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Probiotic and Prebiotic Influence Beyond the Intestinal Tract

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Probiotics and prebiotics have long been appreciated for their positive influences on gut health. Research on the mechanisms and effects of these agents shows that their impact reaches beyond the intestine. Effects on the microecology and pathology of the oral cavity, stomach, and vaginal tract have been observed. Likely mediated through immune influences, systemic effects such as reduced severity of colds or other respiratory conditions, impact on allergy incidence and symptoms, and reduced absences from work or daycare have also been noted. These observations, among others, suggest a broader spectrum of influence than commonly considered for these unique substances.

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INTRODUCTION

That probiotics and prebiotics can have an impact on intestinal targets is well-established.[1,2] However, documentation of their influence in sites beyond the intestinal tract is growing. Resident and transient microbes are capable of influencing many colonized and non-colonized systems of human physiology through interrelated organ function and communication systems. Evidence for prebiotic and probiotic effects on non-intestinal sites spans the range from theoretical to evidence-based, supported by controlled human studies. Endpoints in these studies include pathogen carrier state, infection, tumor incidence, or structural health in targets such as the mouth, vagina, liver, stomach, respiratory system, pancreas, central nervous system, and bone. General health targets include cognitive function, obesity, pediatric growth, and absences from work or school. Although these general health benefits may be the result of improved intestinal function, these findings call attention to the fact that benefits of these dietary agents have broader range than the intestine. This review highlights the research evaluating the evidence for these indications to determine plausible hypotheses for effects, strength of evidence, and identification of targets for future research. It is the result of discussions that took place at the 4th meeting of the International Scientific Association for Probiotics and Prebiotics (www.isapp.net) in June 2006.

INTESTINAL MICROBIOTA: A CENTRAL POINT

Probiotics and prebiotics encounter a diverse microbiota and human cell structure in the gastrointestinal tract and interact with it. Native microbiota represent a huge bacterial community mainly localized in the colon. The number of bacteria exceed by a factor of 10 the number of eukaryotic cells in a human body and the combined genetic information of the microbiota exceeds that in the human genome by at least 100-fold. At least 70% of the bacteria from the microbiota have such sophisticated nutritional or physical requirements that they have not yet been elucidated.[3] Use of the 16S ribosomal sequence and other genetic tools has become essential for the identification and characterization of the microbiota due to their diversity and difficulty in culturing. Each individual has his own microbiota that develops in early childhood, stabilizes in adulthood, and increases in complexity during old age. It is as personal as a fingerprint, but comparisons among individuals suggest a central core of species exists in humans and it is roughly defined by bacterial groups: *Clostridium leptum*, *Clostridium coccoides*, *Bacteroides* and, in smaller part, *Bifidobacterium*.

The protective role of the microbiota is observed in the digestive tract, but clearly extends beyond this realm. The microbiota ferment the non-digested part of the diet producing gas and numerous metabolites, among them short chain fatty acids, which are nutrients for the enterocytes. Some recent experiments have demonstrated that this microbiota fermentor is so efficient it can lead to fat accumulation, and a role for microbiota in obesity has been suggested.[4] The microbiota modifies food residues and, depending on the microbe, can detoxify or generate some oncogenic compounds. The microbiota stimulates the immune system (local and systemic), affording protection from surrounding potential invasions. The microbiota community does not accept bacterial foreigners and exerts an antagonistic effect against pathogenic bacteria assuring protection both within and outside the digestive tract. An improper reaction to the commensal microbiota is thought to lead to the dysregulation expressed by inflammatory bowel disease.[5] The quantities of ingested probiotic bacteria represent a small number compared to the commensal microbiota; however, this minority population has been documented through many controlled studies to exert a health effect. Furthermore, transit of these live microbes through the gastrointestinal tract includes passage through regions that are sparsely colonized (for example the stomach, ileum, and duodenum) and where they may constitute, albeit transiently, the dominant microbial population. Mechanisms are not richly understood but may involve modification of the number or/and the physiology of some key resident species as well as impacting the cross-talk between microbiota and the host. Clearly, by impacting the colonizing microbiota or interacting with host cells, probiotics and prebiotics have the potential to impact health.

PREBIOTIC ACTIVITY AWAY FROM COLON

Prebiotics by definition are not digestible. The host does not secrete enzymes that are able to break down prebiotics into small enough fragments that can be further used by the classical anabolic and catabolic processes that continuously go on in host cells. As a consequence, prebiotics are completely available to the bacteria that reside in the intestinal tract. Prebiotics distinguish themselves from the group of other fermentable carbohydrates by the fact that they selectively interact with the intestinal microbiota.[6] Some groups of bacteria are stimulated, others are not affected, and still others are out-competed. The bacteria that are stimulated coincide with groups of bacteria that are associated with a healthy condition of the host (many of them belong to the same genera as probiotics). As such, prebiotic consumption shifts the composition of the intestinal microbiota.

While prebiotics are selectively interacting with the intestinal microbiota, they are themselves converted into bacterial metabolites. As the composition of the microbiota is modified, the types of bacterial metabolites into which prebiotics are converted are also modified. It is observed that the cecal contents of animals being administered prebiotics contain relatively higher total amounts of short chain fatty acids and the proportional composition of the short chain fatty acids is shifted into the direction of more propionate and butyrate. The complete picture of intestinal bacterial secretions into the chyme is not known; there may be thousands of organisms contributing to this pool. Metabonomics is a relatively new scientific discipline that focuses on the study of these compounds and should shed more light on these aspects in the near future. In the context of the present paper, however, it is important to state that many of these bacterial metabolites are absorbed into the blood of the host and pass the chyme/blood barrier to enter the systemic body space, where they interact with many physiological processes in all vital organs and peripheral tissues of the host.[7]

Anticancer Effects

Anticancer effects induced by prebiotic consumption have been observed in various experimental models. The suppression of carcinogenesis was reproduced in models in which the development of chemically induced

mammary tumors was suppressed.[8] The development of aggressive tumor cells in muscle tissue was slowed down and an increase in life span was induced in the case of ascitic tumors that evolved from intraperitoneally transplanted tumor cells.[9] The suppression of carcinogenesis was also observed by Pierre et al.,[10] in which the spontaneous emerging colon tumors, but also the small intestinal tumors—which are distinct from the colon where prebiotic fermentation takes place—were significantly reduced in numbers. These effects were shown to be valid in human volunteers as well.[11] The Syncan project (www.syncan.be) was the first study to demonstrate significant efficacy of dietary pre- and probiotics against cancer.

Bone Density Effects

In rats it was observed that prebiotic administration strengthens the bone. This was observed in ovariectomised rats[12] tested as a model for post-menopausal women, and in young growing animals whereby whole-body mineral content was increased.[13]

Immune Effects

There are indications from experimental models[14] as well as from human dietary intervention studies[11] that dietary prebiotics modulate various systemic immune markers. Main interactions were at the level of the gut-associated lymphoid tissue but some cellular immune markers were also modified, including an improved T-helper type 1(Th1)/Th2 ratio. The interaction with the immune system in humans translates into improved resistance against infection and improved reaction on suboptimal vaccination against measles.[15]

Other Effects

Another effect quite apart from the colon is suppression of hunger or increasing a feeling of satiety. In experimental models it was demonstrated that prebiotics suppressed ghrelin, the hunger hormone. In an exploratory human intervention study, satiety-inducing effects of prebiotics were demonstrated.[16]

Prebiotic fermentation results in increased production of bacterial biomass, in which nitrogen is fixed. The liver of patients with hepatic encephalopathy is thus relieved from an excess load of ammonia. Prebiotic consumption has resulted in clinical improvement of the disease.[17,18]

Further evidence for prebiotic activity beyond the colon is found in animal nutrition. Old laying hens with declining productivity tend to increase egg-ponding activity when prebiotics are added to their diet. In the carcasses of broilers fed prebiotics, a reduction of adipose tissue deposits has been observed,[19,20] a finding that has been replicated in other animals such as piglets, calves, and fish. A significant improvement of zootechnical performance (growth, feed conversion efficiency) has been observed. These obviously are related to systemic effects of prebiotic feeding.[21] Perhaps the most important aspect in this context is the effect on the small intestine, which is manifest through more pronounced villus structure and an increased length of the small intestine as a whole.[19]

Taken together, these data show there is a causal effect of prebiotic feeding and the occurrence of various physiological effects. The mechanisms behind the observations, however, still need to be elucidated. Novel techniques such as metabonomics seem to be helpful.

