PREBIOTICS & THEIR EFFECT ON BLOOD GLUCOSE PROFILE

Intervention	Fibre	Dose; Duration of the study	Experimental Design	Animal/subjects	Effects	Ref
Blood Glucose	Alginate fiber	5.0-g sodium Alginate supplement (algae isolate, 75% soluble fiber); for two days	Randomized, Placebo controlled	Seven men with type 2 diabetes; mean age of 53 yr	Significantly reduced the postprandial rise in blood glucose (P<0.05) and in serum insulin (P<0.02) by 31% & 42%, resp.	89
	Soy hulls	26 g of soy hulls which Incorporated into 7 slices of bread daily; for 4 weeks	Randomized, double-blind, placebo controlled	10 subjects (5 male & 5 female) with type 2 diabetes; mean age of 65 ± 5.9 yr	Significantly improved the glucose score (P<0.05) & the total area under the glucose curve (P<0.05) by 6.7% & 7.1%, resp.	90

Hypocholesterolemic effect: Hypertension is highly associated with hypercholesterolemia, where a lowering in cholesterol levels has been found to lower blood pressure. In a study evaluating the influence of Prebiotics on cholesterol, Mortensen *et al.* administered a purified diet with 10% of long-chained fructans into male mice for 16 weeks [91]. The control was not fed any Prebiotics. The study involved 40 male mice and the results showed that the supplementation of fructans significantly reduced blood cholesterol by 29.7% (P<0.001), LDL-cholesterol concentration by 25.9% (P<0.01), IDL-cholesterol level by 39.4% (P<0.001) and VLDL-cholesterol concentration by 37.3% (P<0.05) compared to the control group.

Hypertriglyceridemia is often associated with a moderate hyperglycemia and insulinemia [92], and Prebiotics have been found to reduce hepatic triacylglycerol.

TABLE 5: *IN VIVO*- TRIALS SHOWING ASSOCIATION BETWEEN PREBIOTICS & THEIR EFFECT ON LIPID PROFILE

Intervention	Fibre	Dose; Duration of study	Experimental Design	Animal/ Subjects	Effects	Ref
Lipid Profile	Pectin	75 g citrus pectin daily; for four weeks	Randomized, Placebo controlled	Six male adult hypercholesterolemic mini pigs	67.1% decrease in VLDL-cholesterol (P<0.05); 41.1% decrease in LDL cholesterol (P<0.05); 49.4% decrease in total serum cholesterol (P<0.05)	93
	Fiber (*Plantago ovata* husk)	10.5 g *Plantago ovata* husk daily; for eight weeks	Randomized, crossover, placebo controlled, single-blind	Twenty-eight men with myocardial infarction or stable angina	6.7% decrease in plasma triacylglycerol (P<0.02), 6.7% increase in HDL cholesterol concentrations (P<0.006); 10.6% decrease in the total cholesterol /HDL ratio (P<0.002); 14.2% decrease in LDL /HDL ratio (P<0.003)	94

Functional Food Ingredients [95]: As is the case with other dietary fibers, Prebiotics, like Inulin and oligofructose, are resistant to digestion in the upper part of the intestinal tract and are subsequently fermented in the colon. They have a bulking effect due to the increase in microbial biomass that results from their fermentation.

From a quantitative point of view, the bulking effect, expressed as the increase in daily fecal mass, has been reported to vary between 1.5 and 2 g/g of ingested Inulin or oligofructose 23 3o 38, Prebiotics also belong to other categories of "functional food ingredients", which, by reference to the European consensus", should have unique features such as: being part of conventional or everyday foods, to be part of the normal/usual diet, being composed of natural (as opposed to synthetic) components, sometimes in increased concentrations or present in foods that would not normally supply them, and having a positive effect on target function(s) that may enhance well-being and health and/or reduce the risk of disease.

Immunomodulatory effects: Only few studies so far have investigated the Immunomodulatory effects of Inulin (IN)/Oligo fructose (OF) in humans. Recently, 2 clinical trials reported the therapeutic outcome of a prebiotic and synbiotic treatment in subjects with ulcerative colitis and Crohn's disease [96.] In a small randomized, double-blinded controlled trial including subjects with ulcerative colitis, the supplementation with *B. longum*, IN, and OF resulted in an improvement of the full clinical appearance of chronic inflammation. Further, intestinal mRNA levels of the proinflammatory cytokines interleukin (IL)-1b and tumor necrosis factor-α were significantly reduced in synbiotic-treated subjects, whereas no significant differences were seen for the immunoregulatory cytokine IL-10 [97].

