# Thirty Years of *Lactobacillus rhamnosus* GG
## *A Review*

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**Abstract:** *Lactobacillus rhamnosus* GG (LGG) was the first strain belonging to the genus *Lactobacillus* to be patented in 1989 thanks to its ability to survive and to proliferate at gastric acid pH and in medium containing bile, and to adhere to enterocytes. Furthermore LGG is able to produces both a biofilm that can mechanically protect the mucosa, and different soluble factors beneficial to the gut by enhancing intestinal crypt survival, diminishing apoptosis of the intestinal epithelium, and preserving cytoskeletal integrity. Moreover LGG thanks to its lectin-like protein 1 and 2 inhibits some pathogens such as *Salmonella* species. Finally LGG is able to promote type 1 immune-responsiveness by reducing the expression of several activation and inflammation markers on monocytes and by increasing the production of interleukin-10, interleukin-12 and tumor necrosis factor-α in macrophages. A large number of research data on *Lactobacillus* GG is the basis for the use of this probiotic for human health. In this review we have considered predominantly randomized controlled trials, meta-analysis, Cochrane Review, guide lines of Scientific Societies and anyway studies whose results were evaluated by means of relative risk, odds ratio, weighted mean difference 95% confidence interval. The effectiveness of LGG in gastrointestinal infections and diarrhea, antibiotic and *Clostridium difficile* associated diarrhea, irritable bowel syndrome, inflammatory bowel disease, respiratory tract infections, allergy, cardiovascular diseases, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, cystic fibrosis, cancer, elderly end sport were analyzed.

**Key Words:** *Lactobacillus rhamnosus* GG, gut microbiota, probiotics

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The FAO/WHO Expert Committee has defined probiotic strains as "live microorganisms which, when consumed in appropriate amounts in food, confer a health benefit on the host."[1] Some criteria has been also defined for probiotic species:

- Proper taxonomic identification by molecular techniques.
- Deposition in an internationally recognized culture collection.
- Lack of transmissible antibiotic resistance genes.
- Persistence in a viable state in the gastrointestinal (GI) tract.
- Experimentally and clinically demonstrated health benefits.
- Safety for human use.
- Persistence of cell viability and probiotic activities throughout the processing, handling, and storage.

In general, the microorganisms used as probiotics have a long history of safe usage and are considered GRAS (generally recognized as safe); they must be of human origin, alive, and resistant to acidic pH in the stomach as well as to bile and the alkaline pH in the small intestine. They must be capable of attaching to the mucus among other live bacteria (the microbiota) and to perform metabolic activity.

## LACTOBACILLI

The genus *Lactobacillus* currently contains over 180 species gram-positive, facultative anerobic or microaerophilic, rod-shaped, non–spore-forming bacteria, and encompasses a wide variety of organisms[2,3] representing the major part of the lactic acid bacteria group (ie, they convert sugars to lactic acid). In humans, they constitute a significant component of the microbiota at a number of body sites, such as the digestive system, urinary system, and genital system.

### *LACTOBACILLUS RHAMNOSUS* GG

| Genus | Species | Strain |
|---|---|---|
| *Lactobacillus* | *L. rhamnosus* | *L. rhamnosus GG* |
| Genus characteristics | Species characteristics | Strain characteristics |
| Gram-positive | Common morphology | Typical fermentation profile (API50CH) |
| Rod | Similar biochemical characteristics (within limits) | No plasmids |
| In chains | G+C% 45-47 | Genome analysis |
| Homofermentative or heterofermentative | — | Probiotic characteristics: adhesion, colonization, immunologic effects etc. |
| Catalase-negative | — | — |
| G+C% 33-55 | — | — |

The following box is a copy of the original patent and of the summary of the invention that evidentiates the 3 preconditions essential for colonize the human gut, that are:

- The ability to survive and to proliferate at gastric acid pH.
- The ability to survive and to proliferate in medium containing bile.
- The ability to adhere to enterocytes.

The nuclotidic sequencing of the whole genome of LGG with both the phenotypic and genomic characterization is available since 2009.

The knowledge of mechanisms of action of LGG suggests that it depended mainly on the following criteria:

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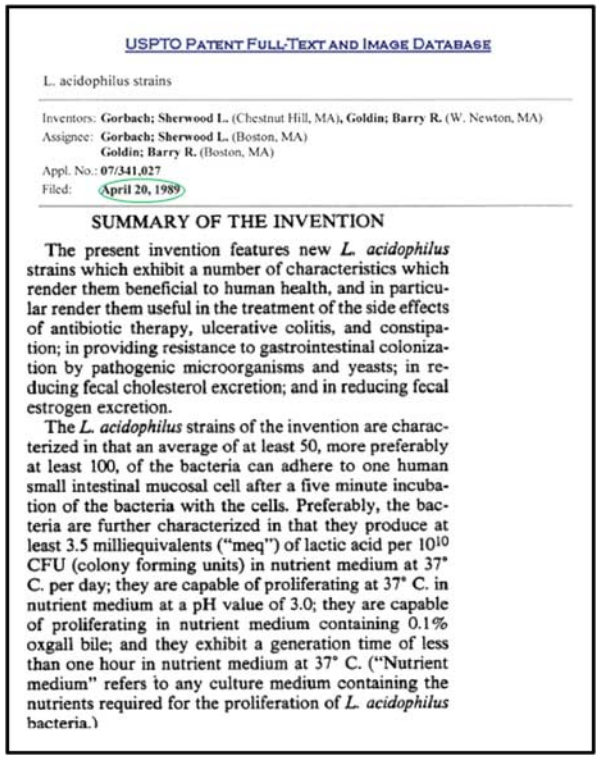
(a) Its high capacity of adhesion to mucosal surfaces thank to his superficial exoplysaccharides and pili.
(b) Its ability to produce >92 proteins in an acid pH milieu.
(c) Its high immune activity.



USPTO PATENT FULL-TEXT AND IMAGE DATABASE

L. acidophilus strains

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Appl. No.: **07/341,027**
Filed: **April 20, 1989**

SUMMARY OF THE INVENTION

The present invention features new *L. acidophilus* strains which exhibit a number of characteristics which render them beneficial to human health, and in particular render them useful in the treatment of the side effects of antibiotic therapy, ulcerative colitis, and constipation; in providing resistance to gastrointestinal colonization by pathogenic microorganisms and yeasts; in reducing fecal cholesterol excretion; and in reducing fecal estrogen excretion.

The *L. acidophilus* strains of the invention are characterized in that an average of at least 50, more preferably at least 100, of the bacteria can adhere to one human small intestinal mucosal cell after a five minute incubation of the bacteria with the cells. Preferably, the bacteria are further characterized in that they produce at least 3.5 milliequivalents ("meq") of lactic acid per $10^{10}$ CFU (colony forming units) in nutrient medium at 37° C. per day; they are capable of proliferating at 37° C. in nutrient medium at a pH value of 3.0; they are capable of proliferating in nutrient medium containing 0.1% oxgall bile; and they exhibit a generation time of less than one hour in nutrient medium at 37° C. ("Nutrient medium" refers to any culture medium containing the nutrients required for the proliferation of *L. acidophilus* bacteria.)

It is especially important to know the following mechanisms:

## ADHESION TO MUCOSAL SURFACES AND NORMALIZATION OF MUCOSAL BARRIER

- LGG versus other *Lactobacilli* has the major adhesion to the mucosal cells.[4]
- LGG adhesion to the mucosa is facilitate by the adhesive protein LGG-0186.[5]
- LGG normalizes the intestinal permeability.[6]
- LGG expresses a long galactose-rich exopolysaccharides (EPS) playing a role in protection against complement-mediated lysis and might be involved in the adhesiveness of the organism.[7,8]
- LGG encode a genome that biosynthesizes a specific *SpaCBA* pili that play a key role in adhesion to mucus, the Caco-2 intestinal epithelial cell line and promote biofilm formation[9]
- LGG genome encodes another pili gene cluster, *spaDEF*.[10]

## IMMUNE ACTIVITY

- LGG stimulates a nonspecific immune response with increase of IgA, IgG, IgM, and enhances intestinal functional maturation and IgA production in neonatal mice.[11–13]
- LGG increases the secretion of interleukin (IL)-6 and the IgA response in splenic cells of rat.[14]
- LGG in vitro generates an effective immune response to antigens.[15]
- LGG inhibits the production of lipopolysaccharides (LPS) and tumor necrosis factor (TNF)-α in murine macrophages.[16]
- LGG soluble factors increases expression of several toll-like receptors (TLRs) in all studied cell types and antigen presentation-associated receptor HLA-DR in macrophages and "intermediate" monocytes, but decreases that of activation markers on monocytes and macrophages and production of IL-10, IL-12, and TNF-α in macrophages.[17]
- LGG in a TLR2/cyclo-oxygenas-2-dependent manner reduces the radiation epithelial lesions.[18]
- LGG expresses 2 genes RS02780 and RS02750 encoding for polypeptides with a N-terminal conserved L-ty lectin designates Llp1 and Llp2 promising bioactive ingredients.[19]
- LGG induces peripheral hyporesponsiveness in stimulated CD4-T cells via modulation of dendritic cells function.[20]
- LGG is sensitive to the human β-defensin-2 but not to the β-defensin-1.[21]
- LGG expresses lipoteichoic acid (LTA) a crucial microbe-associated molecular pattern with proinflammatory activities such as IL-8 induction in intestinal epithelial cells and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) induction in HEK293T cells via TLR2/6 interaction.[22]
- LGG in vitro induced COX2 expression in a time-dependent and concentration-dependent manner in T84 cells, that was inhibited by tyrosine kinase inhibitor genistein (100/μmol), $p^{38}$ mitogen-activated protein kinase (MAPK) inhibitor (SB203580; 1/μmol)and dexamethasone (100/μmol).[23]

## PROTEINS PRODUCTION

- LGG contains genes for 3 secreted LPXTG-like pilins (*spaCBA*) and a pilin-dedicated sortase.[10]
- LGG expresses >90 proteins which are involved in biofilm formation, phage-related functions, reshaping the bacterial cell wall, and immunomodulation.[24]
- LGG produces the soluble protein p40 able to ameliorated cytokine-induced apoptosis in intestinal epithelial cells through activation of the epidermial growth factor (EGF) receptor stimulating a disintegrin and metalloprotease protein 17 (ADAM17) activation and HB-EGF release, which is required for EGF receptor transactivation, prevention of apoptosis, and preservation of barrier function in intestinal epithelial cells.[25–27]
- LGG secretes the major protein Msp1/p75 that can be O-glycosylated with ConA-reactive sugars.[28]
- LGG expresses 2 fluorescent proteins mTagBFP2 and mCherry that could be visualized in mixed-species biofilms and are implemented for the visualization of their adhesion patterns to intestinal epithelial cell cultures.[29]
- LGG-derived soluble proteins p40 and p75 prevent cytokine-induced intestinal epithelial damage and apoptosis and reduce hydrogen peroxide disruption of epithelial barrier. p40 exerts more potent effects than p75.
- p40 regulates cellular responses in intestinal epithelial cells and has protective and therapeutic role in dextran sulfate sodium (DSS)-induced intestinal epithelial injury and acute colitis.
- p40 treatment increased a proliferation-inducing ligand (*APRIL*) gene expression and protein production in small intestinal epithelial cells, fecal IgA levels, IgA+B220+,

IgA+CD19+, and IgA+ plasma cells in lamina propria of Egfrfl/fl. SpaC is necessary for strain GG to adhere to gut mucosa and contributes to strain GG-induced epithelial generation of reactive oxygen species (ROS) playng a role in LGG's capacity to stimulate extracellular signal-related kinase (ERK)/MAPK signaling in enterocytes.[12,30,31]
- LGG is able to form biofilms on abiotic surfaces, in contrast to other strains of the *Lactobacillus casei* group tested under the same conditions. The in vitro biofilm formation is strongly modulated by culture medium factors and conditions related to the GI environment, including low pH, high osmolarity, and the presence of bile, mucins, and nondigestible polysaccharides.[32]

## INFLUENCES ON CYTOKINES ACTIVITY

- LGG prevents cytokine-induced apoptosis in intestinal epithelial cells.[33]
- LGG promotes the production of interferon (IFN)-γ, IL-12, and IL-18.[34]
- LGG alleviates the cytokines proinflammatory effects on mucosa barrier inhibiting NF-κB.[35]
- LGG activates MAPKs and c-Jun N-terminal kinase to induce the transcriptional 1 for *hsp* and increases the mRNA levels of *hsp25* and *hsp72*.[36–38]
- LGG hyperregulates the genes MAPK-related.[39–41]
- LGG stimulates moderately the production of TNF-α and not supports the production of IL-2, IL-12, IL-23, IL-27 in dendritic cells.[42]
- LGG acts on T cells decreasing the production of IL-2, IL-4, and IL-10 in culture medium containing dendritic cells.[43]
- LGG regulates IL-10 signaling in developing murine colon.[44]

## ANTIBACTERIAL ACTIVITIES

- LGG produces microcine, a molecular weight < 1000 bacteriocine resistant to proteases and to heat and 7 peptides which showed anti–gram-negative and anti–gram-positive bactericidal activity.
- Seven peptides were isolated from LGG-conditioned media, which showed anti–gram-negative and anti–gram-positive bactericidal activity.[45–47]

## INFLUENCES On SERT, ROS, $COX_2$

- LGG can upregulate serotonin reuptake transporter (SERT) mRNA and SERT-P levels in intestinal epithelial cells and in mice intestinal tissues.[48,49]
- LGG can induce ROS generation in intestinal epithelia in vitro and in vivo. Intestines from immature mice gavage fed LGG exhibited increased glutathione oxidation and cullin-1 deneddylation, reflecting local ROS generation and its resultant Ubc12 inactivation, respectively. Prefeeding LGG prevented TNF-α-induced intestinal NF-κB activation.
- LGG products activate ROS signaling in a formyl peptide receptors (FPR)-dependent manner and define a mechanism by which cellular ROS influences the ERK pathway through a redox-sensitive regulatory circuit.
- LGG in J774 murine macrophages significantly enhanced ROS generation but also significantly reduced nitric oxide level.[50]
- LGG induce COX2 expression in a time-dependent and concentration-dependent manner in T84 cells. COX2 expression was inhibited by tyrosine kinase inhibitors.[23]

This large number of research data on *Lactobacillus* GG is the basis for the use of this probiotic for human health. Compared with other probiotic strains, LGG showed a better tolerance to conditions in the digestive tract and better survival in functional foods and therefore has been largely utilized in clinics.

Particularly its capacity to adhere to mucosal cells colonizing the gut is determined by its fimbria-like pili and by the production of soluble proteins. Furthermore LGG is able to produces both a biofilm that can mechanically protect the mucosa, and different soluble factors (p75 and p40 proteins, cell wall–associated hydrolase, glyceraldehyde-3-phosphate dehydrogenase, and others) beneficial to the gut by enhancing intestinal crypt survival, diminishing apoptosis of the intestinal epithelium, and preserving cytoskeletal integrity.[7,9,17,25] Moreover, LGG thanks to its lectin-like protein-1 and 2 inhibits some pathogens such as *Salmonella* species or uropathogenic *Escherichia coli*.[19,51] Finally LGG is able to promote type 1 immune-responsiveness by reducing the expressione of several activation and inflammation markers on monocytes and macrophages, and by increasing the production of IL-10, IL-12, and TNF-α in macrophages.[17]

*Lactobacillus* GG taken orally can be recovered from the feces and its colonization capacity seems be significantly better in newborns.[52] Colonic biopsies highlight that LGG can adhere to intestinal mucus[53] suggesting that the colonization continues for longer than indicated by fecal recovery and persist in the descending colon.[54,55]

LGG could also be recovered from the tonsils,[56] vagina,[57] and oral cavity[58] after probiotic therapy.

## *LACTOBACILLUS* GG AND DYSBIOSIS

On the base of these functional properties that distinguish it from other probiotics LGG is able to achieve significant results in the different situations characterized by microbiota dysbiosis.

Dysbiosis[59–61] occurs when bacterial homeostasis is disrupted as a consequence of an imbalance of microbiota composition, a change in metabolic activities and an altered distribution of bacteria in the intestine. On the basis of these elements, dysbiosis shows 3 characteristics:
(1) Numeric loss of beneficial bacteria,
(2) Overgrowth of potentially pathogenic bacteria,
(3) Loss of bacterial diversity.

In most cases, these 3 types of dysbiosis occur simultaneously.