ORAL MICROBIOLOGY

Biotherapeutic intervention to control dental caries has been proposed and is predicated on the hypothesis that non-cariogenic or otherwise beneficial microorganisms can occupy a space in the oral biofilm that otherwise might be occupied by a pathogen. For effectiveness in the oral cavity, probiotics should adhere to dental tissues as a part of the biofilm (or plaque) and compete with the growth of cariogenic bacteria or periodontal pathogens.

The approaches taken are diverse, including consumption of dairy products containing *Lactobacillus* or *Bifidobacterium* probiotics to influence salivary *Streptococcus mutans* levels or incidence of dental caries, construction of a non-cariogenic strain of *S. mutans* to compete with cariogenic colonizers, and delivery of anti-*S. mutans* antibodies to the oral cavity using a probiotic carrier.[22]

A recent study investigated the effect of *Lactobacillus reuteri* ATCC 55730 on the levels of salivary *S. mutans* and lactobacilli in young adults when ingested through two delivery systems. The subjects were 120 healthy young adults (21–24 years), and a placebo-controlled study design with parallel arms was utilized. The subjects were randomly assigned to 4 equally sized groups: group 1 drank 200 ml of water through a prepared straw containing *L. reuteri* ATCC 55730 once daily for 3 weeks; group 2 took 200 ml water through a placebo straw during the same period; group 3 took 1 tablet containing *L. reuteri* ATCC 55730 once daily for 3 weeks; and group 4 took placebo tablets without bacteria. A short-term daily ingestion of lactobacilli-derived probiotics delivered by prepared straws or tablets reduced the levels of salivary mutans streptococci in young adults.[23]

A study of children (ages 1–6 years) in daycare

centers used a randomized, double-blind, placebo-controlled format.[24] Milk (mean daily consumption 218 ml) containing $0.5–1\times10^6$/ml *Lactobacillus rhamnosus* was given to children 5 days/week for 7 months. A statistically significant reduction in both dental caries and *S. mutans* counts in plaque was observed in children aged 3–4 years.

The influence of bifidobacteria on oral ecology has been reported in only one study. The investigation was a double-blind, randomized, cross-over study in which two groups consumed either a probiotic yogurt (200 g daily) containing *B. animalis* DN-173 010 bacteria (7×10^7 cfu/g) or a control yogurt without viable bacteria for 2 weeks.[25] It was concluded that the yogurt with living bacteria significantly diminished cariogenic bacteria and salivary mutans streptococci. Some authors[26,27] suggest that a few species of *Bifidobacterium*, such as *dentium*, may be associated with the generation of deep caries in children. However, no evidence of such association with *Bifidobacterium* species used as probiotics has been established.

Hillman et al.[28] used a biotherapeutic approach by developing a genetically modified *Streptococcus mutans* strain that produced no detectable lactic acid during growth. This strain was able to colonize the mouth and was significantly less cariogenic than the control strain in germ-free and conventional rodent models. Another recombinant approach used antibodies to the adhesion molecule of *S. mutans* with expression in *Lactobacillus zeae*.[29] When this strain was administered in a rat model of dental caries, *S. mutans* bacteria counts and caries scores were markedly reduced. These have not yet been evaluated in humans. In another genetic approach, a vaccine that produced epitopes from *S. mutans* glucosyltransferases and glucan binding protein B had great potential to interfere with the development of caries.[30]

Among the different formats for delivery of probiotics, fermented and unfermented milk products are popular. Milk products per se are considered safe for teeth and have a possible beneficial effect on the salivary microbial composition and caries development due to its natural content of casein, calcium, and phosphorous. Furthermore, a recent epidemiological study by Al-Zahrani[31] documented a lower prevalence of periodontitis for the quintile of individuals with the highest intake of dairy products. Delivery of live bacteria able to reduce colonization of the oral cavity by cariogenic or pathogenic colonizers could further promote oral health.

Additional, larger studies with patient-oriented outcomes such as dental caries or oral infections need to be conducted to better ascertain the value of probiotics in oral health. Table 1 summarizes the probiotic-induced oral effects that have been observed.

STOMACH INFECTION WITH *HELICOBACTER PYLORI*

The Nobel Prize in Medicine or Physiology was awarded in 2005 to Drs. Barry J. Marshall and J. Robin Warren for their discovery and culture of the gram-negative, spiral-shaped, microaerophilic organism *Helicobacter pylori* from the stomach of humans with chronic-active gastritis (nobelprize.org/medicine). Since the successful growth of this organism in the early 1980s, extensive studies with the bacterium have fulfilled each of Koch's postulates, confirming it is indeed a human pathogen.[32]

H. pylori infects over half of the world's human population, primarily those residing in poor socioeconomic circumstances and in developing nations. Infection is acquired in childhood and persists for life, unless specific eradication therapy is initiated. All infected persons develop gastritis, but only a subset will go on to develop disease.[33] It is estimated that the lifetime risk of developing peptic ulceration is roughly 15%. However, this is an exceedingly important disease, because it has serious morbidity (pain, hemorrhage, and perforation) and mortality. The natural history for recurrence of peptic ulcers is completely prevented by successful eradication of the gastric pathogen. *H. pylori* infection is also associated with an increased risk of gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. Indeed, *H. pylori* is the first bacterium to be classified as a class 1 carcinogen by the World Health Organization. Although the final verdict is not yet in, it appears that eradication of infection early in the sequence of events leading to carcinogenesis will prevent the development of malignant transformation.[34] The role of *H. pylori* infection in a variety of extra-gastric symptoms and systemic disease complications is the subject of ongoing investigation.[35]

Eradication of *H. pylori* infection both in animal models and in human subjects is not successful when using antibiotics to which the organism is susceptible in vitro as monotherapy. Current first-line treatment regimens generally employ a potent acid-suppressing agent (for example, a proton pump inhibitor) plus two antibiotics (such as amoxicillin, metronidazole, or tetracycline) twice daily for 7–14 days. However, such combination therapy suffers from suboptimal patient compliance due to the frequent occurrence of adverse effects.