In summary, only few human studies so far have investigated the effects of OF alone on the immune system. The currently available data suggest that the oral intake of IN/OF may modulate the immune system in humans. More human studies including dose-response studies with Prebiotics such as IN/OF are needed, with a special focus on the GALT.

Hepatic Encephalopathy: Substances derived from the metabolism of the gut flora involved in the pathogenesis of hepatic encephalopathy [98, 99, 100]. The therapeutic efficacy of Lactulose has been demonstrated in RCTs. The side effects calculated from 18 studies concerning 298 patients were the following: flatulence 18%, diarrhoea 14.5%, and abdominal pain 13% [100].

The possible mechanisms of action include stimulation of bacterial growth, incorporation of ammonia into bacterial proteins, colon acidification, laxative effect, and possibly a shift in production of medium chain fatty acids to short chain fatty acids [98].

Colon Cancer: Fermentation reduces colonic pH, and may reduce the 7-dehydroxylation of primary bile salts. A role of carbohydrate fermentation in colon cancer prevention has thus been hypothesized [101] and studies in animal models have been encouraging. Several studies have shown that Lactulose administration to healthy volunteers (40-60g/d) lowered fecal concentrations of secondary bile salts [102, 103]. However, in one study, administration of 60ml of Lactulose/d for 12 weeks did not influence crypt cell proliferation assessed in rectal biopsies [104]. Roncucci *et al*., reported in 1993 that Lactulose decreased the recurrence rate of colon adenomas [105]. Two hundred and fifty five patients with colon adenomas were randomized after removal of the adenomas to receive vitamins, Lactulose (20 g/d) or no treatment.

Clinical Studies: Prebiotics like Inulin and Oligofructose have been shown to effect on calcium bioavailability. In adult humans, it appears that as long as the right methodology is used, it is possible to see an improvement of intestinal calcium absorption after Inulin-type fructans consumption [106].

Moreover; such molecules did not alter this process in small intestine, probably because the modulation of mineral absorption by fructans originates mainly in the colon. This is why Ellegard *et al*., have shown that they do not affect mineral digestion in volunteers with an ileostomy, even though another explanation for the lack of effect in such patients could be an increased transit rate, eliciting reduced opportunities of Inulin type fructans to stimulate the fermentation process [107].

In a randomized double-blind crossover trial in 12 postmenopausal women, Tahiri *et al*., failed to show an effect of oligofructose consumption (10g/d for 5 wk) on calcium absorption. However, the length of menopause was quite different among subjects: 8.3 6 7.1 y. This corresponds to huge differences in terms of bone metabolism, and this could explain the lack of any significant effect [108].

If Inulin-type fructans have failed to modulate calcium absorption during the first 5 y after the onset of menopause, they appear to be efficient, later in life, probably because hormonal changes occurring during menopause become less important, leaving some room for other mechanisms of regulation. However, it is important to know if the extra absorbed calcium is deposited in bones. For that purpose, long term studies are needed.

Developing Prebiotics for Specific Probiotic Strains: Prebiotic structure, including chain length, branching, linkage types and the presence of mixtures of different molecules can affect the fermentation specificity of these compounds [109, 110]. As such, small molecular differences in prebiotic structure may induce significant changes in physiological functions. For example, many Fructo-oligosaccharide products are available and it is apparent that products with higher molecular weight may be more slowly fermented and thus persists for longer in the colon.

Combinations of Inulin (DP 10–65) and oligofructose (DP 2-8) may elicit synergistic effects. If it were possible to match Prebiotics with probiotic strains, the physiological benefits may be enhanced [111]. Alternatively, probiotic strains might be selected for their ability to generate prebiotic oligosaccharides, which are then preferentially utilized by the producing probiotics [112, 113, 114].

Synbiotics & Health Benefits:

Pediatric Surgery: Kanamori *et al*., have reported long term efficacy of synbiotic therapy in pediatric patients under surgery for short bowel syndrome that showed repeated episodes of refractory enteritis and bacteremia since birth and *Tracheoesophageal fistula* [115, 116]. For probiotics, live bacterial preparations of *Bifidobacterium breve* strain yakult and *Lactobacillus casei* strain Shirota were used and galactooligosaccharides were supplemented as probiotics.

A high viable probiotic bacterial content was detected in the patients' faeces, the intestinal bacterial flora was improved and the titre of harmful microorganism, Pseudomonas aeruginosa and Candida were reduced. As the intestinal bacterial flora was improved by synbiotic therapy, intestinal peristalsis recovered, intestinal expansion was reduced, and the nutritional condition improved as reflected by gain in body weight.