The typical example of dysbiosis is the use of antibiotics that cause a dysregulation of normal bacterial flora, with an overgrowth of potentially pathogenic and toxic microorganisms, thus leading to a rapid and significant drop in taxonomic wealth, uniformity, and diversity.[62,63]

The previously treated mechanism of action of LGG such as enhancement of the epithelial barrier, increased adhesion to intestinal mucosa, concomitant inhibition of pathogen adhesion,[12,30–32] competitive exclusion of pathogenic microorganisms, production of antimicroorganism substances, and modulation of the immune system[38–47] are the reasons why it was selected as candidate probiotic for the prevention and treatment of every cause of dysbiosis

### GI Infections and Diarrhea

LGG colonizes the gut of newborns significantly better than adults and 2 weeks administration of *Lactobacillus* GG right after birth increases gut *lactobacilli* concentrations and

does not impair the establishment of a normal fecal bacterial microbiota.[64]

At 5 days of age infants of mothers who started consumption of *L. rhamnosus* or placebo 4 weeks before delivery showed a significantly higher presence of *Bifidobacterium breve* and a lower one of *Bifidobacterium adolescentis* than those from the placebo group. In addition, *L. rhamnosus* GG consumption increased the bifidobacterial diversity in infants and reduced the bifidobacterial microbiota similarity between mother and infant.[65,66] Colonization with LGG occurred also in 5 of 24 (21%) infants who weighed <1500 g versus 11 of 23 (47%) heavier infants. There was a paucity of bacterial species at baseline, although heavier infants had more bacterial species [1.59 ± 0.13 vs. 1.11 ± 0.12 (SEM); $P < 0.03$] and higher mean log colony forming units (CFU) (8.79 ± 0.43 vs. 7.22 ± 0.63; $P < 0.05$) compared with infants weighing <1500 g. LGG treatment in infants weighing <1500 g resulted in a significant increase in species number by day 7, with further increases by day 21. No significant changes in species number or quantitative counts were noted after LGG treatment in the infants weighing 1500 to1999 g. LGG was well tolerated in all infants.[67] Infants fed with LGG-enriched formula until the age of 6 months grew better than those fed with regular formula and their changes in their length and weight Standard Deviation Score (SDS) at the end study were significantly higher than those receiving regular formula (0.44 ± 0.37 vs. 0.07 ± 0.06; $P < 0.01$ and 0.44 ± 0.19 vs. 0.07 ± 0.06; $P < 0.005$, respectively). At the end of the study a frequent colonization with *Lactobacilli* was found in the LGG group, 91% versus 76% in the control group ($P < 0.05$).[68]

With this background numerous studies have been carried out on the utilization of LGG in children with acute diarrhea, particularly in developing countries.

In 1991 Isolauri and colleagues studied 71 well-nourished children between 4 and 45 months of age with acute diarrhea (82% rotavirus) who after oral rehydration randomly received either *Lactobacillus GG*-fermented milk product, 125 g ($10^{10\text{-}11}$ CFU) twice daily (group 1); *Lactobacillus GG* freeze-dried powder, 1 dose ($10^{10}$ CFU) twice daily (group 2); or a placebo, a pasteurized yogurt (group 3) The mean (SD) duration of diarrhea after commencing the therapy was significantly shorter in group 1 [1.4 (0.8) d] and in group 2 [1.4 (0.8) d] than in group 3 [2.4 (1.1) d] ($P < 0.001$).[69]

In Pakistan, a prospective, placebo-controlled, triple blind clinical trial was carried out to determine the effect of Lactobacillus GG on the course of acute diarrhea in hospitalized children; 40 children (mean age, 13 mo) were enrolled and after rehydration received either oral *Lactobacillus* GG (n = 21) or placebo (n = 19) twice daily for 2 days. Response was evident on day 2 when the frequency of both vomiting and diarrhea was less in the *Lactobacillus* group: 31% versus 75% ($P < 0.01$).[70]

In the Karelian Republic children receiving LGG had a significantly shorter duration of watery diarrhea [mean (SD), 2.7 (2.2) d] than those receiving the placebo [3.7 (2.8) d; $P = 0.03$].[71]

In Thailandia, 39 children (mean age = 8 mo) were enrolled and following rehydration received either oral Lactobacillus GG (n = 20) as a freeze-dried preparation or placebo (n = 19) twice daily for 2 days; the mean duration of diarrhea was significantly shorter in the *Lactobacillus* group (1.9 d) than in the placebo group (3.3 d) ($P < 0.055$); stool frequency was less on the second day in the *Lactobacillus* group ($P < 0.05$).[72]

In Peru, LGG has been evaluated as prophylactic use of to prevent diarrhea in children at high risk in a randomized, placebo-controlled trial. In total, 204 undernourished children 6 to 24 months old received either LGG or placebo in flavored gelatin once daily, 6 days a week, for 15 months. Subjects in the LGG group had significantly fewer episodes of diarrhea (5.21 episodes diarrhea/child/year) versus 6.02 in the placebo group; $P = 0.028$). The decreased incidence of diarrhea in the LGG group was greatest in the 18 to 29-month age group ($P = 0.004$) and was largely limited to nonbreastfed children (breastfed: 6.59 episodes (of diarrhea)/child/year (ecy) LGG, 6.32 ecy placebo, $P = 0.7$; nonbreastfed: 4.69 ecy LGG, 5.86 ecy placebo, $P = 0.005$).[73] In Europe children 1 month to 3 years of age with acute-onset diarrhea were enrolled in a double-blind, placebo-controlled investigation and randomly allocated to group A, receiving oral rehydration solution (ORS) plus placebo, or group B, receiving the same preparation but *Lactobacillus* GG (at least $10^{10}$ CFU/250 mL). In total, 140 children were enrolled in group A, and 147 in group B. Duration of diarrhea after enrollment was 71.9 ± 35.8 hours in group A versus 58.3 ± 27.6 hours in group B (mean ± SD; $P = 0.03$). In rotavirus-positive children, diarrhea lasted 76.6 ± 41.6 hours in group A versus 56.2 ± 16.9 hours in groups B ($P < 0.008$). Diarrhea lasted longer than 7 days in 10.7% of group A versus 2.7% of group B patients ($P < 0.01$). Hospital stays were significantly shorter in group B than in group A.[74]

Three subsequent meta-analysis studies have discussed the use of LGG for the treatment of acute diarrhea in children. In 2007, Szajewska et al[75] published the first meta-analysis on the treatment of acute diarrhea in children with *Lactobacillus* GG. Eight randomized controlled trials (RCTs) (988 participants) met the inclusion criteria. Compared with controls, LGG had no effect on the total stool volume (2 RCTs, n = 303) (Table 1).

**TABLE 1.** Lactobacillus GG Versus Control

| Studies (References) | WMD (95% CI) |
|---|---|
| Total stool volume (mL/kg) | |
| Costa-Ribeiro et al[76] | −44.69 (−125.06 to 35.28) |
| Salazar-Lindo et al[77] | 52.80 (1.21-104.39) |
| Subtotal | 8.97 (−86.26 to 104.20) |
| Stool volume on day 1 (g/kg) | |
| Raza et al[70] | 13.60 (−13.11 to 40.319) |
| Stool volume on day 2 (g/kg) | |
| Raza et al[70] | 12.40 (−6.39 to 31.19) |

CI indicates confidence interval; WMD, weighted mean difference.
Stool output (modified from Szajewska et al[75]).

However, LGG was associated with a significant reduction in diarrhea duration (7 RCTs, 876 infants, weighted mean difference (WMD) = −1.1 days [95% confidence interval (CI), 1.9 to −0.3] (Table 2), particularly of rotavirus etiology (WMD = −2.1 d, 95% CI = −3.6 to −0.6), risk of diarrhea > 7 days [1 RCT, n = 287, relative risk (RR) = 0.25, 95% CI = 0.09-0.75] and duration of hospitalization (3 RCTs, n = 535, WMD = −0.58, 95% CI = −0.8 to −0.4).

The presence of diarrhea on days 1, 2, > 7, > 10 is reported in Table 3.

## Prevention of Health Care–associated Diarrhea in Children

In 2011, the some authors[81] reviewed systematically data on the efficacy of administering *L. rhamnosus* GG for the prevention of health care–associated diarrhea in children, in particular, due to rotavirus, that may prolong the hospital stay and increase medical costs.

Three RCTs involving 1092 children were included.

**TABLE 2.** Lactobacillus GG Versus Control

| Studies (References) | WMD (95% CI) |
|---|---|
| Duration diarrhea of any etiology | |
| Costa-Ribeiro et al[76] | −0.04 (−0.10 to 0.021) |
| Guandalini et al[74] | −0.57 (−0.88 to −0.26) |
| Guarino et al[78] | −2.60 (−2.99 to −2.21) |
| Isolauri et al[79] | −0.80 (−1.25 to −0.35) |
| Shornikova et al[71] | −1.10 (−1.99 to −0.21) |
| Jasinski et al[80] | −3.00 (−3.84 to −2.16) |
| Salazar-Lindo et al[77] | −0.34 (−0.13 to 0.81) |
| Subtotal | −1.08 (−1.87 to −0.20) |
| Duration of rotavirus diarrhea | |
| Guandalini et al[74] | 0.05 (1.09-0.01) |
| Guarino et al[78] | −3.00 (−3.50 to −2.50) |
| Jasinski et al[80] | −2.40 (−3.34 to −1.46) |
| Subtotal | −2.08 (−3.55 to −0.60) |
| Duration diarrhea by invasive enteropathogens | |
| Guandalini et al[74] | 0.05 (−0.64 to 0.74) |
| Duration diarrhea of unknown cause | |
| Guandalini et al[74] | −0.46 (−0.98 to 0.06) |
| Jasinski et al[80] | −3.00 (−4.24 to −1.75) |
| Subtotal | −1.66 (−4.15 to 0.82) |

CI indicates confidence interval; WMD, weighted mean difference.
Mean duration of diarrhea (h) (modified from Szajewska et al[75]).

Compared with placebo, LGG administration was associated with significantly lower rates of diarrhea (2 RCTs, n = 823, RR = 0.37, 95% CI = 0.23-0.59) and symptomatic rotavirus gastroenteritis (3 RCTs, n = 1043, RR = 0.49, 95% CI = 0.28-0.86). The pooled results showed no significant difference between the LGG and the placebo groups in the incidence of asymptomatic rotavirus infection (2 RCTs, n = 301, RR = 1.39, 95% CI = 0.74-2.62) (Table 4).

The authors themselves in 2013 considered the treatment of acute gastroenteritis in children.[85] Fifteen RCTs (2963 participants) met the inclusion. Combined data from 11 RCTs (n = 2444) showed that LGG significantly reduced the duration of diarrhea compared with placebo or no treatment [mean difference (MD), −1.05 d, 95% CI = −1.7 to −0.4]. LGG was more effective when used at a daily dose $\geq 10^{10}$ CFU (8 RCTs, n = 1488, MD, −1.11 d, 95% CI = −1.91 to −0.31) than when used at a daily dose $<10^{10}$ CFU (3 RCTs, n = 956, MD −0.9 d, 95% CI = −2.5 to 0.69) (Table 5).

LGG was effective in children treated in Europe (5 RCTs, n = 744, MD, 1.27 d, 95% CI = −2.04 to −0.49); in the non-European setting, the difference between the LGG group and the control group was of a borderline statistical significance (6 RCTs, n = 1700, MD, −0.87, 95% CI = −1.81 to 0.08) (Tables 6, 7).

**TABLE 3.** Lactobacillus GG Versus Control

| Studies (References) | RR (95% CI) |
|---|---|
| Diarrhea on day 1 | |
| Raza et al[70] | 0.37 (0.17-0.84) |
| Diarrhea on day 2 | |
| Guandalini et al[74] | 0.61 (0.43-0.85) |
| Isolauri et al[79] | 0.22 (0.05-0.91) |
| Subtotal | 0.56 (0.40-0.78) |
| Diarrhea > 7 d | |
| Guandalini et al[74] | 0.25 (0.09-0.75) |
| Diarrhea > 10 d | |
| Jasinski et al[80] | 0.23 (0.03-1.91) |

CI indicates confidence interval; RR, relative risk.
Presence of diarrhea (modified from Szajewska et al[75]).

**TABLE 4.** Rates of Diarrhea

| Studies (References) | RR (95% CI) |
|---|---|
| Diarrhea | |
| Hojsak et al[82] | 0.42 (0.25-0.71) |
| Szajewska et al[83] | 0.20 (0.06-0.66) |
| Total | 0.37 (0.23-0.59); NNT = 12 (95% CI = 8-21) |
| Rotavirus gastroenteritis | |
| Hojsak et al[82] | 0.19 (0.01-4.04) |
| Mastretta et al[84] | 0.63 (0.35-1.16) |
| Szajewska et al[83] | 0.13 (0.02-1.06) |
| Subtotal | 0.49 (0.28-0.86); NNT = 35 |
| Asymptomatic rotavirus infection | |
| Mastretta et al[84] | 1.30 (0.60-2.80) |
| Szajewska et al[83] | 1.60 (0.52-4.89) |
| Subtotal | 1.39 (0.74-2.62) |

CI indicates confidence interval; NNT, number needed to treat; RR, relative risk.

## Functional GI Disorders in Children

The effect of LGG on abdominal pain-related functional GI disorders in childhood was the object of another meta-analysis.[90] The 3 included studies enrolled 290 patients with irritable bowel syndrome (IBS) (3 studies), functional abdominal pain (2 studies), and functional dyspepsia (1 study). In all of the studies, LGG was compared with placebo. The daily dose of LGG ranged from $10^9$ CFU twice daily to $3\times10^9$ CFU twice daily, for 4 to 12 weeks. For the overall study population with abdominal pain LGG supplementation compared with placebo was associated with a significantly higher rate of responders to the treatment (defined as no pain or a decrease in pain intensity) (3 RCTs, n = 290, RR = 1.31, 95% CI = 1.08-1.59, number needed to

**TABLE 5.** Lactobacillus GG Versus Control

| Studies | Mean Difference (95% CI) |
|---|---|
| $\geq 10^{10}$ | |
| Costa-Ribeiro et al[76] $1\times10^{10}$ | –0.04 (–0.10 to 0.02) |
| Guandalini et al[74] $1\times10^{10}$ | –0.57 (–0.88 to –0.26) |
| Shornikova et al[71] $1\times10^{10}$ | –1.10 (–1.99 to –0.21) |
| Jasinski et al[80] $1\times10^{10}$ | –3.00 (–3.84 to –2.16) |
| Berni Canani et al[86] $1.2\times10^{10}$ | –1.24 (–1.59 to –0.89) |
| Ritchie et al[87] $1.5\times10^{10}$ | 0.05 (–1.07 to 1.17) |
| Isolauri et al[79] $2\times10^{10}$ | –0.80 (–1.25 to –0.35) |
| Basu et al[88] $1\times10^{12}$ | –2.16 (–2.38 to –1.94) |
| Subtotal | –1.11 (–1.91 to –0.31) |
| $\leq 10^{10}$ | |
| Basu et al[88] $1.2\times10^{8}$ | 0.20 (–0.14 to 0.54) |
| Basu et al[88] $1.2\times10^{8}$ | |
| Misra et al[89] $1\times10^{9}$ | –0.31 (–0.64 to 0.02) |
| Guarino et al[78] $6\times10^{9}$ | –2.60 (–2.99 to –2.21) |
| Subtotal | –0.90 (–2.50 to 0.69) |
| Total | –1.05 (–1.70 to –0.40) |

CI indicates confidence interval.
Duration of diarrhea. High dose and low dose (modified from Szajewska et al[85]).

**TABLE 6.** LGG Versus Control

| Studies in Europe | Mean Difference (95% CI) |
|---|---|
| Berni Canani et al[86] | –1.24 (–1.59 to –0.89) |
| Guandalini et al[74] | –0.57 (–0.88 to –0.26) |
| Guarino et al[78] | –2.60 (–2.99 to –2.21) |
| Isolauri et al[79] | –0.80 (–1.25 to –0.35) |
| Shornikova et al[71] | –1.10 (–1.99 to –0.21) |
| Subtotal | –1.27 (–2.04 to –0.49) |
| Studies in non-Europe | |
| Basu et al[88] | 0.20 (–0.14 to 0.54) |
| Basu et al[88] | –2.16 (–2.38 to –1.94) |
| Costa-Ribeiro et al[76] | –0.04 (–0.10 to 0.02) |
| Jasinski et al[80] | –3.00 (–3.84 to –2.16) |
| Misra et al[89] | –0.31 (–0.64 to 0.02) |
| Ritchie et al[87] | 0.05 (–1.07 to 1.17) |
| Subtotal | –0.87 (–1.81 to 0.08) |

CI indicates confidence interval.
Duration of diarrhea in Europe and in non-Europe (modified from Szajewska et al[85]).

treat (NNT) = 7, 95% CI = 4-22). For a subgroup of children with IBS, those in the LGG group were more likely to respond to the treatment than those in the placebo group (3 RCTs, n = 167, RR = 1.70, 95% CI = 1.27-2.27, NNT = 4, 95% CI = 3-8). For the functional abdominal pain group (2 RCTs, n = 103, RR = 1.08, 95% CI = 0.77-1.50), as well as for the functional dyspepsia group (1 RCT, n = 20, RR = 0.83, 95% CI = 0.37-1.85), there was no evidence that LGG supplementation influenced the treatment response (Table 8).