Probiotics have been used successfully to reduce *H. pylori* colonization and diminish the severity of mucosal inflammation in the stomach in a mouse model of infection.[36] However, 4 systematic reviews of the available literature from clinical trials indicate that probiotics are not effective in eradicating established *H. pylori* infec-

Table 1. Studies on the Impact of Probiotics on Oral Microecology and Disease

Reference	Strain (daily dose)	Clinical endpoints	Treatment duration and study design	Results
Çaglar et al. (2006)[23]	*L. reuteri* ATCC 55730 (10^8)	Levels of salivary *S. mutans* and lactobacilli	120 (30/group) healthy adults aged 21–24 y; RPC, parallel; 3 wk duration; 4 treatment groups	↓ Salivary *S. mutans* in both straw ($P<0.05$) and tablets ($P<0.01$), compared to placebo groups
Çaglar (2005)[25]	*B. animalis* DN173-010 (1.4×10^{10} in 200 g yogurt)	Salivary *S. mutans* levels in young adults	21 healthy adults (21–24 yrs); RDB, crossover study; 4 treatment periods; 2 treatment groups	↓ *S. mutans* levels in saliva ($P<0.05$)
Näse et al. (2001)[24]	*L. rhamnosus* GG ($1–2\times10^8$ daily, 5 days/wk delivered in milk)	Dental caries	7 mo duration; ages 1–6 y	Trend toward ↓ dental caries in children 3–4 yrs of age ($P=0.59$)
Hillman et al. (2000)[28]	*S. mutans* BCS3-L1, non-lactic acid-producing recombinant strain	Cariogenicity in germ-free and colonized rats; target: replacement therapy	Animal study	↓ Caries scores in conventional and germ-free rats by 48% and 64%, respectively ($P<0.0001$); Mutant strain was genetically stable and non-cariogentic
Krüger et al. (2002)[29]	Recombinant *L. zeae*	Antibodies to adhesion molecule of *S. mutans* cloned and expressed in *L. zeae*; *S. mutans* levels in dental plaque of rats was assessed	Animal study	↓ *S. mutans* levels in plaque ($P<0.05$) and reduction in caries ($P<0.05$) in rats

Abbreviations: ↔, no change; ↓, reduced; ↑, increased. RDB, randomized, double-blind; RPC, randomized, placebo-controlled

tion in human subjects.[37–40] Gotteland et al.[39] summarize 10 clinical trials (8 in adults, 2 in children) showing that a variety of probiotic agents, when used alone, have limited efficacy in eradicating the organism. This is not too surprising since antibiotics as monotherapy are also not effective in eradicating the gastric pathogen.

On the other hand, in 6 trials that included a total of 607 subjects, probiotics appeared to be effective when employed as adjunctive therapy for reducing the frequency of adverse side effects.[39] In addition, a recent study indicates that the effectiveness of eradication therapy is enhanced by using probiotics.[41] In a randomized, controlled trial of 138 subjects who had previously failed a course of triple therapy, the rate of successful *H. pylori* eradication was significantly higher (85%) in subjects treated with quadruple therapy (omeprazole, amoxicillin, metronidazole, and bismuth subcitrate) plus a yogurt containing a mixture of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Bifidobacterium lactis* ($>10^9$ organisms/ml, with roughly equal amounts of the four strains), compared to patients receiving quadruple therapy without probiotics (71%, $P<0.05$ on an intent-to-treat analysis).

PANCREATITIS

Acute pancreatitis is usually a mild and self-limiting disease, but in a minority of cases it can develop into a severe disease with high morbidity and mortality.[42] The critical initiating event is the premature activation of digestive enzymes within pancreatic acinar cells, leading to tissue autodigestion and a local inflammatory response.[43] Shortly after the initial injury, which can be due to alcohol abuse, gall stones, or an unknown triggering event, inflammatory cells, mainly neutrophils, infiltrate the pancreas, perpetuating the local inflamma-

tory process.[44] In patients with severe acute pancreatitis, this may lead to a systemic inflammatory response syndrome causing damage to remote organs and ultimately multiple organ failure. Mortality in acute pancreatitis follows a biphasic curve. About 50% of overall mortality occurs within the first week of admission due to multiple organ failure induced by the systemic inflammatory response syndrome.[45,46] Patients with a severe attack who survive this initial phase often develop extensive pancreatic necrosis. A refractory state will then commence, which is characterized by general immunosuppression,[47,48] which enables translocated microorganisms from the digestive tract to successfully infect pancreatic necrosis and potentially disseminate to the systemic circulation. Multiple organ failure caused by these infectious complications accounts for the so-called late mortality (>2 weeks).[49]

For several decades, antibiotic prophylaxis has been used to prevent translocation of pathogenic bacteria but with variable success or even conflicting results. Selective decontamination of the digestive tract has shown promising results, but the risk of bacterial multidrug resistance has precluded widespread implementation. In recent years, probiotics have shown promising results in several randomized placebo-controlled trials. A double-blind, placebo-controlled, randomized clinical trial was performed in 45 acute pancreatitis patients.[50] Patients were randomly allocated to receive either viable probiotics (group A) or heat-inactivated probiotics (group B). All patients received a jejunal tube and fiber-enriched enteral feeding for 1 week. In the group treated with probiotics, 1 of 22 (5%) developed infected pancreatic necrosis, mostly gut-derived (positive aspiration culture) compared to 7 of 23 (30%) in the group treated with heat-inactivated probiotics. To further test the protective role of probiotics in acute pancreatitis, a double-blind, placebo-controlled, multicenter trial (PROPATRIA—*pro*biotics in acute *pa*ncreatitis *tria*l) was initiated. Currently, 15 clinical centers are participating in the Dutch Acute Pancreatitis Study Group, including all 8 Dutch university medical centers, and are enrolling patients. The design and rationale of the study have been published previously.[51]

For this study, a disease-specific probiotic was designed based on pathophysiological events in critically ill patients. From a strain collection of 69 different lactic acid bacteria, a primary selection was made of the 14 species showing superior survival in a simulated gastrointestinal environment. Functional in vitro tests, including antimicrobial activity against a range of clinical isolates and cytokine-inducing capacity in cultured human peripheral blood mononuclear cells, were used to further identify potentially useful strains. Based on the in vitro data obtained and general criteria regarding probiotic design and safety, the following selection of 6 strains was made: *B. bifidum* W23, *B. infantis* W52, *L. acidophilus* W70, *L. casei* W56, *Lactobacillus salivarius* W24, and *Lactococcus lactis* W58. In vitro, the combination of these strains into a mixture resulted in a wider antimicrobial spectrum, superior induction of IL-10, and silencing of pro-inflammatory cytokines as compared to the individual components. The potential of this disease-specific, multispecies, probiotic mixture for reduction of bacterial translocation or improvement of clinical outcome has been tested in a rat model of acute pancreatitis. It was found that probiotic treatment significantly reduced bacterial overgrowth of potential pathogens in the duodenum, resulting in significantly reduced bacterial translocation to extra-intestinal organs, including the pancreas (10^5 and 10^3 CFU/g tissue in placebo- and probiotic-treated animals, respectively). Accordingly, health scores of surviving rats treated with probiotics were better throughout the experiment and late-phase mortality was significantly reduced by 44%.

By means of molecular methods, pancreatitis and probiotic-induced changes in intestinal microbiota were determined. During acute pancreatitis the host-specific microbiota was replaced by an acute-pancreatitis-associated microbiota. Although probiotic treatment did not reverse this situation, the presence of an as yet unidentified bacterium was significantly upregulated. The levels of this bacterium were positively and significantly correlated with improved pancreas histology, reduced bacterial counts in duodenal, mesenteric lymph nodes, spleen, liver and pancreatic necrosis, and reduced plasma levels of pro-inflammatory cytokines. Protection and stimulation of this bacterium in the ileum of rats by probiotics resulted in a reduced severity of pancreatitis and associated sepsis. Identification of this bacterial species, which is also present in the human intestine, may turn out to be of key importance in unraveling the mechanisms of probiotic action.

During acute pancreatitis, severe disturbances in mucosal permeability have been reported. A potential risk factor associated with probiotic use might thus be translocation of the administered probiotic microorganisms and subsequent infection of pancreatic necrosis. Probiotics have been applied in a single study with pancreatitis patients and several other studies with critically ill patients. So far, no infections with the probiotic bacteria have been reported. Clearly, more studies are needed to further establish the safe use of these products in critically ill patients.

LIVER FUNCTION

It is well known that the microbiota of the gut influences liver function and vice versa, a relationship

called gut-liver axis.[52] Gut-derived endotoxins and active metabolites like ethanol and acetaldehyde are able to induce or aggravate alcohol- or non-alcohol-related steatohepatitis.[53] Endotoxins may upregulate the production of proinflammatory cytokines and TGF-β resulting in the onset of liver fibrosis. Proinflammatory cytokines also enhance lipid peroxidation, which is an important trigger for the development of steatohepatitis.[54] Therefore, modulation of the gut microbiota may be beneficial in these patients.