Digestive Organ Surgery: Rayes *et al*., compared postoperative complication among groups treated with – (a) antibiotics for intestinal bacterial eradication; (b) synbiotics and (c) killed lactic acid bacteria, in patients with liver transplantation [117]. *Lactobacillus plantarum* 299 as a probiotic and oats as a prebiotic were administered to the synbiotic therapy for 12 days. The incidence of postoperative infections by discharge was 48, 13 and 34% in (a), (b) and (c) group, respectively, showing that the incidence was significantly lower in the intestinal bacterial eradication group [118].

When 4 species of Lactic acid bacteria and 4 types of bacteria were combined in synbiotics after liver transplantation, the incidence of postoperative infection s was further reduced to 3% suggesting that synbiotic therapy was capable of reducing the incidence of postoperative infection that could not be completely checked by antibiotics treatment for control of intestinal bacteria.

Systemic Inflammatory Response Syndrome (SIRS): For emergency treatment, the control of marked systemic inflammatory reactions in response to severe injuries and burns is important. A new concept of inflammatory reaction, systemic inflammatory response syndrome has recently been proposed by a joint meeting of the American college of chest physicians and society of critical care [119]. SIRS represents systemic inflammatory reactions to stresses, and is defined as conditions showing abnormalities in 2 or more of the following 4 body systems (Body temperature, heart rate, respiratory rate and blood cell count. Shimizu *et al*., have investigated fecal microflora in SIRS patients considering disturbance of intestinal microflora in SIRS patients considering disturbance of intestinal flora accompanied by infection and intestinal flora and found that intestinal microflora was markedly disturbed in SIRS patients [120].

Severe Acute Pancreatitis: Forty-five patients were supplemented from a time point in the disease process defined as early as possible with the one LAB/one fibre formula containing either live or heat-killed LAB [121]. The study was interrupted when repeat statistical analysis demonstrated statistically significant differences in infection rate between the two groups. At that time, 22 patients had received treatment with live and 23 with heat-killed Lb plantarum 299. Infected pancreatic necrosis and abscesses were seen in 1/22 (4.5%) in the live LAB group and in 7/ 23 (30%) in the heat-inactivated group (P ¼ 0:023). The only patient in the live LAB group, who developed infection, had signs of urinary infection on the 15th day, e.g. at a time when he had not received treatment during the last eight days. The length of stay was considerably shorter in the live LAB group (13.7 days vs. 21.4 days), but the limited size of the material did not allow statistical significance to be reached.

Liver Transplantation: The 30-day infection rate after liver transplantation is usually above 50%; the most recent study reports a 30-day morbidity of 86% despite or eventually due to extensive and multiple treatment with antibiotics (selective bowel decontamination) [122]. Two randomized studies were conducted in liver transplant patients in collaboration with the University of Berlin. When the one LAB/one fibre composition was supplied, a 30 day infection rate of 13% was observed compared to 34% with heat killed LAB and 48% with selective decontamination. The infection rate was in a second study using the four LAB/four fibre composition further reduced to only 3% (1/33 patients suffered a slight urinary infection) compared to 51% in the group treated with only fibres [123].

Chronic Liver Disease: Pro- and Prebiotics (synbiotics) have the ability to reduce the production and absorption of endotoxin in the intestine, and to down-regulate production of pro-inflammatory cytokines, such as TNF-α. In a study in collaboration with University of Sidney we recently observed that in vitro TNF-α production by peripheral blood mononuclear cells, in response to stimulation by endotoxin or Staph aureus enterotoxin B, is reduced by a median 46% (range: 8–67%) in comparison to pre-supplementation levels in 8/11 (72.7%) cirrhotic patients supplied with the four LAB/four fibre composition [124].

In another study in chronic liver disease either the four LAB/four fibre formula, only the four fibres a placebo consisting in non-fermentable, non absorbable fibre was supplied daily during one month [125]. Significant decreases in the gut content of *Escherichia coli*, *Staphylococcus* and *Fusobacterium*, but not *Pseudomonas* and *Enterococcus*, were observed. Ammonia/s, levels of endotoxin/s and ALT/s fell

significantly in both treatment groups and were accompanied by significant improvements in psychometric tests and in degree of encephalopathy. Supply of synbiotics to patients with various acute and chronic conditions is almost always well tolerated and has no adverse events or adverse changes in general clinical state of the patients.

Synbiotic treatment capable to down regulate the expression of Toll-like receptors and reduce the production of TNF-α seems to have the potential to be a cheap and powerful tool for both long-term treatment of patients with chronic diseases such as liver disease and for treatment of patients with various acute conditions. Efforts must continue to find the most powerful LAB and the most powerful fibres or combinations thereof.

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