The frequency of pain was reduced in those in the LGG group compared with those in the placebo group (2 RCTs, n = 117; RR = −1.04, 95% CI = −1.43 to −0.65) (Table 9).

Compared with placebo, the use of LGG was associated with a significant decrease in the perception of pain intensity in the overall study population with abdominal pain-related functional gastrointestinal disorder (2 RCTs, n = 240; standardized mean difference = 0.44, 95% CI = 0.82-0.05). Similarly, there was a reduction in pain intensity in the subgroup of children with IBS who received LGG compared with placebo (2 RCTs, n = 117; standardized mean difference = 0.60, 95% CI = 0.97-0.23), but not in children with familial adenomatous polyposis and functional dyspepsia (Table 10).

Table 11 summarize data on studies with LGG in diarrhea in pediatric age.

## Antibiotic-associated Diarrhea (AAD)

Another important subject to cover is the effect of antibiotics (AB) on the gut microbiota.

In Europe, about one third of patients receives AB therapy during hospitalization.[94] A common adverse effect

**TABLE 7.** Lactobacillus GG Versus Control

| Studies (References) | Mean Difference (95% CI) |
|---|---|
| Basu et al[88] | 0.10 (–0.09 to 0.29) |
| Basu et al[88] | –3.53 (–3.85 to –3.21) |
| Guandalini et al[74] | –0.73 (–0.94 to –0.52) |
| Shornikova et al[71] | –1.60 (–3.70 to 0.50) |
| Total | –1.42 (–3.05 to 0.21) |

CI indicates confidence interval.
Hospital stay (modified from Szajewska et al[85]).

**TABLE 8.** Primary Outcome

| Studies (References) | Standard Mean Difference (95% CI) |
|---|---|
| Overall | |
| Bausserman and Michail[91] | 1.10 (0.57-2.11) |
| Francavilla et al[92] | 1.34 (1.02-1.74) |
| Gawronska et al[93] | 1.36 (1.00-1.83) |
| Subtotal | 1.31 (1.08-1.59); NNT 7, 95% CI = 4-22 |
| Irritable bowel syndrome | |
| Bausserman and Michail[91] | 1.10 (0.57-2.11) |
| Francavilla et al[92] | 1.76 (1.19-2.59) |
| Gawronska et al[93] | 2.41 (1.31-4.44) |
| Subtotal | 1.70 (1.27-2.27); NNT 4, 95% CI = 3-8 |
| Functional abdominal pain | |
| Francavilla et al[92] | 1.06 (0.61-1.87) |
| Gawronska et al[93] | 1.09 (0.73-1.61) |
| Subtotal | 1.08 (0.77-1.50) |
| Functional dyspepsia | |
| Gawronska et al[93] | 0.83 (0.37-1.85) |
| Subtotal | 0.83 (0.37-1.85) |

CI indicates confidence interval; NNT, number needed to treat.
Effect of Lactobacillus GG on responder rates (modified from Horvath et al[90]).

of AB treatment is the development of AAD defined as ≥ 3 liquid stools in 24 hours that occur in subjects during or even within 6 to 8 weeks after antibiotic.[59–63,95,96] The global prevalence of AAD, with inclusion of the mild to moderate attacks without further clinical diagnostic evaluation, is not well established. Attack rates vary depending on the antibiotic used, the epidemiological setting and the host.[97] The incidence of AAD is estimated as 29% to 60% and is associated with increased costs and length of hospital stay.[95,96]

The effect of AB on small and large intestine microbiota has been evaluated by Ubeda and Pamer[97] (Table 12).

Turck et al[98] published an important paper on risk factors of AAD in children.The incidence of AAD was significantly greater in children below 2 years (61 of 336 = 18%) than in those above 2 years (10 of 314 = 3%; $P < 0.001$). The RR of onset of an episode of diarrhea in a

**TABLE 9.** Secondary Outcome: Effect of Lactobacillus GG on Frequency of Pain

| Studies (References) | Standard Mean Difference (95% CI) |
|---|---|
| Overall | |
| Francavilla et al[92] | –1.07 (–1.43 to –0.71) |
| Gawronska et al[93] | –0.25 (–0.64 to 0.13) |
| Subtotal | –0.67 (–1.46 to 0.13) |
| Irritable bowel syndrome | |
| Francavilla et al[92] | –1.11 (–1.58 to –0.63) |
| Gawronska et al[93] | –0.89 (–1.57 to –0.21) |
| Subtotal | –1.04 (–1.43 to –0.65) |
| Functional abdominal pain | |
| Francavilla et al[92] | 0.16 (–0.37 to 0.69) |
| Gawronska et al[93] | –0.06 (–0.63 to 0.51) |
| Subtotal | 0.06 (–0.33 to 0.45) |
| Functional dyspepsia | |
| Gawronska et al[93] | 0.46 (–0.43 to 1.35) |

CI indicates confidence interval.
Modified from Horvath et al.[90]



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**TABLE 10.** Secondary Outcome

| Studies (References) | Standard Mean Difference (95% CI) |
|---|---|
| Overall | |
| Francavilla et al[92] | –0.62 (–0.97 to –0.28) |
| Gawronska et al[93] | –0.23 (–0.62 to 0.15) |
| Subtotal | –0.44 (–0.82 to –0.05) |
| Irritable bowel syndrome | |
| Francavilla et al[92] | –0.44 (–0.82 to –0.05) |
| Gawronska et al[93] | –0.54 (–1.20 to 0.12) |
| Subtotal | –0.60 (–0.97 to –0.23) |
| Functional abdominal pain | |
| Francavilla et al[92] | –0.22 (–0.80 to 0.35) |
| Gawronska et al[93] | –0.22 (–0.80 to 0.35) |
| Subtotal | –0.32 (–0.71 to 0.07) |
| Functional dyspepsia | |
| Gawronska et al[93] | 0.68 (–0.23 to 1.59) |

CI indicates confidence interval.
Effect of Lactobacillus GG on intensity/severity of pain.
Modified from Horvath et al.[90]

child below 2 years was 1.81 (range = 1.50 to 2.14). In the group of children above 2 years, the incidence of AAD was greater in the youngest of them (2 to 7 y; 9 of 253 = 4%) than in the older patients (> 7 y; 1 of 61 = 2%), but the difference was not significant. Children with an episode of AAD were younger than those who did not have episodes of diarrhea (13.4 ± 14.7 vs. 36.6 ± 34.5 mo; $P < 0.001$). The rate of onset of AAD differed significantly ($P = 0.012$) according to the type of antibiotic prescribed:

- Penicillins G and V, 3% penicillins A and M (except amoxicillin/clavulanate), 11%
- Amoxicillin/clavulanate, 23%
- Cephalosporins, 9%
- Macrolides, 8%
- Trimethoprim/sulfamethoxazole, 6%
- Erythromycin/sulfafurazole, 16%

## Meta-Analysis on Probiotics in AAD

Specific probiotics have been considered as the best intervention to control dysbiosis. Some meta-analyses considered studies with efficacy of the administration of probiotics on AAD:

(1) McFarland[99] included 25 RCTs (n = 2810) obtaining a RR = 0.43, 95% CI = 0.31-0.58, $P < 0.001$ in favor of probiotics. The probiotic strains that showed significant efficacy were *Saccharomyces boulardii*: RR = 0.37, 95% CI = 2.26-0.52, $P < 0.0001$ and *L. rhamnosus* GG: RR = 0.31, 95% CI = 0.13-0.72 ($P = 0.006$).

(2) Johnston et al[100] in a meta-analysis of randomized placebo-controlled trials included 6 studies (total n = 707 patients). The results showed significant benefit for the use of probiotics over placebo with a RR = 0.43 (95% CI = 0.25-0.75). A subgroup analysis on 4 studies provided evidence that at least $5^9$ CFU daily (range, 5.5 to $40\times10^9$ ) of single-strain (*Lactobacillus GG, L. sporogens* or *S. boulardii*) showed strong evidence with narrow CIs for the preventative effects of probiotics for AAD with RR = 0.36 (95% CI = 0.25-0.53).

(3) Videlock and Cremonini[101] found a RR of AAD = 0.53 (95% CI, 0.44-0.63) when compared with placebo, with a NNT = 8 (95% CI, 7-11).

(4) Hempel et al[108] included 63 RCT (11,811 participants) with a RR = 0.58 (95% CI = 0.50-0.68) to develop diarrhea in probiotic group compared with a control group; the pooled risk difference of developing AB-associated diarrhea was statistically significant (RR = −0.07, 95% CI = −0.10 to −0.05) with NNT = 13 (95% CI = 10.3-19.1).

(5) Ritchie and Romanuk[102] evidentiates the efficacy in prevention and treatment of several pathologies selecting 74 controlled randomized studies with a large number of patients. For AAD they evidentiated a RR = 0.43 (95% CI = 0.32-0.56).

(6) Pattani et al[103] in a further meta-analysis evaluated the efficacy of probiotics administered with AB demonstrating a RR = 0.61 (95% CI = 0.47-0.79, NNT = 11 6. A Cochrane Review[104] analyzed 23 studies (3938 participants). Trials included treatment with either *Bacillus* spp., *Bifidobacterium* spp., *Clostridium butyricum*, *Lactobacilli* spp., *Lactococcus* spp., *Leuconostoc cremoris*, *Saccharomyces* spp., o or *Streptococcus* spp., alone or in combination. The incidence of AAD in the probiotic group was 8% (163/1992) compared with 19% (364/1906) in the control group with RR = 0.46 (95% CI = 0.35-0.61). The pooled estimate suggests a precise probiotic

**TABLE 11.** Therapeutic Effect of LGG in Acute and Persistent Diarrhea

| Causa Dell'infezione | No. (Age) Patients | Dose | Clinical Effect (Controlls/ Treated) |
|---|---|---|---|
| Not documented | 71 (children) | Fermented milk, $10^9$ CFU/mL for 5 d | < Length acute diarrhea (2.4/ 1.4 d)[79] |
| Rotavirus | 49 (children) | Polvere, $10^{10}$-$10^{11}$ CFU twice daily for 5 d | < Length acute diarrhea (2.7/ 1.8 d); > IgA[92] |
| Rotavirus | 40 (children) | Polvere$10^{10}$-$10^{11}$ CFU×2/di for 5 d | < No. patients with acute diarrhea (75/31%) at day 2[73] |
| Rotavirus | 123 (children) | Polvere $10^9$ CFU× 2 /ORS | < Length acute diarrhea (30.4/ 17.7 h)[92] |
| Rotavirus, enteric bacteria | 204 (children) | $3.7\times10^{10}$ CFU×1/d for 6 d/wk for 15 mo | Prophylactic effect on diarrhea incidence[73] |
| Rotavirus | 287 (children) | Polvere $10^{10}$ CFU/mL ORS until stop | < Length acute diarrhea (76.6/ 57.2 h)[74] |
| Rotavirus | 81 (children) | $6\times10^9$ CFU/mL for 2 d in hospital | Prophylactic effect on diarrhea incidence[83] |
| Rotavirus (27.5) enteric bacteria (36%) | 179 (infants) | Fermented milk $10^9$ CFU/mL for day+ORS | Not significative difference on diarrhea length[77] |
| Not documented | 192 (children) | $6\times10^9$ CFU/mL/ d+ORS | < Length acute diarrhea (115.5/ 78.5 h)[86] |
| Rotavirus, enteric bacteria | 64 (children) | $5\times10^9$ CFU/mL 3 times a day for 3 d+ORS | < Length acute diarrhea (115.5/ 78.5 h)[88] |

Controlled studies in pediatric age.
ORS indicates oral rehydration solution.

**TABLE 12.** Antibiotic-induced Changes on the Gut Microbiota

| Antibiotic | Effect on the Microbiota | Effect on Immunity |
|---|---|---|
| Amoxicillin | Lactobacillus spp. depletion in SI<br>↓aerobic and anerobic bacterial numbers in the colon | ↓ MHC I and MHC II expression in SI and LI<br>↓ AMPs expression in SI<br>↑ mast cell proteases expression in SI |
| Metronidazole, neomycin, and vancomycin | ↓ Bacterial numbers in SI and LI<br>Multiple effects on composition, including:<br>↓Bacteroidetes ↑ Enterobacteriaceae | ↓ Reg3γ expression in SI |
| Metronidazole | Bacteroidales and *Clostridium coccoides* depletion ↑ Lactobacilli | ↑ Reg3γ and IL-25 expression in colon<br>↑ numbers of macrophages and NK cells in<br>Colon ↓ mucus |
| Colistin | ND | ↓ numbers of ILFs |
| Ampicillin, neomycin, metronidazole, vancomycin | Microbiota depletion<br>↓ peptidoglycan levels in serum | ↓Neutrophil-mediated killing of pathogenic bacteria<br>↓Reg3γ expression by γδ T cells<br>↓pro-IL-1β, pro-IL-18, NLRP3 |
| Amoxicillin/clavulanate | ND | ↓ IgG serum levels |
| Ampicillin, gentamicin, metronidazole, neomycin, vancomycin | ↓ Bacterial numbers in LI<br>Multiple effects on composition, including in LI:<br>↓luminal Firmicutes<br>↓ mucosal associate Lactobacillus | ↓ IFN-γ and IL-17 production by CD4 + T cells in SI<br>↑ IgE serum levels<br>↑ basophils in blood |
| Vancomycin | ↓ Gram-positive bacteria<br>↑ Enterobacteriaceae | ↓ Treg cells in colon<br>↓ Th17 in SI<br>↓ ILFs to a lesser extent than colistin |

IL indicates interleukin; IFN, interferon; ILF, inducible lymphoid organs; LI, large intestine; MHC, major histocompatibility complex; ND, not determined; NK, Natural Killer; SI, small intestine; ↑, increase; ↓, decrease.
Modified from Ubeda and Pamer.[97]

effect with a RR = 0.46 (95% CI = 0.35-0.61, NNT = 10). Among the various probiotics evaluated, *L. rhamnosus* or *S. boulardii* at 5 to $40^9$ CFU/d may be appropriate given the modest NNT and the likelihood that adverse events are very rare.

(7) Blaabjerg et al[105] in a recent meta-analysis included data from 17 studies with a total of 3631 patients showing that the probiotics may reduce the risk of AAD by 51% (RR = 0.49; 95% CI = 0.36-0.66) with a a NNT = 11 (95% CI = 6-13). The most effective probiotic strain was *L. rhamnosus* GG (RR = 0.29; 95% CI = 0.15-0.57; 307 participants), followed by *S. boulardii* (RR = 0.41, 95% CI = 0.30-0.57; 1139 participants). It was provided a preliminary evidence of a possible dose-response relationship considering that higher doses were associated with fewer ADD events (higher than $5\times10^9$ CFU = 3.6% vs. $<5\times10^9$ CFU = 8.9%; $P < 0.002$). These data confirm those based on 25 studies with 13 probiotics in which a dose cut-off point was determined: in studies with a dose below $10^{10}$ CFU, probiotics tended to be ineffective.[106]

(8) Hawrelak et al[107] have made a meta-analysis to evaluate the effectiveness of *Lactobacillus GG* in preventing AAD. Six trials that met eligibility were included, but significant statistical heterogeneity of the trials precluded meta-analysis. Four of the 6 trials found a significant reduction in the risk of antibiotic-associated diarrhea with coadministration of *Lactobacillus* GG (Tables 13–15).

## Clostridium difficile–associated Diarrhea (CDAD)

AB may lead to reduced resistance to pathogens such as *C. difficile* that is the leading cause of nosocomially acquired intestinal infection affecting virtually all cases of pseudomembranous colitis and up to 20% of cases of AAD. Even after receiving antibiotic treatment with either metronidazole or vancomycin, 20% of patients will have recurrent *C. difficile* diarrhea. The use of probiotics to protect gut microbiota by AB has been hypothesized.

Some preliminary studies have investigated this possibility highlighting a positive effect of probiotics on CDAD also with *Lactobacillus* GG.[118–121]

A meta-analysis of 3 studies that used the probiotic combination *Lactobacillus acidophilus* CL1285 and *L. casei* LBC80R and a combined analysis of those studies with 4 studies that used *S. boulardii*, showed lower CDAD rates in recipients of probiotics compared with recipients of placebo (RR = 0.39; 95% CI = 0.19-0.79).[122]

A recent Cochrane Review[123] in a complete case analysis (ie, participants who completed the study) among trials investigating CDAD (31 trials, 8672 participants) suggests that probiotics reduce the risk of CDAD by 60%. The incidence of CDAD was 1.5% (70/4525) in the probiotic

**TABLE 13.** Risk Reduction of AAD With Probiotics in Published Meta-Analysis

| Meta-Analyis AAD | RR (95% CI); NNT (95% CI) |
|---|---|
| McFarland[99] | 0.43 (0.31-0.58), $P < 0.001$ |
| Johnston et al[100] | 0.43 (0.25-0.75) |
| Videlock and Cremonini[101] | 0.53 (0.44-0.63); NNT = 8 (7-11) |
| Hempel et al[108] | 0.58 (0.50-0.68); NNT = 13 (10.3-19.1) |
| Rirchie[102] | 0.43 (0.32-0.56) |
| Pattani et al[103] | 0.61 (0.47-0.79); NNT = 11 (8-20) |
| Goldenberg et al[104] | 0.46 (0.35-0.61) |
| Blaabjerg et al[105] | 0.49 (0.36-0.66 ); NNT = 11 (6-13) |

AAD indicates antibiotic-associated diarrhea; CI, confidence interval; NNT, number needed to treat; RR, relative risk.