So far, few data on the use of pre-, pro-, or synbiotics in patients with liver disease are available. In a mouse model for nonalcoholic fatty liver disease, the probiotic combination VSL#3 (containing three species of *Bifidobacterium*, four species of *Lactobacillus* and *Streptococcus thermophilus*) as well as TNF-antibodies led to decreased hepatic steatosis and inflammation compared to controls.[55] The authors speculated that VSL#3 acted via modulation of hepatic insulin sensitivity.

Two clinical studies were performed in patients with hepatitis C virus-related chronic hepatitis, alcoholic cirrhosis, and nonalcoholic steatohepatitis. Synbiotic treatment (a combination of *L. acidophilus*, *Bifidobacterium*, *L. rhamnosus*, *L. plantarum*, *L. salivarius*, *L. bulgaricus*, *L. lactis*, *L. breve* plus fructo-oligosaccharides) resulted in significant decreases of target liver enzymes in the patients with nonalcoholic fatty liver disease and alcoholic cirrhosis,[56] but not in the patients with hepatitis C virus. In the alcoholic cirrhosis group, there was also an improvement of liver function. The mode of action was partly explained by a decrease of tumor necrosis factor α and markers of lipid peroxidation (malondialdehyde, 4-hydroxynenal). In a second study,[57] the three patient groups were treated with VSL#3 for 3 months. Serum levels of liver enzymes decreased significantly in all patients and liver function improved significantly in the alcoholic cirrhosis group. VSL#3 led to a normalization of increased cytokine levels (TNF-α, IL-6 and IL-10) and markers of lipid peroxidation (malondialdehyde, 4-hydroxynenal and S-nitrosothiol). A prospective, randomized study in patients with liver cirrhosis and minimal hepatic encephalopathy[58] showed that a synbiotic mixture of four probiotic non-urease-producing strains (*L. plantarum*, *L. paracasei*, *Leuconostoc mesenteroides*, *Pediococcus pentosaceus*) and four fibers (betaglucan, resistant starch, inulin, pectin) resulted in recolonization of fecal microbiota with non-urease-producing *Lactobacillus* species, lowering of the urine pH, lowering of serum ammonia levels as well as serum endotoxin levels, and reversal of the minimal hepatic encephalopathy in 50% of the patients. Surprisingly, liver function improved significantly in 50% of patients treated with the synbiotic combination.

MAJOR ABDOMINAL SURGERY

Despite antibiotic prophylaxis and advanced technique, bacterial infections remain a major cause of morbidity and mortality following abdominal surgery.[59] One important pathogenic mechanism for these infections is bacterial translocation from the gut, which is enhanced due to surgical trauma, portal hypertension, liver insufficiency, and immunosuppression[60] leading to a disturbed colonic microbiota. Restoring the gut microbiota and the innate immune response with synbiotics might help to prevent bacterial infections in these patients.

Four prospective, randomized studies with synbiotics have been performed in surgical patients. In the first trials,[61,62] liver transplant recipients as well as patients with major abdominal surgery treated with *L. plantarum* 299 and oat fiber had significantly fewer bacterial infections and a trend towards shorter antibiotic therapy and shorter hospital stay compared to patients who received selective bowel decontamination. The same probiotic was able to prevent the occurrence of infected pancreas necrosis in patients with acute pancreatitis compared to placebo.[50] Most of the reported infections were gut-derived.

A highly concentrated synbiotic combination of four fibers and four probiotics (Synbiotic 2000 consisting of 10^{10} *L. plantarum*, *L. paracasei*, *L. mesenteroides*, and *P. pentosaceus*, and 2.5 g each of betaglucan, resistant starch, inulin, and pectin) led to a significant reduction of infections (3% vs. 48%) and antibiotic therapy compared to fibers only in liver transplant recipients.[63] No severe adverse events were noted in these trials, especially no infections caused by the probiotics.

Despite the limited experience so far, synbiotics seem to be a safe and relatively inexpensive tool for reducing postoperative bacterial infection rates in these patients.

KIDNEY STONES

The gut microbiota has been hypothesized to play a role in oxalate accumulation in the urine. The absence of *Oxalobacter formigenes* from fecal microbiota has been shown to be a risk factor in the development of kidney stones.[64] Manipulation of the gut microbiota has been proposed to reduce the risk of kidney stones. No studies in humans have documented that probiotic administration reduces the incidence of kidney stones. However, animal and human studies have documented that *O. formigenes* can establish in the gut and reduce the urinary oxalate concentration.[65,66]

VAGINAL AND URINARY TRACT INFECTIONS

Since the normal microbiota of the vagina consists predominantly (10^8–10^9 cfu/ml vaginal fluid) of lactoba-

cilli and since disruption of this normal colonization is associated with infections, probiotic lactobacilli have been studied for the prevention and treatment of urogenital infections in women. Glycogen metabolism by commensal lactobacilli reduces the vaginal pH to 4–5 and plays a central role in colonization resistance of the vagina. Hydrogen peroxide production, bacteriocin production, coaggregation of lactobacilli and arginine deaminase production may also contribute to this function.

Although the intestine is the source of vaginal lactobacilli, it is also a major source of many urogenital pathogens. Bacterial vaginosis is not only associated with discomfort for the patient, it is also strongly associated with an increased risk of preterm delivery and increased risk of acquiring sexually transmitted diseases, including HIV infection; this extends to increased risk for mother-to-child transmission of HIV during delivery.

Some controversy remains regarding which lactobacilli are characteristic for healthy vaginal microbiota. Almost 2 decades ago, Giorgi et al.[67] showed that the predominant vaginal *Lactobacillus* species were *L. crispatus*, *L. gasseri*, *L. jensenii* (*L. delbrueckii* group), but not *L. acidophilus* as is often suggested, even today, in commercial products and some publications. While most epidemiological studies report *L. crispatus* as the predominant species in undisturbed vaginal microbiota, followed by *L. jensenii*, a finding confirmed by cloning studies,[68] Reid et al.[69] report *L. jensenii* or *L. iners* to be the dominant species. In the study of Anukam et al.,[70] 64% of 185 Nigerian women were colonized with *L. iners* and only 4% with *L. crispatus*, but 51% of women presented with intermediate vaginal microbiota and 14% with bacterial vaginosis (BV), reflecting the high incidence of BV in black African women, also outside of Africa.

UTI are predominantly caused by uropathogenic *Escherichia coli*,[71,72] gram-negative organisms,[72,73] and *Enterococcus faecalis*.[72] The role of probiotics in managing UTIs has been published.[74] UTI is a condition that is common in pre- and postpubertal women, an observation which, in itself, is already a strong indication for the role of lactobacilli in the protection against this class of infections. Symptomatic UTI is often painful and can last several days. Just as is the case for BV, UTI may develop into a recurrent condition, poorly responding to antibiotic treatment, which also has been linked to biofilm formation, in the case of UTI due to uropathogenic *E. coli* in the bladder. Increasing drug resistance among uropathogens is reported and may be partially explained by biofilm formation.