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**TABLE 14.** Risk Reduction of Antibiotic-associated Diarrhea With LGG in Published Meta-Analysis

| Trial | LGG, CFU/d | Antibiotic Studied | % of Subjects With Diarrhea LGG Group | % of Subjects With Diarrhea Placebo Group | Relative Risk (95% CI) |
|---|---|---|---|---|---|
| Siitonen et al[109] | LGG yoghurt | Erythromycin | 2 d | 8 d† | Not determined |
| Vanderhoof et al[110] | $1\times10^{10}$ to $2\times10^{10}$/10 | Various | 8 | 26* | 0.29 (0.13-0.63) |
| Arvola et al[111] | $4\times10^{10}$/7-10 | Various | 5 | 16* | 0.32 (0.09-1.11) |
| Thomas[115] | $2\times10^{10}$/14 | Various | 29 | 30 | 0.98 (0.68-1.4) |
| Armuzzi[113] | $1.2\times10^{10}$/14 | Rabeprazole, clarithromycin, and tinidazole | 3 | 27* | 0.13 (0.02-0.94) |
| Cremonini[116] | $1.2\times10^{10}$/14 | Rabeprazole, clarithromycin, and tinidazole | 5 | 30* | 0.17 (0.02-1.27) |
| Mc Farland[99] | — | — | — | — | 0.31 (0.13-0.72) |
| Blaabjerg et al[105] | — | — | — | — | 0.29 (0.15-0.57) |

*Statistically significant difference ($P < 0.05$).
†Analyzed by mean days of diarrhea in each group.
CI indicates confidence interval.

group compared with 4.0% (164/4147) in the placebo or no treatment control group (RR = 0.40, 95% CI = 0.30-0.52), NNTB = 42 patients (95% CI = 32-58).

## LGG in Necrotizing Enterocolitis (NEC)

NEC is the most common serious acquired disease of the GI tract in preterm infants, characterized by bowel wall necrosis, of various length and depth. Bowel perforation occurs in one third of the affected infants.[124,125] The incidence of NEC varies across countries and neonatal centers. It has been reported to affect up to 10% of very low–birthweight infants (VLBW).[126] Intestinal ischemia and colonization of the intestine by pathologic bacteria are considered the causes of NEC. The immaturity of the intestinal barrier is among the major etiological factors that may be modulated by probiotic administration. Most probiotic trials in preterm infants have focused on the impact on intestinal colonization[67] and recent critical reviews and meta-analyses justified this kind of intervention.[127–130] A Cochrane Review[131] included in the analysis 24 eligible trials highly variable with regard to enrollment criteria (ie, birth weight and gestational age), baseline risk of NEC in the control groups, timing, dose, formulation of the probiotics, and feeding regimens. In the meta-analysis of trial data (Table 16), enteral probiotics supplementation significantly reduced the incidence of severe NEC (stage II or more), RR = 0.43 (95% CI = 0.33-0.56) (20 studies, 5529 infants) and mortality RR = 0.65 (95% CI = 0.52-0.81) (17 studies, 5112 infants).

**TABLE 15.** Effect of *Lactobacillus rhamnosus* GG for Preventing Antibiotic-associated Diarrhea

| Studies | RR (95% CI) |
|---|---|
| Antibiotics for infections in children | |
| Vanderhoof et al[110] | 0.29 (0.13-0.63) |
| Arvola et al[111] | 0.32 (0.09-1.11) |
| King et al[115] | 0.66 (0.22-1.97) |
| Vaisanen et al[116] | 1.17 (0.47-2.95) |
| Subtotal (95% CI) | 0.52 (0.25-1.05) |
| Antibiotics as part of *H. pylori* eradication therapy in children | |
| Szajewska[118] | 0.29 (0.06-1.35) |
| Antibiotics for infections in adults | |
| Thomas et al[112] | 0.98 (0.68-1.42) |
| Subtotal (95% CI) | 1.13 (0.64-1.99) |
| Antibiotics as part of *H. pylori* eradication therapy in adults | |
| Armuzzi et al[113] | 0.12 (0.04-0.34 |
| Cremonini et al[114] | 0.16 (0.02-1.20) |
| Armuzzi et al[113] | 0.29 (0.10-0.82) |
| Padilla et al[117] | 0.69 (0.22-2.19) |
| Subtotal (95% CI) | 0.26 (0.11-0.59) |
| Total (95% CI) | 0.49 (0.29-0.83) |

CI indicates confidence interval; *H. pylori*, *Helicobacter pylori*; RR, relative risk.

Table 17 point out data obtained in the trials with *Lactobacillus* GG.

The ESPGHAN Working Group for Probiotics, Prebiotics & Committee on Nutrition in 2017[135] has published a systematic review and network meta-analysis of RCTs investigating probiotics in preterm infants reporting as outcomes data on mortality, NEC, late-onset sepsis (LOS), or time until full-enteral feeding. In total, 51 RCTs involving 11,231 preterm infants were included.

Seven treatments reduced NEC incidence, 2 reduced LOS, and 3 reduced time until full-enteral feeding.

In 7 trials has been utilized the *Lactobacillus* GG:

(1) Chrzanowska-Liszewska et al[136] compared the stool of bottle-fed preterms, randomized to receive LGG $6\times10^9$ (21 babies) or placebo (26 babies) with formula feeding. Fecal sampling was performed at day 7, 21, 42. Presence of LGG colonization, somatic growth, and length of hospital stay were recorded The number of *Lactobacillus* were significantly higher ($P = 0.014$) on day 7, and 21 ($P = 0.024$) in the study group, and so was the number of *Enterobacteriaceae* on all study days ($P = 0.004$, 0.000, 0.000, respectively), and *Enterococcus* spp on day 21 ($P = 0.000$).
(2) Dani et al[132] evaluate the effectiveness of *Lactobacillus* GG supplementation in reducing the incidence of urinary tract infections, bacterial sepsis, and NEC in preterm infants. In total, 585 patients were studied. The duration of *Lactobacillus* GG and placebo supplementation was 47.3 ± 26.0 and 48.2 ± 24.3 days, respectively. Bacterial sepsis was more frequent in the probiotics

**TABLE 16.** Probiotics Versus Control (All Infants)

| Outcome or Subgroup Title | No. Studies | No. Participants | Statistical Methods |
|---|---|---|---|
| Severe NEC (stage II-III) | 20 | 5529 | RR (95% CI) = 0.43 (0.33-0.56) |
| Mortality | | | |
| All causes | 17 | 5112 | RR (95% CI) = 0.65 (0.52-0.81) |
| NEC-related mortality | 7 | 2755 | RR (95% CI) = 0.39 (0.18-0.82) |
| Hospitalization (d) | 11 | 3713 | Mean difference (95% CI) = −3.71 (−4.32 to −3.11) |
| Severe NEC or sepsis | 1 | 367 | RR (95% CI) = 0.54 (0.37-0.79) |
| Severe NEC: *Lactobacillus* | 5 | 1955 | RR (95% CI) = 0.45 (0.27-0.75) |
| Mortality: *Lacctobacillus* | 4 | 1734 | RR (95% CI) = 0.72 (0.47-1.10) |

CI indicates confidence interval; NEC, necrotizing enterocolitis; RR, relative risk.
Modified from AlFaleh and Anabrees.[131]

group (4.4%, n = 11) than in the placebo group (3.8%, n = 9), but the difference was not significant.

(3) Manzoni et al[133] evaluated the effectiveness of LGG 6 $\times 10^9$ CFU/d in the prevention of GI colonization by *Candida* species in preterm, VLBW (ie, <1500 g) neonates during their stay in a neonatal intensive care unit. During the first 3 days of life, the neonates were randomly assigned to receive either an oral probiotic added to human (maternal or pooled donors') milk (group A) or human milk alone (group B) for 6 weeks or until discharge from the neonatal intensive care unit. On a weekly basis, specimens obtained from various sites (ie, oropharyngeal, stool, gastric aspirate, and rectal specimens) were collected from all patients for surveillance culture, to assess the occurrence and intensity of fungal colonization in the GI tract. The incidence of fungal enteric colonization was significantly lower in group A than in group B (23.1% vs. 48.8%; RR = 0.315, 95% CI = 0.120-0.826; $P = 0.01$). The numbers of fungal isolates obtained from each neonate ($P = 0.005$) and from each colonized patient ($P = 0.005$) were also lower in group A than in group B. LGG was more effective in the subgroup of neonates with a birth weight of 1001 to 1500 g.

(4) Manzoni et al[137] in a following study assigned 743 VLBW to receive orally either bovine lactoferrin (BLF) 100 mg/d alone (group LF; n = 247) or with LGG $6 \times 10^9$ CFU/d or placebo (control group; n = 258) from birth until day 30 of life. NEC incidence was significantly lower in groups BLF and BLF+LGG [5/247 (2.0%)] and 0/238 (0%), respectively] than in controls [14/258 (5.4%)] (RR = 0.37, 95% CI = 0.136-1.005; $P = 0.055$ for BLF vs. control; RR = 0.00; $P < 0.001$ for BLF+LGG vs. control). The incidence of death-and/or-NEC was significantly lower in both treatment groups (4.0% and 3.8% in BLF and BLF+LGG vs. 10.1% in control; RR = 0.39, 95% CI = 0.19-0.80; $P = 0.001$ and RR = 0.37, 95% CI = 0.18-0.77; $P = 0.006$, respectively). No adverse effects or intolerances to treatment occurred.

**TABLE 17.** *Lactobacillus* GG Versus Control (Species of Probiotic), Outcomes

| Studies | Probiotics (n/N) | Control (n/N) | RR (95% CI) |
|---|---|---|---|
| Severe NEC | | | |
| Dani et al[132] | 4/295 | 8/290 | 0.49 (0.15-1.61) |
| Manzoni et al[133] | 1/39 | 3/41 | 0.35 (0.04-3.23) |
| Manzoni[134] | 0/151 | 101/168 | 0.05 (0.00-0.90) |
| Mortality | | | |
| Dani et al[132] | 0/295 | 2/290 | 0.20 (0.01-4.08) |
| Manzoni et al[133] | 5/39 | 6/41 | 0.88 (0.29-2.64) |
| Manzoni[134] | 6/151 | 121/168 | 0.56 (0.21-1.45) |

CI indicates confidence interval; NEC, necrotizing enterocolitis.
Severe NEC, mortality (modified from AlFaleh and Anabrees[131]).

(5) Millar et al[138] al aimed to find out whether or not the probiotic *Lactobacillus* GG can colonize the immature bowel of premature infants. Twenty preterm infants were randomized to receive LGG $10^8$ CFU twice a day for 2 weeks. Fecal short chain fatty acids (SCFAs), ethanol, and urinary 2,3-butanediol were measured in parallel with microbiological studies. LGG colonized 9 babies. From 1 to 28 days of age fecal SCFAs did not differ significantly from controls. Ethanol was detected in more fecal samples from treated babies (65% vs. 37%), and at higher concentration [6.3 (trace-40) vs. 3.3 (0.6 to 8.8) (mmol/g). 2,3-butanediol was found in 66% of urine samples from treated babies and 58% from controls. Orally administered *Lactobacillus* GG was well tolerated and did colonize the bowel of premature infants.

(6) Pärtty et al[139] randomized 94 preterm infants (age 32 to 36 wk and birth weight > 1500 g) randomized to receive prebiotics (mixture of galacto-oligosaccharide and polydextrose 1:1), probiotics (*L. rhamnosus* GG), or placebo during the first 2 months of life (follow-up for 1 y) to evaluate the impact of early prebiotic and probiotic intervention on preterm infants' well-being, crying, growth, and microbiological programming. A total of 27 of 94 infants (29%) infants were classified as excessive criers, significantly less frequently in the prebiotic and the probiotic groups than in the placebo group (19% vs. 19% vs. 47%, respectively; $P = 0.02$). The placebo group had a higher percentage of *Clostridium histolyticum* group bacteria in their stools than did the probiotic group (13.9% vs. 8.9%, respectively; $P = 0.05$). There were no adverse events related to either supplementation.

(7) Rougé et al[140] enrolled 45 infants in a double-blind RCT to receive enteral probiotics (*Bifidobacterium longum* BB536 and *L. rhamnosus* GG) and 49 to receive placebo. The primary endpoint was the percentage of infants receiving > 50% of their nutritional needs via enteral feeding on the 14th day of life. The primary endpoint was not significantly different between the probiotic (57.8%) and placebo (57.1%) groups ($P = 0.95$). However, in infants who weighed > 1000 g, probiotic supplementation was associated with a shortening in the time to reach full-enteral feeding ($P = 0.04$).

(8) Another recent meta-analysis has been published on behalf of the Italian Society of Neonatology.[141] In total,



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25 studies were included in the meta-analysis. Overall, probiotic supplementation resulted in a significantly lower incidence of LOS (RR = 0.79, 95% CI = 0.71-0.88; $P < 0.0001$). According to feeding type, the beneficial effect of probiotics was confirmed only in exclusively human milk-fed preterm infants (RR = 0.75, 95% CI = 0.65-0.86; $P < 0.0001$). Among human milk-fed infants, only probiotic mixtures, and not single-strain products, were effective in reducing LOS incidence (RR = 0.68, 95% CI = 0.57-0.80; $P < 0.00001$). The results of the present meta-analysis show that probiotics reduce LOS incidence in exclusively human milk-fed preterm infants.

## LGG in Respiratory Tract Infections

Upper respiratory tract infections (URTI) in adults and children have a high incidence and thus form a major health threat. Furthermore, they form a common reason for antibiotic prescription in the clinical practice, particularly in children. Yet, unnecessary prescribing of AB is costly, leads to serious unintended side effects, and increases the risk of developing antibiotic resistance. Recent researches on the human microbiome composition and functions have aroused a great interest for a target of a probiotic application and development to prevent acute respiratory infections. LGG is able to inhibit adherence of pathogenic bacteria to human epithelial cells in vitro and induces an antigen-specific immune response in mice.[15,142] The lymphoid tissue in the adenoid is the body's first line of immune defence and is important in both local and regional immune functions. Because of their location and function, adenoids harvest multiple bacteria and viruses. Picornaviruses (rhinovirus and enterovirus) can be found frequently in the lymphoid ring of the naso-oropharynx, especially during the cold months.[143] Oral administration of LGG reduced the incidence of rhinovirus-induced respiratory infections in preterm infants.[144], but only few studies have examined the presence of probiotics in the human naso-oropharynx. In 1 trial *Lactobacillus plantarum* DSM 9843 has been seen to adhere to tonsil surface after oral administration and *Streptococcus salivarius* K12 has been cultured from nasopharynx of young otitis-prone children (n = 19) after a 10-day intervention.[145,146] Tonsillar recovery of LGG after oral consumption was studied in 57 young adults in a placebo-controlled and randomized trial. LGG was recovered in 40% of the LGG groups' tonsillar samples and in 30% of the placebo groups' samples.[147] A double-blind, placebo-controlled, randomized study[148] was conducted with the aim to evaluate the presence of LGG in the adenoid tissue of children referred for adenotomy after a 3-week oral administration of 3 capsules/day (8 to $9\times10^9$ CFU) versus placebo. LGG was recovered in the adenoid sample in 100% of children in the LGG group and in 76% in the placebo group ($P = 0.07$). Probiotics have proven themselves able to reducing the risk of acute respiratory infections in infancy.[149,150] Moreover, previous studies suggest that LGG has the potential to reduce the severity and duration of upper respiratory infection symptoms,[151] as well as the number of days with respiratory symptoms in healthy day care children.[152,153] In a randomized, double-blind, placebo-controlled trial 523 children aged 2 to 6 years attending day care centers in Finland received either normal milk or the same milk with GG on 3 daily meals for 28 weeks. Number of days with at least 1 respiratory symptom in all subjects was 5.03/month (95% CI = 4.92-5.15) in the GG group and 5.17/month (95% CI = 5.05-5.29) in the placebo group, incidence rate ratio = 0.97 (95% CI = 0.94-1.00; $P = 0.098$). In the completed cases, the figures were 4.71 days/month (95% CI = 4.52-4.90) in the GG group and 5.67 days/month (95% CI = 5.40-5.94) in the placebo group (RR = 0.83, 95% CI = 0.78-0.88; $P < 0.001$).[154]

Interestingly, DNA-based microbiome research suggests an inverse correlation between the presence of LAB and the occurrence of potential pathogens, such as *Moraxella catarrhalis*, an important URT pathogen linked to otitis media, sinusitis, and chronic obstructive pulmonary disease. A study[155] investigated the direct antipathogenic effects of *Lactobacillus* species, on *M. catarrhalis* using agar-based assays, time course analysis, biofilm assays, and minimal inhibitory concentration (MIC) testing. A proportion of *Lactobacillus* strains, including *L. rhamnosus* GG, showed a strong and direct activity against *M. catarrhalis*, at least in vitro, with mean MIC values for D- and L-lactic acid varying between 0.5 and 27 g/L depending on the pH. Furthermore, LGG also decreased the adhesion of *M. catarrhalis* to human airway epithelial Calu-3 cells with > 50%, and the expression of mucin MUC5AC, proinflammatory cytokines IL-8, IL-1β, and TNF-α at least 1.2-fold.

A meta-analysis[156] has evaluated the effectiveness of LGG for prevent respiratory infections in children. Four RCTs involving 1805 participants met the inclusion criteria showing a reduced incidence of acute otitis media, of upper respiratory infections and of antibiotic treatments. There was no significant difference between the LGG and the control groups in the incidence of lower respiratory infections and in the risk of overall respiratory infections except that in a subgroup analysis of 2 studies on children older than 1 year that showed significant reduction in the risk of overall respiratory infections (2 RCTs, n = 794, RR = 0.73, 95% CI = 0.57-0.92; NNT = 8, 95% CI = 5-14).

Adverse effects were similar in both groups. No serious adverse events were reported.

A successive Cochrane Review[157] considered 12 trials, which involved 3720 participants including children and adults. Probiotics were better than placebo when measuring the number of participants experiencing episodes of acute URTI, the mean duration of an episode of acute; reduced antibiotic prescription rates and cold-related school absence (Tables 18, 19).

In subsequent years numerous studies have been conducted on the topic and have been the subject of at least 3 meta-analysis.[158–160] In the first[158] total of 23 trials involving 6269 children (from infants to 18 y olds) were eligible for inclusion. Probiotics prescription to children reduced morbidity. The number of patients with 1 acute respiratory infection episode (RR = 0.89, 95% CI = 0.82-0.96; $P = 0.004$) was much lower, with a total decrease of sick days (MD = –0.16, 95% CI = –0.29 to –0.02; $P = 0.03$). Children with a probiotic administration were absented in school or needed in a day-patient treatment for fewer days (MD = –0.94, 95% CI = –1.72 to –0.15; $P = 0.02$). The second meta-analysis[159] was carried out on 30 trials that enrolled 2972 patients in intensive care. In the analysis, a decrease in nosocomial infection incidence (RR = 0.80, 95% CI = 0.68-0.95; $P = 0.009$) and a significant reduction in the incidence of ventilator-associated pneumonia was found (RR = 0.74, 95% CI = 0.61-0.90; $P = 0.002$) and an increase in the incidence for ventilator-associated pneumonia in patients with artificial lung ventilation (RR = 0.74, 95% CI = 0.61-0.90;

**TABLE 18.** Overall Risk Ratio in Studies With LGG in Respiratory Infections

| Studies | No. Children Included | Overall: RR (95% CI) |
|---|---|---|
| Otitis media | 1805 | 0.76 (0.64-0.91); NNT = 17 (11-46) |
| Hatakka (2001) | — | 0.81 (0.64-1.03) |
| Kumpu et al[154] | — | 0.63 (0.27-1.47) |
| Kukkonen[151] | — | 0.79 (0.59-1.05) |
| Rautava et al[149] | — | 0.44 (0.21-0.90) |
| Upper respiratory infections | 281 | 0.62 (0.50-0.78) |
| Hojsak et al[82] | — | — |
| Lower respiratory infections | — | 0.82 (0.22-2.98) |
| Hojsak et al[82] | 281 | — |
| Antibiotic treatments | 1805 | 0.80 (0.71-0.91) |
| Hatakka (2001) | — | 0.86 (0.72-1.01) |
| Kumpu et al[154] | — | 0.69 (0.43-1.11) |
| Kukkonen[151] | — | 0.82 (0.66-1.03) |
| Rautava et al[149] | — | 0.52 (0.71-0.91) |
| Children (> 1 y) | 794 | 0.73 (0.57-0.92); NNT = 8 (5-14) |
| Children (< 2 mo) | 1011 | 1.02 (0.93-1.11) |

CI indicates confidence interval; NNT, number needed to treat; RR, relative risk.
Modified from Hao et al.[157]

$P = 0.002$) were confirmed. No effect on mortality, LOS, or diarrhea was observed. Subgroup analysis indicated that the greatest improvement in the outcome of infections was in critically ill patients receiving probiotics alone versus synbiotic mixtures, although limited synbiotic trial data currently exist.

On other meta-analysis performed in Brazil confirmed these data.[160]

The last meta-analysis[161] just published evaluated 12 RCTs with 4527 children in day care settings (aged 3 mo to 7 y). Compared with placebo, LGG significantly reduced duration of respiratory tract infections (3 RCTs, n = 1295,

**TABLE 19.** OR of Probiotics Versus Placebo for Upper Respiratory Tract Infections

| | |
|---|---|
| At least 1 episode | OR = 0.53 (95% CI = 0.37-0.76) $P < 0.001$ |
| At least 3 episodes | OR = 0.53 (95% CI = 0.36-0.80) $P = 0.002$ |
| Mean duration of an acute episode | MD = −1.89 (95% CI = −2.03 to −1.75) $P < 0.001$ |
| Reduced antibiotic prescription | OR = 0.65 (95% CI = 0.45-0.94) |
| Cold-related school absence | OR = 0.10 (95% CI = 0.02-0.47) |

CI indicates confidence interval; MD, mean deviation; OR, odds ratio.
Modified from Hao et al.[157]

**TABLE 20.** Effect of LGG for Preventing Total Respiratory Tract Infections

| | Probiotics | | Control | | |
|---|---|---|---|---|---|
| References | Events | Total | Events | Total | Risk Ratio (95% CI) |
| Kumpu et al[154] | 97 | 252 | 123 | 261 | 0.82 (0.67-1.00) |
| Hojsak et al[82] | 60 | 139 | 96 | 142 | 0.64 (0.51-0.80) |
| Kumpu et al[154] | 121 | 251 | 122 | 250 | 0.99 (0.82-1.18) |
| Subtotal | — | 642 | — | 653 | 0.81 (0.63-1.03) |
| Total events | 278 | | 341 | | |

CI indicates confidence interval.
Modified from Pilmann Laursen and Hojsak.[161]

MD = −0.78 d, 95% CI = −1.46 to −0.09) (Table 20). On the basis of the results from 2 studies (n = 343), *Bifidobacterium animalis subsp. lactis* BB-12 showed no effect on duration of RTIs or on absence from day care. Meta-analyses on other strains or their combination were not possible due to limited data and different outcome measures (Table 20).

Some studies have shown that nasally administered immunobiotics had the potential to improve the outcome of influenza virus infection. However, the capacity of immunobiotics to improve protection against respiratory syncytial virus (RSV) infection was not investigated before.

A study has been performed to evaluate whether the nasal administration of *L. rhamnosus* CRL1505 (Lr05) and *L. rhamnosus* CRL1506 (Lr06) are able to improve respiratory antiviral defenses and beneficially modulate the immune response triggered by TLR3/RIG-I activation and to investigate whether viability of Lr05 or Lr06 is indispensable to modulate respiratory immunity and to improve the resistance of infant mice against RSV infection.

Nasally administered Lr05 and Lr06 differentially modulated the TLR3/RIG-I-triggered antiviral respiratory immune response. Lr06 administration significantly modulated the production of IFN-α, IFN-β, and IL-6 in the response to poly(I:C) challenge, whereas nasal priming with Lr05 was more effective to improve levels of IFN-γ and IL-10. Both viable Lr05 and Lr06 strains increased the resistance of infant mice to RSV infection while only heat-killed Lr05 showed a protective effect similar to those observed with viable strains. Therefore the nasal administration of immunobiotics is able to beneficially modulate the immune response triggered by TLR3/RIG-I activation in the respiratory tract and to increase the resistance of mice to the challenge with RSV.[162] These data require to be confirmed in human studies.

## Anti-Infective Activities of Lactobacillus GG

By producing bacteriocins, resident bacteria have bacteriostatic or bactericidal effects against pathogens, playing a fundamental role in the chemical barrier effect of the gut microbiota.[163–165] Bacteriocins in nanomolar range develop antibacterial activities both in vitro and in vivo acting upon the cell envelopes of target pathogens or within the cell affecting its gene expression.[166,167] It is also important to remember that in bacteria an intercellular communication process called quorum sensing (QS) is based on the synthesis and secretion of small hormone-like molecules, termed autoinducers, coordinated mainly in response to the bacterial population density.[168] A QS mechanism regulates the production of bacteriocins by lactic acid



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bacteria via secreted bacteriocin-like peptide pheromones.[169] Interestingly, Lactobacillus QS molecules controlling bacteriocin production have been found to be activated in response to infection.[170,171] With these premises it is necessary to consider a direct anti-infective activity of LGG:

- A loss of about 4 log CFU/mL of *Shigella sonnei* viability has been observed after 4 hours of exposure to LGG.[172,173]
- LGG reduced *Salmonella enterica serovar typhimurium and Salmonella typhimurium* C5 adhesion and cytotoxicity during epithelial cell stress.[174]
- LGG reversed the rotavirus-induced increase in intestinal barrier permeability.[175]
- LGG in combination with anti-rotavirus antibodies reduced both the duration and the severity of the resulting diarrhea and the histopathologic changes and virus load in the intestine.[176]
- LGG shortened the duration of diarrhea and decreased epithelium vacuolation in the jejunum.[177]
- LGG decreased the viability of enterovirulent *E. coli* by 3 to 4 log CFU/mL after 4 hours of direct contact.[173,174]
- The viability of *S. typhimurium* was dramatically lowered, by about 5 log CFU/mL, after 4 hours of exposure to the *L. rhamnosus* GG.[172,174,178]
- LGG produced molecules reduce the levels of Shiga toxin stx2A mRNA of enterohemorrhagic *E. coli* O157:H7[179]
- LGG is able to form biofilms on abiotic surfaces. In vitro biofilm formation by *L. rhamnosus* GG is strongly modulated by culture medium factors and conditions related to the GI environment, including low pH, high osmolarity; and the presence of bile, mucins, and nondigestible polysaccharides. In addition, phenotypic analysis of mutants affected in exopolysaccharides (*wzb*), lipoteichoic acid (*dltD*), and central metabolism (*luxS*) showed their relative importance in biofilm formation.[32]
- LGG has been shown to promote the production of intestinal mucus mediating the upregulation of epithelial mucin MUC2 and MUC3 mRNAs or proteins in Caco-2 cells and HT-29 cells, which is accompanied by a concomitant inhibition of adhesion of enteropathogenic *E. coli* and enteropathogenic *E. coli*.[180]

It is important to remember that LGG produce a low–molecular-weight, heat-stable, nonproteinaceous bactericidal substance, active at acidic pH against a wide range of bacterial species and that the spent culture supernatant of LGG grown in De Man, Rogosa and Sharpe agar medium contain 5 compounds (porcine serpine protease inhibitor, p75 and p40 proteins, cell wall–associated hydrolase, glyceraldehyde-3-phosphate dehydrogenase) and others able to enhance intestinal crypt survival and to diminish apoptosis and preserve cytoskeletal integrity.[45] Others 7 small peptides have been identified from LGG cultured media retaining the antibacterial activity exerted against gram-negative (*E. coli* EAEC 042 and *Salmoella typhi*) and, with less potency, gram-positive (*Staphylococcus aureus*) bacteria.[47]

Table 21 shows an overviews of in vitro antibacterial effects of probiotic *Lactobacillus* GG against gastric or enteric pathogens

## Lactobacillus GG Antibiotic Resistance and Susceptibility

The significance of antimicrobial resistances in bacteria and the possible transmission of the resistance factors, such as plasmids or insertion sequence elements, to pathogenic microorganisms[194] it has become of great importance. Also a LAB strain resistant to antimicrobials might transfer the antimicrobial resistance factor to harmful bacteria. Any way probiotics belonging to species included in the EFSA QPS list[195,196] have excellent safety records, and detrimental effects produced as a consequence of their ingestion are very scarce. Currently, it is generally accepted that the possibility of transfer is related to the genetic basis of the resistance mechanism, that is, whether the resistance is intrinsic, acquired as a result of a chromosomal mutation(s), or acquired by horizontal gene transfer Acquired resistance can be due either to acquired genes (genes acquired by the

**TABLE 21.** Overviews of In Vitro Antibacterial Effects of Probiotic *Lactobacillus* GG Against Gastric or Enteric Pathogens

| Pathogens | Experimental Conditions | Observed Effect(s) | References |
|---|---|---|---|
| *Shigella* | Direct contact | Bactericidal | 172,173 |
| Enterovirulent | Direct contact | Bactericidal | 181 |
| *Escherichia coli* | Peptides with NPSRQERR and PDENK sequences | Bactericidal | 182 |
| | Direct contact | Decrease of Shiga toxin | 183–185 |
| | Direct contact | Inhibition of adhesion | 185 |
| | Direct contact | Inhibition of TJ lesions | 179,186,187 |
| | Direct contact | Inhibition of IL-8, CCL, and CXCL production | 26 |
| | Direct contact | Increased MUC2 and MUC3 mRNA | — |
| *Salmonella typhimurium* | Direct contact | Bactericidal | 172,173 |
| | Peptides with NPSRQERR PDENK sequences | Bactericidal | 186,188,189 |
| | Direct contact | Inhibition of adhesion | 189,190 |
| | Direct contact | Inhibition of cell-entry into enterocyte-like cells | 190 |
| | Direct contact | Inhibition of interleukin-8 production | — |
| | Direct contact | — | — |
| *Helicobacter pylori* | Direct contact with LB-SCS | Low bactericidal activity | 191 |
| | Direct contact with a produced bacteriocin | Bactericidal activity | 192 |
| | Direct contact | Inhibition of adhesion onto gastric cells | 193 |
| | Direct contact with LB-SCS | Absence of inhibitory effect against adhesion onto mucus secreting cells | 191 |

LB-SCS indicates spent culture supernatant of strain LB.

bacteria via gain of exogenous DNA) or to the mutation of indigenous genes.[197,198] When resistance to an antimicrobial is inherent to a bacterial species, it is generally referred to as "intrinsic resistance" (sometimes called "natural resistance") and is typical of all the strains of that species. In contrast, when a strain of a typically susceptible species is resistant to a given antimicrobial drug, it is considered to be "acquired resistance." Acquired resistance can be due either to added genes (genes acquired by the bacteria via gain of exogenous DNA) or to the mutation of indigenous genes.[198] Horizontal transfer of resistance/virulence genes between bacteria may occur by different mechanisms: (1) the acquisition of exogenous DNA containing resistance/virulence genes by transformation; (2) the acquisition of resistance/virulence genes by transduction mediated by bacteriophages; and (3) the acquisition/virulence of resistance genes on mobile genetic elements such as plasmids or transposons by conjugation. There are no reports concerning virulence factors in LGG.