Selection of Probiotics for Urogenital Applications

Different characteristics have been considered important when selecting probiotics for urogenital applications. Among them is the ability to establish, grow, and produce anti-pathogenic compounds, including H_2O_2, lactic acid, and bacteriocins in the vaginal environment. A very comprehensive study describing selection criteria and methodology for testing vaginal probiotic strains was published.[75] Although rapid and efficient lactic acid production is probably the basic factor of importance in maintaining the lactobacillar ecosystem, vaginal epithelium itself and many bacteria also acidify the environment to a certain degree.[76] Hydrogen peroxide production has been put forward in numerous publications as a potent antibacterial mechanism,[77] but Famularo et al.[78] and Pybus and Onderdonk[79] have listed several arguments to question the importance of hydrogen peroxide production in this ecosystem. *L. crispatus* strains are generally among the strongest producers. With regard to persistence of probiotics applied to the vaginal ecosystem, Antonio et al.[80] showed that administration of *L. crispatus* CTV-05 colonized 7 of 9 women, without displacement of other endogenous lactobacilli. Both women who were not successfully colonized by the probiotic strain were already colonized by H_2O_2-producing *L. crispatus* strains at baseline, suggesting that colonization by an exogenous strain of *L. crispatus* may be less successful in persons already having predominant H_2O_2-producing lactobacilli. Osset et al.[81] showed there is considerable variation among *Lactobacillus* species regarding their adherence to uroepithelium, blockage of uropathogen attachment, and inhibition of uropathogen growth, with *L. crispatus* showing greater capacity to block uropathogen adherence than *L. jensenii* and unspecified lactobacilli (61.9%, 49.5%, and 52.6% of blockage, respectively).

Famularo et al.[78] reviewed extensively the advantages that might be offered by the production of arginine dihydrolase or arginine deiminase. Since arginine is an important nitrogen source for the vaginal pathogens, depletion of arginine by strong arginine deiminase activity might not only inhibit the metabolism of the pathogens but also reduce the clinical symptoms. Rousseau et al.[82] showed that a *L. jensenii* strain that produced arginine deiminase was the most effective in inhibiting other species. The original description of *L. jensenii* mentions arginine dihydrolase production, which is absent in the vaginal *L. acidophilus* group species.

Clinical Evaluations

The concept of vaginal instillation of lactobacilli dates back to at least 1920,[83] but until recently few controlled human studies have been conducted. Studies have been conducted with both oral[84] and vaginal[85] administration routes. Targets have included prevention of BV,[86] treatment of BV,[87-92] prevention of recurrent

BV,[91] treatment of recurrent BV,[93] prevention of UTI, treatment of UTI in adult women[94] and in preterm infants,[95] and treatment and prevention of recurrent UTI.[96,97] A notable recent finding also documented that probiotics could serve as effective co-therapy for BV. After preliminary assessments showed that *L. rhamnosus* GR-1 and *L. reuteri* RC-14 administered in milk could pass through the intestine, ascend to the vagina, and restore a normal lactobacilli microbiota in women prone to infections,[96] these strains were delivered in yogurt to African women with bacterial vaginosis and shown to improve therapeutic outcome.[70,83]

Given the importance of mother-to-child transmission of HIV in developing countries, and considering the increased risk of HIV transmission when normal vaginal microbiota is disturbed vaginal probiotics should be urgently tested for their possible contribution to reducing perinatal HIV transmission. It has additionally been suggested that vaginal probiotics be applied for the prevention of preterm birth.[98]

Vaginal Probiotics and Cancer

Intestinal probiotic strains are thought to reduce the risk of gastrointestinal cancers through the inhibition of bacterial enzymes like nitroreductase and glucuronidase, which are intestinal carcinogenic enzymes. It can be expected that inhibition of nitroreductase will also be inhibitory for vaginal anaerobes. Since BV has been suggested to facilitate infection with human papilloma virus,[99] a major cause itself of cervix carcinoma, probiotics effective against BV may indirectly reduce the risk of cervix carcinoma. The production of reactive oxygen species by vaginal lactobacilli has been proposed as a direct means by which these bacteria may reduce the risk for vaginal cancer.[100] It has also been suggested that probiotics have potential in the prevention of gynecological neoplasms.[101]

ALLERGIC DISEASE

Since probiotic bacteria have an immunomodulatory effect, they have also been used in the treatment of atopic disorders such as eczema, asthma, and allergies. Atopy is distinct from atopic disease. Technically, a child with atopy is sensitized to a specific allergen, [i.e., produces specific immunoglobulin E (IgE) antibodies upon exposure to a specific allergen]. This level of sensitization can be measured by objective tests. Atopic diseases, such as eczema, allergic rhinitis, and asthma, have characteristic symptoms. Children with atopic diseases, like asthma, may or may not have atopy; and children with atopy may or may not have atopic disease.

The effectiveness of probiotics has been examined in several different contexts of atopic disease. Primary prevention is the attempt to avert the occurrence of a disease through specific interventions. Secondary prevention includes attempts to slow or halt the progression of a disease through screening. Tertiary prevention includes actual treatment of the disease.[102] Attempts to utilize probiotics for allergic disease can be differentiated as primary prevention versus tertiary prevention.

Treatment of Eczema and Atopic Dermatitis

There has been limited success in the use of probiotics for the treatment of allergic disease. A comparison of studies is difficult, as the studies differed regarding the probiotic evaluated, indications for use, dosing, study design, and outcome measurements. There are several studies that have examined the use of probiotics for the treatment of atopic dermatitis.

Isolauri et al.[103] used a double-blind placebo-controlled trial design to study the effect of *L. rhamnosus* GG-supplemented formula or *B. lactis*-supplemented formula compared to controls on the severity of eczema. Subjects included 27 infants with a history of eczema that began during breastfeeding. After exposure for 2 months, SCORAD (SCORing Atopic Dermatitis) levels improved in both groups that received the probiotic supplement. Viljanen et al.[104] conducted a randomized double-blinded trial to test the effect of *L. rhamnosus* GG on atopic eczema with 230 infants. Although there were no differences between treatment groups at 4 weeks in general, symptoms were alleviated in those infants with IgE-associated atopic eczema (i.e., infants with positive skin prick tests or elevated IgE).

Using a cross-over, double-blind, placebo-controlled trial design, Rosenfeldt et al.[105] tested the effectiveness of *L. rhamnosus* or *L. reuteri* for 6 weeks for the treatment of atopic dermatitis. Although there was subjective improvement in symptoms, the SCORAD level, which objectively quantifies severity, was unchanged. Although there was no objective improvement overall, the treatment seems to be useful for a small subset of patients with an 'allergic constitution' characterized by positive skin prick tests and elevated IgE levels at baseline.

Brouwer et al.[106] used a randomized controlled trial design to examine the effectiveness of probiotic treatment on atopic dermatitis. The 3-arm, 50-subject study evaluated either hydrolyzed whey-based formula as placebo, or the same formula supplemented with one of two different *L. rhamnosus* strains for 3 months. Subjects were infants diagnosed with atopic dermatitis at less than 5 months of age. The results showed no differences in SCORAD level, sensitization, or other inflammatory parameters such as blood eosinophilia.

Preliminary studies have also examined the impact of *L. rhamnosus* GG for the management of atopic eczema and cow's milk allergy. Using a randomized controlled trial design, Kirjavainen et al.[107] treated 35 infants with atopic eczema and cow's milk allergy with extensively hydrolyzed whey formula that was supplemented with viable *L. rhamnosus* GG, heat-inactivated *L. rhamnosus* GG, or placebo. Preliminary results suggest some improvement based on SCORAD levels for those infants fed viable *L. rhamnosus* GG.

In general, although there has been some success in the use of probiotics for atopic disease, the results are more positive for patients with more severe disease who are treated at younger ages. Certain subgroups may benefit from probiotic treatment for atopic dermatitis.

Treatment of Allergic Rhinitis

Wang et al.[108] conducted a double-blind, placebo-controlled trial to examine the effectiveness of *Streptococcus thermophilus* and *L. bulgaricus* yogurt compared to yogurt with *L. paracasei*, as well as *S. thermophilus*, *L. bulgaricus* yogurt. Subjects were 80 children, less than 5 years of age, who were diagnosed with allergic rhinitis for more than 1 year. The results showed no differences in symptom frequency but some differences in subjective outcomes, such as "level of bother" and quality of life.