It is important to remember that *Lactobacillus* species and in particular LGG are sensible to some AB and in particular penicillin and amoxicillin, the AB accounting for >60% of a prescriptions in children aged below 5 years.[199,200] Therefore their ability to colonize the gut and act as a probiotic may be limited when antibiotic therapy is administered. However, LGG was isolated in 57% of fecal specimens of subjects exposed to penicillin.[201]

In a double-blind RCT *L. rhamnosus* GG long-term supplementation was confronted with placebo. AB were administered to 44% of the placebo group and to 40% of the LGG group. *L. rhamnosus* GG showed an influence on the composition of the intestinal microbiota, causing an increase in the abundance *of Prevotella*, *Lactococcus*, and *Ruminococcus*, and a decrease in *Escherichiae* with simultaneous reduction in antibiotic use. Moreover, the prevalence of sulfonamide-trimethoprim use was significantly reduced in the *L. rhamnosus* GG group (RR = 0.34, 95% CI = 0.14-0.85). During the 3-year follow-up period after the intervention, the difference in antibiotic use between the groups gradually increased with a significant difference in the proportion of children treated with macrolides (RR = 0.68, 95% CI = 0.46-1.02) and sulfonamide-trimethoprim (RR = 0.6, 95% CI = 0.36-0.99). By the end of the 3-year follow-up, the *L. rhamnosus* GG group had received 48% fewer macrolide and 36% fewer sulfonamide-trimethoprim courses per person.[202,203]

The colonization of LGG in the gut of 7 children treated with amoxicillin/clavulanate using fresh fecal samples collected before (T0) and after 10 days (T1) of administration of both the antibiotic and LGG $3\times10^9$ CFU has been evaluated.[204] At T0 no patients carried LGG. After 10 days (T1) of antibiotic treatment, the species-specific 16S rRNA analysis pointed out the presence of *L. rhamnosus* in 6 of the 7 patients. The further evaluation through Rep-PCR profiles demonstrated the presence of the target research microorganism LGG in 4 of the 7 children. This support the potential of enteric colonization by LGG even during oral administration of one of the most common antibiotic treatment used in children.[205] Bacteria belonging to genus *Lactobacillus* are intrinsically resistant to vancomycin, which means vancomycin-susceptible strains of these species do not exist. Particularly LGG have not been shown to contain *van* genes, which encode[206,207] for resistance. In clinical microbiology, the emergence of vancomycin-resistant enterococcal (VRE) strains has caused a serious therapeutic problem, since *Enterococci* may contain several other antibiotic resistance genes, vancomycin is often the only effective antibiotic for treatment. Furthermore, many concerns have been expressed about the possible transfer of *van* genes to *Staphylococci*. There is no indication that intrinsically vancomycin-resistant lactobacilli can transfer vancomycin-resistance genes to other species. In a recent study in adults it has been determined whether eating LGG as yoghurt (100 g daily of yoghurt containing LGG for 4 weeks vs. standard pasteurized yoghurt) improves clearance of VRE in fecal samples obtained 3 times at about weekly intervals. All 11 patients in the treatment group who completed the study cleared VRE; 3 subjects reverted VRE positivity after using AB to which LGG is sensitive, while all others remained negative for at least 4 weeks after trial completion.[208] In another study children (0 to 18 y old) diagnosed with GI carrier state of VRE were randomized to receiving $3\times10^9$ CFU LGG/day or placebo for 21 consecutive days. A total of 61 children completed the study (32 in the treatment group and 29 in the control group). Rectal swabs for VRE and *Lactobacillus* spp. were collected at baseline, during supplementation at weekly intervals and 1 month after supplementation. Antibiotic supply was controlled throughout the duration of the analysis. ) The VRE carrier state was lost by 20 of 32 participants in the treatment group and 7 of 29 in the control group ($P = 0.002$).[209]

## MIC of Lactobacillus GG

As a basic requirement, the MIC of the antimicrobials expressed as mg/L or μg/mL should be determined at least for each of the following substances: ampicillin, vancomycin, gentamicin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline, chloramphenicol. MIC has been evaluated for *Lactobacillus* GG in some studies (Table 22).[110,201,206,210]

**TABLE 22.** The Antibiotic Sensitivity of Lactobacillus GG in MIC

| | MIC (μg/mL) | | | |
|---|---|---|---|---|
| **Antibiotic** | **Saxelin[201]** | **Vanderhoof et al[110]** | **Klein et al[206]** | **Salminen et al[210]** |
| Benzylpenicillin | 0.19 | 1.0 | 0.25 | 1.0 |
| Ciprofloxacin | 2.0 | 0.2 | >4.0 | 1.0 |
| Gentamicin | 24.0 | — | >32.0 | — |
| Ampicillin | 0.5 | 0.5 | 1.0 | 1.0 |
| Piperacillin | — | — | — | 1.0 |
| Imipenem | 20 | — | 2.0 | 2.0 |
| Doxicycline | 0.125 | — | >64 | 0.50 |
| Vancomycin | >258 | — | — | >256 |
| Ceftriaxone | — | — | — | >256 |
| Cefuroxime | — | — | — | 8 |
| Cefotaxime | 4.0 | 0.25 | 0.4 | — |
| Erythromycin | 0.094 | — | — | 0.25 |
| Amoxycillin/ clavulanate | 0.5 | 0.5 | — | — |
| Cefalotin | — | 16.0 | 4.0 | — |
| Tetracyclin | — | 2.0 | <2.0 | — |
| Trimethoprim/ sulphamethoxazole | — | — | >4.0/ >76 | — |
| Oxacillin | — | — | 1.0 | — |
| Clindamycin | — | — | 0.5 | 0.25 |
| Chloramphenicol | — | — | <4 | — |
| Netilmicin | — | — | — | 4.0 |
| Tobramaicyn | — | — | — | 16 |

MIC indicates minimal inhibitory concentration.



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## LGG and IBS

IBS is a functional disorder classified into IBS with constipation (IBS-C), IBS with diarrhea (IBS-D), mixed IBS with both constipation and diarrhea, and unsubtyped IBS with neither constipation nor diarrhea using the Rome III criteria. The pathophysiology of IBS is related to alterations in GI motility, visceral hypersensitivity, dysfunction of the brain-gut axis or certain psychosocial factors. Recently intestinal dysbiosis[211] has been linked to the IBS, thought that numerous data support that the composition of luminal and mucosal microbiota differs among specific subgroups of IBS patients and healthy individuals.[211,212] Moreover, dysbiosis is also associated with significant alterations in intestinal transit time.[213]

In particular infection and AB may alter the population of *Lactobacilli* and *Bifidobacteria* and may increase *Firmicutes*/*Bacteroidetes* ratios[214,215] with a significant decrease in *Roseburia*, a predominant butyrate-producing genus.[216] Moreover, postinfectious IBS with a reported incidence between 5% and 32%[217] and post-AB IBS suggest a pathophysiological mechanisms including increased intestinal permeability, altered motility, and persistent intestinal inflammation[218,219] due to microbiota imbalance. Another important active factor to consider in the pathogenesis of IBS is serotonine ~90% of which is located in the enterochromaffin cells in the GI tract, where it is used to regulate intestinal movements.[220] Enterocytes express also the SERT, which terminate the action of 5-HT. Human microbiota and high concentrations of particular luminal microbial metabolites promote 5-HT biosynthesis from colonic enterochromaffin cells increasing colonic and blood 5-HT in germ-free mice.[221] Serum levels of serotonin (5-HT) decrease in patients with IBS-C and increase in patients with IBS-D,[222] but is inactivated after reuptake by SERT in intestinal or nerve cells. Downregulation of SERT is implicated in the pathophysiology of IBS[48,49,222,223] found that SERT mRNA was lower in children with IBS than in the control.

## PROBIOTICS AND IBS

Taking in consideration the link between gut microbiota and IBS numerous trials were dedicated to the possible role of probiotics in this situation. Indeed subjects with IBS represent an interesting target patient population for probiotic use, and this is reflected by the number of articles and clinical trials assessing the efficacy of these products in IBS. Some mata-analysis have been carried out on this matter (Table 23).

To date, the published studies provide stimulating results but raise important questions yet to be determined. The meta-analysis of human clinical trials concluded that probiotics, were more beneficial than placebo in reducing pain and symptom severity scores. Overall some studies enrolled a small number of patients for a short duration of observation and with significant design flaws.

## *LACTOBACILLUS* GG AND IBS

The conflicting results and the heterogenous therapeutic response obtained in IBS trials with different strains of probiotics could be due to differences in host specificity between strains and species of probiotics.It has been suggested that host specificity could be one of the selection criteria for probiotics.[230] An analysis of 100 *L. rhamnosus* strains identified that the production of functional mucus binding pili *SpaCBA* by *L. rhamnosus* GG[231] may provide a colonization advantage in the intestinal tract. This observation along with documented activity of LGG to upregulate serotonin, transporter (SERT) mRNA and SERT-P levels in intestinal epithelial cells and in mice intestinal tissues[48,49,222,223] are certainly valid requirements to believe that this probiotics should be considered particularly suitable for treating IBS. It is particularly interesting to remember that serotonin is an important GI hormone that modulates intestinal fluid secretion, gut motility, and GI sensation. Moreover, LGG produce factors, identified as "postbiotic" mediators, able to protect human colonic smooth muscle cells from LPS-induced induced morphofunctional alterations of muscle cells, that is, cell shortening and inhibition of contractile response. Novel insights have been provided for the possibility that LGG-derived products could reduce the risk of progression to postinfective motor disorders.[232]

Some recent studies have tested LGG in IBS in children and adults. Table 24 summarize the results of 7 recent trials.

These results confirm the activity of LGG on symptoms of IBS both in children and adults.

## LGG AND INFLAMMATORY BOWEL DISEASE (IBD)

Crohn's disease (CD) and ulcerative colitis (UC) have distinct features. UC is characterized by inflammation with superficial ulcerations limited to the mucosa of the colon. Inflammation normally starts in the rectum and continuously spreads throughout the large intestine. CD, however, is characterized by a discontinuous pattern, potentially affecting the entire GI tract. In contrast to UC, inflammation in CD patients is transmural with large ulcerations, and occasionally granulomas are observed. In IBD, the gut microbiota is characterized by

**TABLE 23.** Results of 6 Recent Meta-Analysis on Probiotics in Irritable Bowel Syndrome

| References | No. Studies/No. Subjects | Results |
|---|---|---|
| Nikfar et al[224] | 8/1011 | Clinical improvement vs. placebo RR = 1.22 (95% CI = 1.07-1.4) $P = 0.0042$ |
| Hoveyda et al[225] | 7/895 | Overall symptoms improvement OR = 1.6 (95% CI = 1.2-2.2) |
| | 6/657 | SMD = 0.23 (95% CI = 0.07-0.38) |
| | 6/850 (only adults) | Overall symptoms OR = 1.59 (95% CI = 1.19-2.13) |
| Moayyedi et al[226] | 10/918 | RR of not improving patients vs. placebo = 0.71 (95% CI = 0.57-0.88); NNT = 4 (95% CI = −12.5) |
| Ford et al[227] | 23/2575 | RR = 0.79 (95% CI = 0.70-0.89); NNT = 7 (95% CI = 4-12.5) |
| Didari et al[228] | 15/1793 | RR of responders to therapies vs. placebo = 1.96 (95% CI = 1.14-3.36) $P = 0.01$ |
| | — | RR improvement of general symptoms vs. placebo = 2.14 (95% CI = 1.08-4.26) $P = 0.03$ |
| Hu et al[229] | 17/1700 | Overall symptoms improvement SMD = −0.20 (95% CI = 0.33 to −0.07) $P = 0.002$ |
| | — | Abdominal pain improvement SMD = −0.19 (95% CI = 0.29 to −0.09) $P < 0.0001$ |
| | — | Abdominal distension improvement = −0.16 (95% CI = 0.28 to −0.03) $P = 0.020$ |
| | — | Defecation discomfort improvement = −0.22 (95% CI, 0.42 to −0.02) $P = 0.030$ |

CI indicates confidence interval; NNT, number needed to treat; OR, odds ratio; RR, relative risk; SMD, standardized mean difference.

**TABLE 24.** Studies on *Lactobacillus* GG in Irritable Bowel Syndrome

| Studies | N/Duration (wk) | Results |
|---|---|---|
| O'Sullivan and O'Morain[233]* | 24/8 | < No. unformed bowel motions in patients with diarrhea |
| Bausserman and Michail[91]† | 24/8 | No effect |
| Gawronska et al[93]† | 37/4 | RR = 33% vs. 5% (95% CI = 1.2-38); NNT = 4 (95% CI = 2-36) |
| Francavilla et al[92]† | 48/12 | Reduction frequency and severity abdominal pain ($P < 0.01$) At week 12 success in 48 vs. 37 ($P < 03$) children |
| Kajander et al[234]‡ | 103/12 | Symptoms score = −7.7 (95% CI = 13.9-1.6) <placebo ($P = 0.015$) |
| Kajander[235]‡ | 55/24 | Gut microbiota stable during the trial; no changes in short chain fatty acids |
| Kajander[236]‡ | 86/20 | Symptoms score decreased 14 points (95% CI = −19 to −9) vs. 3 points (95% CI = −8 to 1) with placebo ($P = 0.0083$) |

*LGG.
†In children.
‡Multispecies probiotic consisting of LGG, *Lactobacillus rhamnosus* Lc705, *Propionibacterium freudenreichii* ssp. *shermanii* JS and *Bifidobacterium breve* Bb99.
CI indicates confidence interval; NNT, number needed to treat; RR, relative risk.

dysbiosis with a decrease in diversity and in abundance of some dominant commensal members (such as Clostridium IV and XIVa) and an increase in detrimental bacteria.[237,238] The most consistent observations of altered composition of the gut microbiota in IBD patients are a reduction in Firmicutes and an increase in Proteobacteria (as shown in Table 25).

The dysbiosis potentially contributes to the pathogenesis of IBD by augmenting host proinflammatory immune responses.[248–254] Moreover, dysbiosis can also alter the production of bacterial products, such as SCFAs, and the host gene expression profile thereby troubling mucosal defense.[255,256] Therefore dysbiosis could be not simply a result of inflammation, but rather a functionally defect contributing to inflammation.[256,257] Considering their possible role on IBD dysbiosis probiotics have been tested as treatment IBD. The mechanism of action of probiotics in IBD may be due to competition and suppression of pathogen, stimulation of an immune response, enhancement of barrier activity and induction of T-cell apoptosis. Overall probiotics have demonstrated some efficacy in IBD. Nevertheless the high number of review and editorial articles, the number of published well-designed clinical trials of probiotics in IBD is small and the results are discordant. Three Cochrane Reviews[239,247,258] concluded that there is insufficient evidence to make any conclusions about the efficacy of probiotics for induction of remission in CD and for maintenance of remission in UC. According as well to Joint ECCO and ESPGHAN Evidence-based Consensus Guidelines there is not sufficient evidence to recommend routine probiotic therapy to ambulatory pediatric patients with UC for induction or maintenance of remission.[240] Probiotics, however, may be considered in children with mild UC intolerant to 5-acetylsalicylic acid, or as an adjuvant therapy in those with mild residual activity despite standard therapy.

**TABLE 25.** Gut Microbiota in Inflammatory Bowel Disease

| | Sample Number | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sample Source | CD | UC | C | Diversity | Firmicutes | Bacteroidetes | Actinobacteria | Proteobacteria |
| Stool[239] | 6 | — | 6 | ↓in CD | ↓in CD | →in IBD | — | — |
| Biopsy[240] | 6 | 5 | 5 | — | ↓in CD | ↑ in CD | ↑in IBD | ↑in CD |
| Surgical tissue[241] | 35 | 55 | 34 | — | ↓Lachnospiraceae | ↓ in IBD | ↑Bifidobacteriaceae | ↑in IBD |
| Stool[242] | 29 | 16 | 35 | ↓in CD | ↑ in iCD<br>↑Ruminococcaceae in cCD<br>↓ Ruminococcaceae in iCD | — | — | ↑Enterobacteriaceae in CD |
| Biopsy[243] | 6 | 6 | 5 | ↓in IBD | ↓in CD | ↑in IBD | — | ↑Enterobacteriaceae in CD |
| Biopsy, stool[244] | 121 | 75 | 27 | — | ↓in CD | — | ↑in IBD | ↑Enterobacteriaceae in CD |
| Endoscopic lavage[245] | 16 | 16 | 32 | ↓ in IBD | ↓ in IBD | ↑in IBD | — | ↑in IBD |
| Stool[246] | 21 | 34 | 21 | — | ↓ *C. coccoides C. leptum* in IBD<br>↑ Lactobacillus in CD<br>↓*F. prausnitzii* in IBD | ↑in IBD | ↑Bifidobact. in UC | ↑ *E. coli* in CD |
| Biopsy[246] | 29 | 15 | 21 | — | ↓*C. coccoides* in CD<br>↓*C. leptum* in IBD ↑ Lactobacillus in CD ↓ *F. prausnitzii* in IBD | ↑in IBD | ↓Bifidobacteriaceae In CD | ↑ *E. coli* in CD |

Modified from Butterworth et al.[247]
cCD indicates colonic CD; CD, Crohn's disease; *C. coccoides*, *Clostridium coccoides*; *C. leptum*, *Clostridium leptum*; *E. coli*, *Escherichia coli*; *F. prausnitzii*, *Faecalibacterium prausnitzii*; IBD, inflammatory bowel disease; iCD, ileal CD; UC, ulcerative colitis; ↑, increase; ↓, decrease.



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**TABLE 26.** *Lactobacillus rhamnosus* GG Activities in Inflammatory Bowel Disease Research

| |
|---|
| Stimulus of nonspecific IgA, IgG, IgM immune response[11–13] |
| Inhibition of the production of LPS and TNF-α in murine macrophages[16] |
| Increased expression of several toll-like receptors[17] |
| Hyporesponsiveness in stimulated CD4-T cells via modulation of DC function[20] |
| Prevention of cytokine-induced apoptosis in gut epithelial cells[35] |
| Promotion of the production of IF-γ, IL-12, and IL-18[36] |
| Control of the cytokines proinflammatory effects on mucosa barrier inhibiting NF-κB[37] |
| Moderate stimulation of the production of TNF-γ[44] |
| Decreased production of IL-2, IL-4, and IL-10 in culture medium containing DC[45] |
| Production of microcine and 7 other peptides with anti–gram-negative and anti–gram-positive bactericidal activity[48] |
| Preservation of mucosal barrier function in an EGF receptor-dependent manner[259,260] |
| Inhibition of TNF-α production[260] |
| Protein p40 activate EGF receptor in colon epithelial cells upregulating a disintegrin and metalloproteinase domain-containing protein 17 (ADAM17) catalytic activity[260,261] |

DC indicates dendritic cell; IL, interleukin; LPS, lipopolysaccharides; TNF, tumor necrosis factor;.

Recently 2 meta-analysis[241,242] have been performed on this topic: (1) In 6 RCTs, a total of 721 participants were enrolled and the maintenance effect of probiotics (n = 364) versus that of aminosalicylates was evaluated (n = 357). No significant difference was observed between probiotics and aminosalicylate groups (RR = 1.08, 95% CI = 0.91-1.28; $P = 0.40$).[241] (2) In 22 RCTs, there was no benefit of probiotics over placebo in inducing remission in active UC (RR of failure to achieve remission = 0.86; 95% CI = 0.68-1.08). However, when only trials of VSL#3 were considered there appeared to be a benefit (RR = 0.74, 95% CI = 0.63-0.87). However, probiotics appeared equivalent to 5-ASAs in preventing UC relapse (RR = 1.02, 95% CI = 0.85-1.23).[242] However considering the activities in basic research (Table 26), LGG has been considered an important tool for the possible treatment of IBD. Nevertheless only a small number of studies were conducted to highlight the efficacy of LGG in IBD.

A healthy volunteers study documents a direct effect by LGG on the cellular immune system with increased response of CD4+ T lymphocytes and the decreased secretion of TNF-α, IL-6, and IFN-γ by peripheral blood cells.[243]

In the only double-blind study dedicated to UC, 187 patients with quiescent disease were randomized to receive LGG $18\times10^9$ CFU/d (65 patients), mesalazine 2400 mg/d (60 patients) or LGG+mesalazine (62 patients). Overall analysis showed no difference in relapse rate at 6 ($P = 0.44$) and 12 months ($P = 0.77$) among the 3 treatment groups. However, the treatment with LGG seems to be more effective than standard treatment with mesalazine in prolonging the relapse-free time ($P < 0.05$).[244] In CD LGG ($10^{10}$ CFU×2/d for 10 d) was investigated to evaluate the IgA immune response. Mean number of specific antibody secreting cells to β-lactoglobulin in the IgA class increased significantly from 0.2 (95% CI = 0.04-1.3) to 1.4 (95% CI = 0.3-6.0)/$10^6$ cells and to casein from 0.3 (95% CI = 0.1-1.4) to 1.0 (95% CI = 0.2-4.8)/$10^6$ cells indicating that orally administered *Lactobacillus* GG has the potential to increase the gut IgA immune response and thereby to promote the gut immunologic barrier.[245] In 20 patients (10 LGG, 10 placebo) with a previous history of pouchitis and endoscopic inflammation LGG 0.5 to $1\times10^{10}$ CFU bd for 3 months changed the pouch intestinal flora by increasing the ratio of total fecal *lactobacilli* to total fecal anaerobes ($P = 0.03$) and enhancing the frequency of *lactobacilli*-positive cultures in the pouch and afferent limb mucosal biopsy samples.[246] In another study,[262] pouchitis was delayed by LGG 1 to $2\times10^{10}$ providing significant clinical benefit, without side effects. The first episodes of pouchitis were observed less frequently in patients with a daily intake of LGG (cumulative risk at 3 y: 7% vs. 29%; $P = 0.011$). In a pediatric study, 75 children with CD in remission were randomized to either LGG $10^{10}$ CFU and inulin 295 mg daily (n = 39) or 355 mg inulin as placebo (n = 36) and followed for up to 2 years. The median time to relapse was 9.8 months in the LGG group and 11 months in the placebo group ($P = 0.24$). In total, 31%(12/39) of patients in the LGG group developed a relapse compared with 6/36 (17%) on the placebo group[263] In a short observation period in patients with CD LGG $2\times10^9$ CFU/d or placebo was given for 6 months. In 2/5 patients of the LGG group relapse occurred in week 12, at the end of the tapering steroid medication. This was also seen in the placebo group, wherein 2 patients suffered a relapse at week 4 and week 8, respectively, and therefore is probably due to the fact of reducing the steroid medication. Two on 5 patients receiving LGG and 2/6 patients in the placebo group achieved and maintained remission. Because of the small sample size and 1 patient not finishing the trial in the placebo arm, we are not able to comment on the effect of *LGG* to maintain remission once the steroid medication had ceased.[264] In a larger double-blind trial,[265] 45 consecutive patients (29 men and 16 women) operated on for Crohn's were allocated random to receive LGG $12^9$ CFU/d[23] or placebo[22] for 1 year. Clinical recurrence was ascertained in 3 LGG group (16.6%) patients and in 2 (10.5%) placebo group. Nine of 15 patients in clinical remission on LGG (60%) had endoscopic recurrence compared with 6 of 17 (35.3%) on placebo ($P = 0.297$).These nonbrilliant results could probably in part due to the ileal localization of disease in 69.6% of patients receiving LGG and in 86.4% of those receiving placebo. In fact, it is well known that LGG is able to colonize colonic mucosa, but there are not enough data on the adhesion to ileal cells.[55] The small intestine and the colon differ profoundly not only in their bacterial loads but also in the components of the epithelial innate immune defence related to defensins production both in health and during inflammation.[266] As a whole, these data indicate that although in UC (and to a lesser extent in colonic CD) the innate immune response is enhanced, it is constitutively depressed in ileal CD.[25,27,267–269] New and more numerous studies methodologically correct with appropriate sample size and dose finding are necessary.

## Probiotics, Blood Pressure (BP), and Heart Failure (HF)

By reducing the production of angiotensin II and inhibiting the degradation of bradykinin the angiotensin-converting-enzyme (ACE) inhibitor is an important tool for BP control.

Certain probiotic strains such as *Lactobacilli* and *Bifidobacteria* can effectively produce not only SCFAs, conjugated linoleic acid, γ-aminobutyric acid, but also ACE-inhibitory peptides, which are released during protein hydrolysis[270,271] and have shown potential hypotensive effects.[272] ACE-inhibitory peptides can be derived from a variety of products, including cheese milk soymilk and yogurt, fermented by various starter microorganisms.[273] Upon fermentation, the proteinases of various probiotics are capable of releasing ACE-inhibitory peptides and thus a BP-lowering effect can be derived from the milk proteins.[274,275]

Similar to ACE-inhibitory peptides, other peptides, casokinins-derived milk proteins, and lactokinins derived whey proteins, are also being released during enzymatic proteolysis and microbial fermentations.[273] Moreover, the SCFAs produced by gut microbes, in particular propionate, modulates BP levels via Gpr41 and *Olfr78* receptors. Furthermore, *Olfr78* knockout mice with reduced gut microbial biomass upon antibiotic treatment showed elevated BP levels.[276] Moreover, reduced microbial richness and diversity has been observed in spontaneously hypertensive rats, with an increase in *Firmicutes*/*Bacteroidetes* ratio and decrease in acetate, butyrate-producing microbes.[277–287] *Lactobacillus helveticus* is capable of releasing antihypertensive tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) which are ACE-inhibitory from milk protein casein[271] and also *B. longum* and *L. acidophilus* strains showed ACE-inhibitory activity during growth.[288] Recent research studies have also shown that soy peptides with inhibitory activity against ACE could be produced by fermentation with probiotics.[288–290]

To confirm that the protein p75 released by *L. rhamnosus* GG has effect on ischemia/reperfusion (I/R) induced heart cell injury in a rat model. The pretreatment of rats with the purified p75 protein isolated from *L. rhamnosus* GG 30 minutes before I/R surgery significantly attenuated heart tissue infarction in a dose-dependent manner. This phenotype was reportedly generated by enhanced expression of heat shock proteins with p75 pretreatment[291] suggesting that proteins produced by LGG have a direct cardioprotective effect against ischemic injury.

The biological benefits and clinical effects of probiotics and fermented foods based on in vitro and in vivo studies are reported in the Table 27.

**TABLE 27.** Antihypertensive Effect of Probiotics or Probiotic Fermented Foods

| Effect | Subjects | Strains | Dose (CFU) | Result (mm Hg) |
|---|---|---|---|---|
| Reduced SBP[290] | 60 prediabetic patients (25-65 y old) | *L. casei*, *L. acidophilus*, *L. rhamnos*, *L. bulgar.* *Bifidobacterium breve*, *longum Strept. thermophilus* | $7\times10^9$,$2\times10^9$ 1,5 $\times10^9$, $2\times10^8$ $2\times10^{10}$, $7\times10^9$ $1.5\times10^{10}$ | SBP 3.10 ± 2.2 |
| Hypotensive effect[288] | 702 subjects | *S. thermophiles L delbrueckii* *L. acidophilus L. kefiri* | NA | SBP 3.1 ± 1.56 DBP 1.09 ± 0.06 |
| Antihypertensive effect[291] | 46 hypertensive men (aged 23-59 y) | *L. helveticus S. cerevisiae* | Sour milk 169 g/d | SBP 5.2 ± 8.1 DBP 1.7 |
| Reduced BP[292] | 28 hypert. subjects 14M, 14W | *L. casei* | 400 mg cell lysate (LEx) | SBP 9 ± 2 DBP 6 ± 2 |
| Reduced BP[293] | 36 hypertensive subjects aged 40-80 y | *L. helveticus Sacch. cerevisiae* | Fermented milk 95 mL/d | 4 wk SBP 9.4 ± 3.6 8 wk SBP 14.1 ± 3.1 DBP 6.9 ± 2.2 |
| Reduction in high BP levels[294] | Total 80 subjects 40 high-normal BP 40 MH | *L. helveticus* CM4 | 12 g/d tablet | High-normal group = SBP no change DBP 5.0 ± 0.1 MH group = SBP 11.2 ± 4.0 DBP 6.5 ± 0.1 |
| Reduced BP[295] | 17 mild-hypertensive subjects | *L. helveticus* LBK-16H | 150 mL/d fermented milk | 7.3% reduction |
| Lowering BP[29] | 39 MH patients 16 W 23 M Mean age 54.2 y | *L. casei* Shirota *Lactococcus lactis* | 100 mL/d fermented milk | SBP 17.4 ± 4.3 DBP 7.2 ± 5.7 |
| Lowering BP[296] | 60 subjects (36 M 24W) | *L. helveticus* LBK-16H | 150 mL/d fermented milk | 10 wk (mean) SBP 2.3 DBP ± 0.5 |
| Reduced BP[297] | 70 healthy, overweight, and obese subjects 20 males 50 females 18-55 y old | Group 1 *S thermophilus* + *L. acidophilus* Group 2 *S.thermophilus* + *Enterococcus faecium* Group 3 *S. thermophiles* + *L. rhamnosus* | 450 mL/d fermented milk | 8 wk mean Group 1 ΔSBP 4.4 ± 1.8 ΔDBP 3.4 ± 1.5 Group 2 ΔSBP 8.0 ± 2.3 ΔDBP 4.0 ± 2.3 Group 3 ΔSBP 2.6 ± 3.1 ΔDBP 0.8 ± 2.0 |
| < SBP, cholest triglyceride levels[298] | 20 healthy adults | *S. thermophilus L. casei* | $6.8\times10^8$ mL and $2.6\times10^7$ CFU in 250 mL fermented milk | Significant reduction in SBP ($P = 0.05$) |
| Reduced BP[299] | 40 sybjects | *Lactobacillus plantarum* TENSIA | 50 mg/d probiotic cheese | Morning ΔSBP 12.2 ± 1.5 ΔDBP 4.0 ± 0.9 Evening ΔSBP 8.8 ± 0.9 ΔDBP 1.6 ± 1.2 |
| Fung[300] | 30 hypertensive rats | *L. helveticus* LBK-16H | Sour milk containing 2.5-3.5 mg/kg/d of | < SBP 17 |
| Hata[301] | 30 hypertensive men | *L. helveticus Sacch. cerevisiae* | 95 mL sour milk | < SBP 14.1 < DBP 6.9 |
| Seppo[302] | 39 hypertensive men | *L. helveticus* | 150 mL/d sour milk | < SBP 6.7 ± 3.0 < DBP 3.6 ± 1.9 |

In vivo studies.

BP indicates blood pressure; DBP, diastolic blood pressure; *L. casei*, *Lactobacillus casei*; M, men; MH, mild hypertension; NA, not available; SBP, systolic blood pressure; W, women.

**TABLE 28.** Probiotics Effect on SP and DP in Men

| Studies | Mean Difference (95% CI) SP | Mean Difference (95% CI) DP |
|---|---|---|
| Agerholm-Larsen[305] | −5.80 (−7.30 to −4.30) | −2.50 (−1.38 to −1.14) |
| Chang[306] | −1.98 (−5.92 to 1.96) | 0.11 (−3.51 to 3.73) |
| Hata[301] | −9.70 (−12.25 to −7.25) | −4.40 (−6.11 to −2.69) |
| Jones[307] | 1.36 (−2.61 to 5.33) | −1.30 (−2.97 to −0.37) |
| Jones[308] | 1.88 (−2.43 to 6.19) | 0.20 (−2.41 to 2.81 |
| Naruszewicz[310] | −11.00 (−21.45 to −0.55) | −1.00 (−11.13 to 9.13) |
| Savard[311] | −1.70 (−7.65 to 4.25) | −2.20 (−6.22 to 1.82) |
| Sharafedtinov[312] | −0.80 to 0.28 | −2.38 (−3.84 to −0.93) |

CI indicates confidence interval; DP, diastolic pressure; SP, systolic pressure.

To better define the effects of probiotics on BP a meta-analysis[292] of RCTs was drawn up including 9 trials.[293–298,303,304] Probiotic consumption significantly changed systolic BP by −3.56 mm Hg (95% CI = −6.46 to −0.66) and diastolic BP by −2.38 mm Hg (95% CI = −2.38 to −0.93) compared with control groups.

Furthermore, subgroup analysis of trials with daily dose of probiotics $<10^{11}$ CFU did not result in a significant effect (Table 28).

Recent years have highlighted the impact of the human gut microbiota on cardiovascular diseases (CVD), including HF suggesting a causal link between increased plasma levels of trimethylamine-N-oxide (TMAO) and increased risk of CVD. Briefly, nutrients such as lecithin, choline, and L-carnitine which are abundant in animal-derived products such as red meat, egg yolk, and full-fat dairy products when consumed are processed by gut bacteria resulting in the release of various metabolites including TMA (trimethylamine) into the blood. TMA is then transported to the liver where it is enzymically oxidated into TMAO by flavin-containing monooxygenase-3 (FMO3). This metabolic pathway of dietary carnitine to TMAO is gut microbe dependent as confirmed by 2 studies involving ingestion of either isotope-labeled phosphatidylcholine or isotope-labeled carnitine as a tracer before and after exposure to an oral cocktail of poorly absorbed AB to suppress intestinal microbes.[299,313] In human study individuals receiving oral AB for a week before consuming red meat experienced a complete suppression of endogenous TMAO production. The same study also reported that vegetarians and vegans had significantly lower fasting baseline TMAO levels, significantly higher abundance of *Bacteriodes* and lower abundance of *Prevotella* species in the gut microbiota compared with omnivores and a decreased risk for coronary heart disease and the traditional risk factors for CVD such as hypertension, atherosclerosis, peripheral artery disease, and stenosis.[299–301] Plasma levels of TMAO were assayed in patients with chronic HF compared with control subjects showing highest values in individuals with ischemic HF, followed by those with stable coronary artery disease and nonischemic HF.[302] TMAO levels were also involved in prediction of risk for thrombotic events in human subjects and TMAO enhances submaximal stimulus-dependent platelet activation. Direct exposure of platelets to TMAO enhanced submaximal stimulus-dependent platelet activation from multiple agonists through augmented $Ca^{2+}$ release from intracellular stores.[313] Animal model studies using dietary choline or TMAO, germ-free mice, and microbial transplantation collectively confirm a role for gut microbiota and TMAO in modulating platelet hyperresponsiveness and thrombosis potential and identify microbial taxa associated with plasma TMAO and thrombosis potential. In mice, for example, the proportion of *Allobaculum*, a high-choline diet characteristic taxa, was significantly positively associated with TMAO levels and shortened internal carotid artery occlusion times. In contrast, alternative bacterial taxa that showed significant reduction in proportion, such as *Candidatus arthromitus* or *Lachnospiraceae*, were associated with both lower TMAO levels and an antithrombotic phenotype.[314] Finally, an association between plasma TMAO levels and both the extent of coronary atherosclerotic plaque burden and CVD risks has been observed in multiple distinct clinical studies.[301,302,305,313–316] It is also important to remember that CVD and kidney diseases are closely interrelated, the so-called cardiorenal syndrome.[306] It is well known that the composition of gut microbiota is markedly altered in CKD patients,[307,308] leading to an influx of circulating urea and other uremic toxins into the gut lumen.[309] Within the intestinal tract, urea is hydrolyzed by microbial urease to form large quantities of ammonia, which is then converted to ammonium hydroxide. Ammonia and ammonium hydroxide disrupt the intestinal epithelial tight junctions causing intestinal epithelial barrier dysfunction in CKD that allows the translocation of gut bacterial DNA and uremic toxins into systemic circulation, resulting in systemic inflammation.[309] Therefore, the gut could be a target of treatment with probiotics of cardiorenal syndrome in conjunction with efforts to improve dialysis techniques to better remove these uremic toxins.