Xiao et al.[109] conducted a randomized, placebo-controlled trial of yogurt-supplemented *B. longum* BB536 for the treatment of Japanese cedar pollinosis. There were limited, if any, effects for adult patients. The results showed no changes in itching, rhinorrhea, and throat symptoms; however, there was some change in eye symptoms.

Helin et al.[110] conducted a double-blind, placebo-controlled trial on 39 adolescents with a history of respiratory, eye, and oral allergy symptoms. Patients were assigned to *L. rhamnosus* supplementation each day or placebo. Based on oral challenge tests, medication use, and frequency of symptoms, there were no differences in outcomes.

Overall, there are limited numbers of studies that examine the effect of probiotics on allergic rhinitis. However, current evidence suggests the probiotics have limited effectiveness for allergic rhinitis.

Treatment of Asthma

There are limited data on the effectiveness of probiotics for the treatment of asthma once the disease has been diagnosed. Wheeler et al.[111] conducted a small, cross-over, double-blind trial with 15 patients with a history of asthma, who required daily asthma medications. Subjects were assigned to 1 month of 250 g of yogurt with *L. acidophilus*, *L. bulgaricus*, and *S. thermophilus* or 1 month of the same yogurt without *L. acidophilus*. Based on pulmonary function tests and quality of life assessment, there were no differences in daily peak flow or spirometry values, and quality of life measures were unchanged.

Primary Prevention

The hygiene hypothesis suggests that early environmental factors can affect immune system development and lead to atopic conditions. Specifically, the theory suggests that the absence of endotoxin exposure leads to an unfavorable balance between type 1 Th cells and type 2 Th cells. As a result, probiotics may be a promising and practical exposure that may lead to a Th phenotype that is not associated with atopic conditions.[112]

A double-blind, randomized, controlled trial of 62 mother-infant pairs to evaluate the effect of probiotic supplementation to the pregnant and lactating mothers suggested that probiotic supplementation decreased the infant's risk of developing atopic eczema during the first 2 years of life.[113,114] Extended follow-up of the cohort suggests that such effects are sustained past infancy.[115] Despite these promising results, there are some caveats regarding the applicability of the results. For example, the placebo group had an unusually high prevalence (46%) of atopic dermatitis, which would increase the likelihood that the intervention would demonstrate an effect. Furthermore, although there is a difference in the level of clinical disease, there is no difference in the rate of sensitization between the control and intervention groups. Additional studies are needed to replicate these findings with different populations in different settings.

Attempts to utilize probiotics in allergic disease can be differentiated as primary prevention versus tertiary prevention, the actual treatment of the disease. The use of probiotics for the treatment of atopic disease has been most successful for the treatment of atopic dermatitis, compared to allergic rhinitis and asthma. Some studies also suggest a potential benefit of probiotics for the primary prevention of atopic dermatitis. Table 2 summarizes the human studies conducted on probiotics and prevention and treatment of symptoms of allergic disease.

AIRWAY INFECTIONS

Although few human studies investigating the effect of oral probiotic intake on common cold and flu have been performed, a beneficial effect of oral probiotic intake on the duration and severity of respiratory tract infections has been suggested in a few recently and well-performed studies.[116-118] These studies found that

Table 2. Studies on the Impact of Probiotics on Allergy

Reference	Strain (daily dose)	Clinical endpoints	Treatment duration and study design	Results
Primary prevention				
Kalliomaki et al. (2003)[113]	*L. rhamnosus* GG (10^{10})	Prevent atopic eczema at 2 y	2–4 wk before delivery to mother and 6 mo to infant or breastfeeding mother after delivery; 132 infants; DBPC	↓ Incidence of atopic eczema at 2 y by 46% ($P=0.008$)
Kalliomaki et al. (2001)[115]	*L. rhamnosus* GG (10^{10} administered during age 0–6 mo)	Atopic eczema: skin prick test; asthma; exhaled nitric oxide (bronchial inflammation marker)	4-y follow up to earlier study[113]; 107 of 132 subjects from initial report	↔ Skin prick test or asthma ($P=0.30$); ↓ exhaled nitric oxide ($P=0.03$); ↓ atopic eczema by 57% at 4 y (relative risk; 0.57; 95% confidence interval 0.33, 0.97)
Rautava et al. (2002)[114]	*L. rhamnosus* GG ($2x10^{10}$)	Anti-inflammatory transforming growth factor β1 and β2 (TGFβ2) in breast milk; IgE in cord blood; clinical states	62 Mother/infant pairs; mothers consumed probiotic; DBPC	↑ TGFβ2 in breast milk ($P=0.018$); infants with elevated cord blood IgE levels defined the group most responsive to treatment; clinical data from 57 of 62 infants – ↓ incidence of atopic eczema ($P=0.0098$)
Treatment				
Isolauri et al. (2000)[103]	*L. rhamnosus* GG ($3x10^8$/g formula) or *B. lactis* BB-12 (10^9/g formula)	Atopic eczema (SCORAD and subjective index); growth/nutrition; serum cytokines/ chemokines; soluble cell surface adhesion molecules; urinary methyl-histamine and eosinophilic protein X	27 infants (mean age 4.6 mo) with atopic eczema during exclusive breast-feeding; infants weaned to EHF with *L. rhamnosus* GG, Bb-12 or no probiotic; DBPC	At 2 mo: Improved SCORAD scores in both probiotic-supplemented formulas ($P=0.002$); ↓ serum CD4 and urinary eosinophilic protein X ($P=0.005$); ↔ at 6 mo.
Rosenfeldt et al. (2003)[105]	*L. rhamnosus* 19070-2 (2×10^{10}); *L. reuteri* DSM 122460 (2×10^{10})	SCORAD (total score combination of intensity, itch and extent); skin prick test; subjective perception of symptom severity; serum eosinophil cationic protein; serum IgE; PBMC cytokines	6 wk; 1–13 y age, atopic eczema; 43 children; DBPC crossover	↔ Total SCORAD in probiotic group ($P=0.06$), but ↓ extent scores ($P=0.02$); ↔ IL-2, IL-4, IL-10, IFN-γ; ↓ eosinophil cationic protein; perception of symptoms improved in probiotic group; response greater in subset of subjects with positive skin prick test and elevated serum IgE

Table 2. (Cont'd) Studies on the Impact of Probiotics on Allergy

Reference	Strain (daily dose)	Clinical endpoints	Treatment duration and study design	Results
Kirjavainen et al. (2003)[107]	*L. rhamnosus* GG ($\sim 3\times10^{10}$/kg body weight in EHF; heat-killed cells as control)	SCORAD; sIgA; fecal microbiology (FISH)	DBPC; 35 infants with suspected cow's milk allergy (3.5–6.8 mo); 3 groups: placebo (8), heat-killed (13) and viable (14)	Extensively-hydrolyzed formula with heat-killed *L. rhamnosus* GG resulted in intestinal symptoms, so study prematurely terminated; no adverse incidents in other 2 groups; all 3 groups showed reduction in SCORAD compared to baseline; no difference between groups; no fecal microbiology changes observed
Helin et al. (2002)[110]	*L. rhamnosus* GG (5×10^9)	Symptom (nasal, eye and lung) and medication diaries kept by subjects; skin prick test	DBPC; 28 teens and young adults with allergy to birch pollen and apple; probiotic taken 2.5 mo before through 2 mo after pollen season (5.5 mo total)	↔ Between placebo and probiotic group for symptoms before or during allergy season (*P* value range: 0.10 to 0.90); negative results
Wheeler et al. (1997)[111]	*L. acidophilus* (8×10^8/g yogurt, 450 g yogurt/d) + *S. thermophilus* (3×10^8/g); *L. bulgaricus* ($3x10^8$/g); strain designations not provided	Pulmonary function tests; quality-of-life index	15 asthmatic ; 1-mo treatment phase; DBPC crossover; yogurt with or without *L. acidophilus*	↔ Pulmonary function tests; ↔ quality of life index
Viljanen et al. (2005)[104]	*L. rhamnosus* GG (5×10^9 CFU). The mixture group received *L. rhamnosus* GG, (5×10^9 CFU) *L. casei* LC705 (5×10^9 CFU), *B. breve* Bbi99 (2×10^8 CFU) and *Propionbacterium freudenreichii* ssp. *shermanii* JS (2×10^9 CFU)	Atopic dermatitis severity	DBPC; 1-mo treatment phase; 230 infants with suspected cow's milk protein allergy; randomized to one of three groups: *L. rhamnosus* GG (N=80); a mixture of probiotics (N=76) or placebo (N=74)	↔ Atopic dermatitis severity; in subgroup analysis, the *L. rhamnosus* GG group showed improvement for those infants with IgE-associated atopic dermatitis ($P=0.036$)

Table 2. (Cont'd) Studies on the Impact of Probiotics on Allergy

Reference	Strain (daily dose)	Clinical endpoints	Treatment duration and study design	Results
Brouwer et al. (2006)[106]	Nutrilon Pepti with *L. rhamnosus;* Nutrilon Pepti with *L. rhamnosus* GG or, Nutrilon Pepti with placebo (5×10^9 CFU/ 100 mL formula)	Severity of atopic dermatitis; total IgE; food-specific IgE; skin prick test for cow's milk; eosinophil; eosinophil protein X in urine; fecal alpha-1-antitrypsin; IL-4; IL-5; IFN-gamma	DBPC; 3-mo treatment phase; 50 infants (<5 mos of age); exclusively formula-fed with atopic dermatitis and suspected of having cow's milk protein allergy; randomized to one of three groups: Nutrilon Pepti with *L. rhamnosus* (N=17); Nutrilon Pepti with *L. rhamnosus* GG (N=16) or, Nutrilon Pepti with placebo (N=17)	↔ Atopic dermatitis severity; ↔ total IgE, food-specific IgE, fecal alpha-antitrypsin and eosinophils; ↔IL-4, IL-5 and IFN-gamma
Wang et al. (2004)[108]	Fermented yogurt formula with or without *L. paracasei* (200 mL/bottle) 1×10^7 CFU/ mL formula	Symptom frequency, quality-of-life scores; 'level of bother' score	DBPC; 1-mo treatment; 80 children (>5 years of age); diagnosed with allergic rhinitis for more than 1 year; sensitization to house dust mites	↔ Symptom frequency; improved scores for overall quality of life ($P=0.037$); ↓ 'level of bother' ($P=0.022$)
Xiao et al. (2006)[109]	*B. longum* (BB536) 2×10^7 cfu (measured at the end of the intervention period)	Subjective symptom scores; total IgE; JCP-specific IgE; IFN-gamma; IL-10; eosinophil rate levels	Randomized DBPC with 2 week run-in period; 40 adult volunteers with a > 2 year clinical history of Japanese cedar pollinosis (JCP) and the presence of serum JCP-specific IgE; 14-week treatment phase; yogurt with or without BB536	↓ Subjective eye symptoms ($P=0.044$); ↔ subjective symptoms; differences in IFN gamma, IL 10, eosinophil rate over time, but not between groups

Abbreviations: ↔, no change; ↓, reduced; ↑, increased. DBPC, double-blind, placebo-controlled; EHF, extensively hydrolyzed whey formula; FISH, fluorescent in situ hybridization; PBMC, peripheral blood mononuclear cells.

both the severity and duration of symptoms may be beneficially affected, as demonstrated by the reduction of total symptom scores, days with fever, and absence from daycare. There is less evidence for the reduction of incidence. Differences in doses, probiotic strains, and target populations may explain some of the differences in outcome or level of statistical significance, but these types of studies are an indication of potential probiotic efficacy on infections beyond the gastrointestinal tract. Underlying mechanisms for this 'long-distance efficacy' may be immune modulation, as probiotics have been shown to enhance the numbers of T-lymphocytes in recent studies of the common cold,[116,117] and to enhance phagocytosis, natural killer cell activity, and IgA production in several other studies.[119–123] Moreover, a probiotic oral intervention study has demonstrated a reduction in potential nasal pathogens.[124]

An alternative approach to the classic oral intake of probiotics for the modulation of airway infections is the use of airway commensals applied through nasal or oral

sprays. It has been shown consistently in a few multicenter studies that a 10-day course of spraying with alpha-streptococci reduces the recurrence rate of pharyngotonsillitis and otitis media.[125–127] The mechanisms underlying this bacterial interference are thought to relate to colonization resistance and comprise competition for nutrients and attachment sites and the production of bacterial toxins and metabolites with antibacterial activity.[128]

Studies show the existence of microbial imbalance in individuals prone to otitis, sinusitis, and tonsillitis, i.e., they have relatively more potential pathogens and fewer protective bacteria with interfering capacity such as alpha-streptococci.[129,130] Antibiotic treatment often reinforces this imbalance. Bacterial interference with alpha-streptococci may restore balance in the airway microbiota, provide competition with potential pathogens for nutrients and attachment-sites, and produce antipathogenic metabolites and enzymes, thereby enhancing colonization resistance. The impact of probiotics on common colds and influenza in children is summarized in Table 3.

Thus, although the results are limited but promising, and some contradictory results have been reported, these types of studies demonstrate the potential of probiotics to act on sites beyond the gastrointestinal tract, e.g. the airways. It is hypothesized that immune modulation and reduction of airway pathogenic load through colonization resistance may account for the observed effects.

SOCIOECONOMIC CONSIDERATIONS: ABSENCES FROM DAYCARE AND WORKPLACES

In consideration of the role of probiotics and prebiotics in promoting human health, the analysis of their possible impact on the pharmaco- and socioeconomic burden associated with studied health disorders may be taken into account. Recent studies have documented the efficacy of probiotics on endpoints such as the following: 1) absences from work, whether this is directly related to the health state of the employee or to the need to stay home due to illness of a child that normally attends a daycare center; 2) incidence of infections; 3) length of stay in hospital for different kinds of pathology; and 4) use of antibiotics and other intervention procedures. If further substantiated, such effects could contribute to important quality-of-life and socioeconomic measures.

The incidence of infections can be influenced by certain daily life environments leading to an increased level of exposure and thus a higher risk for transmission of community-acquired pathologies, such as in daycare centers.[131] Studies on the relationship between the ingestion of probiotics and the direct and indirect costs of the most frequent diseases, such as gastrointestinal disorders and respiratory tract infections have been conducted. Hatakka et al.[118] studied the long-term consumption of probiotic milk in a group of 282 healthy children as compared to a control group of 289 and documented a modest reduction in the duration of absences, and in the incidence of respiratory infections and antibiotic use.

A meta-analysis conducted by Van Niel et al.[132] concluded that *Lactobacillus* therapy for acute infectious diarrhea in children was effective. The authors provided an economic context for this effect indicating that "A 48-hour course of a *Lactobacillus* product is commercially available for approximately US$10 and on average could save approximately 17 hours of caring for a sick child with diarrhea, and 1 to 2 diapers."

A double-blind, placebo-controlled, randomized trial carried out by Weizman et al.[133] in 14 daycare centers compared the effect of 2 probiotics delivered in infant formula in 201 healthy infants aged between 4 and 10 months. The authors reported a significant decrease in the number of days with fever, clinic visits, childcare absences, and antibiotic prescriptions.

In another double-blind, placebo-controlled, randomized trial on children attending daycare centers, a dairy probiotic product supplementation reduced the incidence of acute diarrhea by about 30% as compared to a yogurt-supplemented control group.[134] With the same regimen, a significant decrease in the duration of acute diarrhea was also observed in children.[135] This decrease corresponds to a 20% reduction as compared to yogurt consumption and almost 50% as compared to a jellied milk control. These data support the notion that probiotic products can positively impact the attendance of children in daycare centers, hence the cost to the parents and the associated public health issues.

Beyond the focus of probiotics, other publications address the question of costs related to healthcare. Between September 1996 and November 1997 Carabin et al.[136] followed the attendance of 273 toddlers in 52 daycare centers over a 6-month period in order to estimate the direct and indirect costs caused by common infections. The direct costs of medication and visits to a physician and the indirect costs of alternative care provided by a family member, babysitter, or employed parent who missed work were tracked. The evaluation was based on calendars filled in by parents on the occurrence of colds, diarrhea, and vomiting and any actions taken with respect to these occurrences. The overall adjusted average cost per child incurred to the parents and society amounted to US$260.70.

In a recent paper on infectious disease in pediatric out-of-home care,[137] it was estimated that in the United States 13 working days are lost per year by parents who have children in childcare. The interest of the area in general is underscored by a survey reported by Kahan et al.[138] in which a questionnaire completed by 173 pedia-

Table 3. Impact of Probiotics on Common Colds and Influenza (Symptoms)

Reference	Design and probiotic/ prebiotic	Population and Treatment	Parameters	Results	Comments
de Vrese et al. (2005)[116]	Randomized DBPC parallel intervention, prospective; 3 mo or 5.5 consumption in winter/springtime; immune parameters before and after 2 weeks intervention; *L. gasseri* PA 16/8, *B. longum* SP 07/3, *B. bifidum* MF 20/5	479 Healthy adults (18–67 y); vitamin supplement with probiotics: Multibionta (Merck) with 3 strains (daily dose): *L. gasseri* PA 16/8 ($4x10^7$); *B. longum* SP 07/3 ($5x10^6$); *B. bifidum* MF 20/5 ($5x10^6$); or vitamin supplement alone (placebo)	Self assessment with questionnaire; valuation of: single specific symptoms, total symptom score (primary parameter), duration and incidence; subsample: immune cell number, phagocytic activity, type of virus, fecal lactobacilli and bifidobacteria count	↓ Total symptom score ($P=0.056$), most notably in nasal, pharyngeal and bronchial symptoms (all sign or near sign.); ↓ duration from 8.9 to 7.0 days ($P=0.045$); ↓ number of days with fever from 1.0 to 0.24 days ($P=0.017$) - ↔ incidence; ↑ CD8+ T cells	Use of probiotics may reduce duration and symptom-severity of common colds; no effect on incidence
Winkler et al. (2005)[117]	Randomized DBPC parallel intervention, prospective; 3 mo or 5.5 consumption in winter/springtime; immune parameters before and after 2 weeks intervention with *L. gasseri* PA 16/8, *B. longum* SP 07/3 and *B. bifidum* MF 20/5	477 Healthy adults (18–70 y); vitamin supplement with probiotics: Tribion harmonis (Merck) with 3 strains (daily dose): *L. gasseri* PA 16/8 ($4x10^8$), *B. longum* SP 07/3 ($5x10^7$), *B. bifidum* MF 20/5 ($5x10^7$), or vehicle only (placebo)	Self assessment with questionnaire; evaluation of single specific symptoms, total symptom score (primary parameter), duration and incidence; subsample: immune cell number and phagocytic activity	↓ Symptom scores for headache, conjunctivitis, number of days with fever, ↔ nasal, pharyngeal and bronchial symptoms, ↓ incidence ($P=0.07$); mean duration slightly reduced ($P=0.19$), ↑ T cells (CD4 and CD8)	Use of supplement with vitamins, minerals and probiotics may reduce some symptoms of RTI; weaker effects than in previous study (116)
Turchet et al. (2003)[139]	Randomized open intervention pilot study; 3 week consumption period; *L. casei* DN-114 001	360 Elderly >60 y; consumption of 2x100 ml Actimel ($1x10^8$ *L. casei* DN-114 001 cfu/ml) or no study product as control	Incidence of respiratory tract and intestinal symptoms, influenza syndrome (ear/ nose/throat symptoms and respiratory diseases)	↓ Duration of pathologies in total ($P=0.024$); ↓ maximal temp ($P=0.01$); ↔ incidence	Actimel may reduce severity of winter pathologies; no placebo, not blinded short intervention

Table 3. (Cont'd) Impact of Probiotics on Common Colds and Influenza (Symptoms)

Reference	Design and probiotic/ prebiotic	Population and Treatment	Parameters	Results	Comments
Hatakka et al. (2001)[118]	Randomized DBPC, parallel intervention, prospective; consumption during 7 mos over the winter	571 healthy Finnish children (1–6 y) attending day-care centers; consuming milk with 5–10x10^5/ml *L. rhamnosus* GG or standard milk, average 260 ml/day (~2.6x10^8)	Number of days with respiratory or intestinal symptoms; upper respiratory tract infections with complications; lower respiratory tract infections; antibiotic use; absence from day care	↔ Number of days with intestinal or respiratory infections; ↓ days of absence (P=0.03, age adj. P=0.09); relative reduction in number of children with respiratory tract infections with complications (mainly otitis) and lower respiratory tract infections (P=0.05, age adj P=0.13); relative reduction in antibiotic courses (P=0.03, age adj. P=0.08)	Long-term consumption of *L. rhamnosus* GG may reduce the severity and complications of RTI; differences in age distribution among the groups

Abbreviations: ↔, no change; ↓, reduced; ↑, increased. DBPC, double-blind, placebo-controlled.

tricians showed that about half of the physicians felt pressured by parents to provide antibiotic therapy for ill children to accelerate the child's return to its daycare center.

Several other health conditions have been studied to establish the possible contribution of probiotics in terms of healthcare economics. Among these are trials on winter infections in the elderly,[139] common colds,[116,117] and a recently reported work on increasing workplace healthiness, in which there was a 55% reduction of sick-leave in the studied population and particular interest in the well-being of shift-workers.[140]

Other areas that might be promising in terms of cost reduction, with a potential benefit from the use of probiotics, are multiple and possibly include otitis (both acute and chronic),[141,142] growth and osteoporosis, rheumatoid arthritis,[143] cognitive development, stress, and even critical-care management.

However, in order to clearly determine whether the regular intake of probiotics really has an impact on the socio- and pharmacoeconomic burden of current health management and to what extent it may contribute to improving the cost-effectiveness, further research is needed both in developed and developing countries, including among individuals at risk of being confronted with diminished health conditions or impaired well-being because of their daily living conditions. Such studies should be based on validated tools in terms of decision-analysis models.

CONCLUSION

Evidence is accumulating on the health effects of probiotic bacteria and prebiotic carbohydrates. Predecessors to the modern forms of probiotics and prebiotics have been part of humans' daily food since ancient times, but research on the role of these bioactive food compounds is recent. The majority of studies conducted so far have mainly concentrated on the gut. But more recently results are showing solid evidence of the useful role probiotics and prebiotics may play beyond the gut. Although it is common knowledge that the intestinal microbiota may be involved with numerous body functions, relatively few investigators have looked into the importance of these microbial inhabitants of our digestive tract and their interactions with probiotics and prebiotics on systems that are more or less distant from the gastrointestinal tract.

This review presents evidence of the expanding realm of probiotic and prebiotic effects. It remains critically important that further randomized, controlled trials be conducted to determine the nature, extent, reproduc-

ibility, and mechanisms of action of probiotic and prebiotic effects.

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