## LGG AND ALCOHOLIC LIVER DISEASE (ALD), NONALCOHOLIC FATTY LIVER DISEASE (NAFLD), NONALCOHOLIC STEATOHEPATITIS (NASH)

ALD is a term that encompasses the liver manifestations of alcohol overconsumption, including fatty liver, alcoholic hepatitis, and chronic hepatitis with liver fibrosis or cirrhosis. NAFLD is defined by pathologic accumulation of fat in the liver due to causes other than excessive alcohol use. NASH is defined as an inflammatory response to hepatic fat accumulation, resulting in chronic liver damage, scarring, and fibrosis that may progress to cirrhosis. Because only 30% of alcoholics develop ALD, a factor other than heavy alcohol consumption must be involved in the development of induced liver injury in ALD, but also in NAFLD and NASH. Animal and human studies suggest that bacterial products, such as endotoxins, are the second key cofactors, and leaky gut is one of the sources of endotoxemia. Indeed chronic alcohol consumption in humans causes bacterial overgrowth and dysbiosis, highlighted by the jejunal bacterial overgrowth in chronic alcoholics that might contribute to functional and/or morphologic abnormalities of the small intestine commonly found in patients with chronic alcohol abuse.[310] In animal models the intestinal dysbiosis may potentially contribute to the pathogenesis of liver disease by

altering intestinal barrier integrity, resulting in intestinal hyperpermeability and increased production of proinflammatory factors that could both promote liver pathology[311,312] an important role of dysbiosis in alcohol-induced endotoxemia.[317] Tereforeand probiotics could be activated to combat this phenomenon effectively. In man small intestinal bacterial overgrowth (SIBO) may contribute to the development of NASH, perhaps by increasing intestinal permeability and promoting the absorption of endotoxin or other enteric bacterial products. A study on this topic showed that SIBO was present in 50% of patients with nonalcoholic steatosis and 22% of control subjects ($P=0.048$). Mean TNF-α levels in NASH patients and control subjects were 14.2 and 7.5 pg/mL, respectively ($P=0.001$).[318] *L. rhamnosus* GG prevents cytokine-induced apoptosis in mouse or human colon intestinal epithelial cell models. Culture of LGG activates the antiapoptotic Akt/protein kinase B and inhibits activation of the proapoptotic p38/MAPK by TNF, IL-1α, or IFN-γ. Furthermore, products recovered from LGG culture broth supernatant show concentration-dependent activation of Akt and inhibition of cytokine-induced apoptosis.[33]

An attempt has been made in rat model to reduce both circulating endotoxin and liver injury by administering LGG which it has been shown to be capable to provide a potential form of therapy for both endotoxemia and ALD.[319] Male Sprague-Dawley rats gavaged with alcohol twice daily (8 g/kg) for 10 weeks were also treated with once daily gavage of either $2.5\times10^7$ LGG (Alc+LGG) or vehicle (Alc+V). ALC+LGG-fed rats had significantly ($P\leq0.05$) less severe alcoholic steatohepatitis, reduced alcohol-induced gut leakiness and significantly blunted alcohol-induced oxidative stress and inflammation in both intestine and the liver than ALC+V-fed rats.[320] It is also been hypothesized that alcohol impairs the adaptive hypoxia-inducible factor (HIF) and that probiotic supplementation could attenuate this impairment, restoring barrier function in a mouse model of ALD by increasing HIF-responsive proteins (eg, intestinal trefoil factor) and reversing established ALD. Actually in mice LGG supplementation significantly reduced alcohol-induced endotoxemia and hepatic steatosis and improved liver function reducing HIF-2α and intestinal trefoil factor levels. In addition, in vitro studies using the Caco-2 cell culture model showed that the addition of LGG supernatant prevented alcohol-induced epithelial monolayer barrier dysfunction. Furthermore, gene silencing of HIF-1α/2α abolished the LGG effects, indicating that the protective effect of LGG is HIF-dependent.[321] The effects of *L. rhamnosus* GG culture supernatant (LGG-s) on the acute alcohol-induced intestinal integrity and liver injury has been evaluated in a mouse model measuring intestinal permeability and alcohol-induced liver injury by the activity of alanine aminotransferase (ALT) in plasma, and liver steatosis by triglyceride content and Oil Red O staining of the liver sections. LGG-s pretreatment restored alcohol-induced reduction in ileum mRNA levels of claudin-1, intestine trefoil factor, P-glycoprotein (P-gp), and cathelin-related antimicrobial peptide, which play important roles on intestinal barrier integrity.[322] In mice fed with Lieber-DeCarli liquid diet containing 5% alcohol for 8 weeks LGG treatment reduced alcohol-induced hepatic inflammation by attenuation of TNF-α production via inhibition of TLR4- and TLR5-mediated endotoxin activation.[323] Moreover, in mice LGG-s culture decreased ethanol-elevated miR122a level increasing occludin expression.[324] In a subsequent study in C57BL/6 mice the Lieber-DeCarli diet containing 5% alcohol for 10 days induced an elevation in liver enzymes, steatosis, and morphology changes, while LGG supplementation attenuated these changes significantly improving intestinal barrier function reflected by increased mRNA expression of tight junction proteins and villus-crypt histology in ileum, and decreased *E. coli* protein level in liver. Importantly, flow cytometry analysis showed that alcohol reduced Treg-cell population while increased TH17 cell population as well as IL-17 secretion, which was reversed by LGG-s administration[325] (Table 29).

In human evidence on the gut microbiota association and involvement in development of liver injury is accumulating. The liver blood supply comes from the intestine exposing the hepatocytes to a multitude of intestinal metabolites and food products.[333] It has also been shown that gut dysbiosis and SIBO are more evident in patients with NASH than in healthy contols.[334] Intestinal dysbiosis facilitate the translocation of microbial products as pathogen-associated molecular patterns (PAMPs) from the gut lumen through the lamina propria to the blood stream. The concomitant activation of TLRs causes hepatic fibrogenesis and systemic inflammation. Furthermore the gut microbiota reduces fasting-induced adipose factor expression and free fatty acids uptake. Moreover, the gut microbiota is able to gather energy from complex polysaccharides into monosaccharides and SCFA,which are substrates for hepatic lipogenesis and gluconeogenesis.[325]

Some studies were conducted in man to investigate microbiota involvement in the development of NAFLF and NASH[335–341] (Table 30).

**TABLE 29.** Animal Studies on Activity of LGG

| Studies (References) | Animals | Outcome |
|---|---|---|
| Mutlu et al[323] | Male Sprague-Dawley rats 10 wk | Supplementation of *L. rhamnosus* GG prevented alcohol-altered colonic mucosa-associated microbiota composition in rats |
| Nanji[326] | GG Male Wistar rats 1 mo | Probiotic feeding reduced alcohol-induced endotoxemia and liver injury |
| Forsyth[327] | Male Sprague-Dawley rats 10 wk | *L.* GG reduced alcohol-induced gut leakiness and blunted alcohol-induced oxidative stress and inflammation both in the intestine and liver |
| Wang[328,329] | Male C57BL/6N mice Last 2 wk of the 8-wk feeding | *L.* GG supplementation reduced alcohol-induced endotoxemia and hepatic steatosis |
| Wang[330] | Male C57BL/6N mice 5 d | Bacteria-free *L.* GG culture supernatant ameliorated acute alcohol-induced gut leakiness and liver injury |
| Zhao[331] | Mice | < intestinal ethanol-elevated miR122a level > occludin expression |
| Chen[332] | C57BL/6 mice | > mRNA expression of tight junction proteins > villus-crypt histology in ileum < *Escherichia coli* protein level in liver.<br>Reversion by LGG of alcohol reduced Treg-cell population and increased TH17 cell population |

**TABLE 30.** Human Studies Investigating Microbiota Involvement in the Development of NAFLD and NASH

| Studies | Subjects | Samples | Results |
|---|---|---|---|
| NAFLD | | | |
| Michail[342] | 13 obese children with NAFLD<br>11 obese children no NAFLD<br>26 healthy children | Stool | Obese children with NAFLD:<br>> Gammaproteobacteria<br>> Epsilonproteobacteria > Prevotella |
| Spencer[343] | 15 individuals:<br>10 d normal diet (baseline),<br>42 d choline-depleted diet | Stool | Baseline samples:<br>> Gammaproteobacter at baseline correlates to lower risk of developing fatty liver on low-choline diet.<br>> Erysipelotrichia at baseline correlates to higher risk of developing fatty liver on low -choline diet |
| Raman[344] | 30 obese NAFLD patients<br>30 healthy control | Stool | Obese NAFLD vs. healthy controls:<br>> Lactobacillus < Firmicutes |
| NASH | | | |
| Zhu[345] | 22 NASH children<br>25 obese children<br>16 healthy controls | Stool | Obese and NASH vs. healthy controls:<br>> Bacteroidetes > Prevotella<br>NASH vs. obese and healthy controls<br>> Proteobacter > Enterobacteriaceae |
| Wong[346] | 16 NASH patients<br>22 Healthy controls | Stool | NASH vs. healthy controls: < Firmicutes<br>No change e Bacteroidetes<br>> Parabacteroides > Allisonella<br>< Faecalibacterium <Anaerosporobacter |
| Boursier[347] | 22 NAFLD 35 NASH patients | Stool | NASH vs. NAFLD: > Bacteroidetes |
| Mouzaki[348] | 50 adults: 22 NASH on biopsy<br>11 Simple steatosis on biopsy<br>17 Healthy control | Stool | > *C. coccoides* in NASH vs. simple steatosis < Bacteroidetes: in NASH vs. simple steatosis |

On this basis probiotics have been utilized in NADH and in NAFLD.

*C. coccoides* indicates *Clostridium coccoides*; NADH, nicotinamide adenine dinucleotide (reduced form); NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

On this basis probiotics have been utilized in nicotinamide adenine dinucleotide (reduced form) and in NAFLD.

A 2007 Cochrane Review concluded that even if the results from pilot studies seem promising, there is no evidence to support or refute probiotics for patients with NAFLD and randomized clinical trials are necessary to asses the clinical implication of probiotics therapy in these situations.[349] Some studies have been performed in the following years. In patients with NAFLD *Lactobacillus bulgaricus* and *Streptococcus thermophiles* in a RCT versus placebo after 3 months of treatment improved liver aminotransferases and gGT levels versus baseline values (ALT: 67.7 ± 25.1 vs. 60.4 ± 30.4 UI/L $P < 0.05$; aspartate transaminase: 41.3 ± 15.5 vs. 35.6 ± 10.4 UI/L; $P < 0.05$) (gGT: 118.2 ± 63.1 vs. 107.7 ± 60.8 UI/L; $P < 0.05$). In the placebo group all liver function parameters remained unchanged.[326] In another study, *B. longum* with Fos significantly reduces TNF-α, CRP, serum aspartate transaminase levels, HOMA-IR, serum endotoxin, steatosis, and the NASH activity index.[327] In an interesting study patients with histology-proven NASH were randomized to receive probiotics *L. plantarum*, *Lactobacillus deslbrueckii*, *L. acidophilus, L. rhamnosus*, and *Bifidobacterium bifidum* (n = 10) or usual care (n = 10) for 6 months. Intrahepatic triglyceride content as measured by proton-magnetic resonance spectroscopy (IHTG) decreased from 22.6% ± 8.2% to 14.9% ± 7.0% in the probiotic group ($P = 0.034$) but remained static in the usual care group (16.9% ± 6.1% to 16.0% ± 6.6%; $P = 0.55$). Six subjects in the probiotic group had IHTG reduced by > 30% from baseline, compared with 2 subjects in the usual care group ($P = 0.17$). The probiotic group also had greater reduction in serum aspartate aminotransferase level ($P = 0.008$).[328] These data have been confirmed in a pediatric study in which 20 obese children (age 10.7 ± 2.1 y) with persisting hypertransaminasemia and ultrasonographic bright liver were enrolled in a double-blind, placebo-controlled pilot study receiving either probiotic *L. rhamnosus* GG (12 billion CFU/d) or placebo for 8 weeks. Multivariate analysis after probiotic treatment revealed a significant decrease in ALT (average variation vs. placebo, $P = 0.03$) and in anti-peptidoglycan-polysaccharide antibodies (average variation vs. placebo, $P = 0.03$) irrespective of changes in body mass index score and visceral fat.[330] A meta-analysis on the effects of probiotics in NAFLD has been carried out including 4 randomized trials involving 134 NAFLD/NASH patients.[329] The results showed that probiotic therapy can significantly reduce liver aminotransferases, total-cholesterol, TNF-α, and improve insulin resistance in NAFLD patients (Table 31).

**TABLE 31.** Overall Data of Meta-Analysis on Probiotics in Nonalcoholic Fatty Liver Disease

| |
|---|
| ALT: WMD = −23.71 (95% CI = −33.46 to −13.95, $P < 0.00001$) |
| AST: WMD = −19.77 (95% CI = −32.55 to −7.00, $P = 0.002$) |
| Total chol: WMD = −0.28 (95% CI = −0.55 to −0.01, $P = 0.04$) |
| HDL: WMD = −0.09 (95% CI = −0.16 to 0.01, $P = 0.03$) |
| TNF-α: WMD = −0.32 (95% CI = −0.48 to −0.17, $P < 0.0001$) |
| HOMA-IR: WMD = −0.46, 95% CI = −0.73 to −0.19, $P = 0.0008$ |

ALT indicates alanine aminotransferase; AST, aspartate transaminase; CI, confidence interval; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment insulin resistance; TNF, tumor necrosis factor; WMD, weighted mean difference.

Any way studies assessing microbial contribution to disease pathogenesis in animal models and in human will surely supply valuable data to understand the pathogenesis of both NAFLD and NASH and the possible role of probiotics. A combination of multiomics approaches should be applied to identify bacterial community and host changes on the level of species abundance (16S ribosomal RNA gene sequencing), gene abundance (shotgun metagenomics sequencing), transcript abundance (bacterial and host RNA sequencing), and metabolite abundance (metabolomics profiling).

## LGG AND CYSTIC FIBROSIS (CF)

CF is an inherited multisystemic disease affecting mainly the respiratory system but also the digestive system. Chronic inflammation is present in the CF gut and many CF-related conditions like pulmonary inflammation are associated with systemic inflammation in which the gut microbiota may play an important role. Intestinal dysbiosis is well-documented in people with CF[331,332] and emerging evidence suggests that it occurs within the first year of life and then progresses further.[331,350,351] Furthermore, intestinal dysbiosis may also be associated with impaired innate (inherent) immunity in CF children. It has been also reported an enhanced output of the inflammatory proteins, albumin, IgG, IgM, eosinophilic cationic protein, neutrophil elastase, IL-1β and IL-8 in the gut lavage of children with CF[342,351] and an increased mononuclear cell infiltration in the lamina propria of duodenal mucosal specimens that resulted in increased expression of IL-2, IFN-γ, IL-2R, ICAM-1, and transferrin receptors.[343,344]

Therefore, CF is one interesting area of application for bacterial therapy with probiotics. A prospective, randomized, placebo-controlled, crossover study was performed.[345] Nineteen children received LGG for 6 months and then shifted to ORS for 6 months. In parallel 19 received ORS and then shifted to LGG. Patients treated with LGG showed a reduction of pulmonary exacerbations (median 1 vs. 2, range, 4 vs. 4, median difference 1, 95% CI = 0.5-1.5; $P = 0.0035$) and of hospital admissions (median 0 vs. 1, range, 3 vs. 2, median difference 1, 95% CI = 1.0-1.5; $P = 0.001$) compared with patients treated with ORS. LGG resulted in a greater increase in forced expiratory volume [3.6% (75.2) vs. 0.9% [76]; $P = 0.02$] and body weight [1.5 kg (71.8) vs. 0.7 kg (71.8); $P = 0.02$]. Overall LGG reduces pulmonary exacerbations and hospital admissions in patients with CF. For its relationship with intestinal inflammation also the composition of intestinal microbiota was analyzed before and after LGG administration in children with CF with and without antibiotic treatment. In total, 22 children with CF were enrolled in the study (median age, 7 y; range, 2-9 y). Fecal calprotectin and rNO levels were higher in children with CF than in healthy controls ($184 \pm 146$ vs. $52 \pm 46$ μg/g; $18 \pm 15$ vs. $2.6 \pm 1.2$ μmol/L $NO_2^-$, respectively; $P < 0.01$). Compared with healthy controls, children with CF had significantly different intestinal microbial core structures: the levels of *Eubacterium rectale*, *Bacteroides uniformis*, *Bacteroides vulgatus*, *B. adolescentis*, *Bifidobacterium catenulatum*, and *Faecalibacterium prausnitzii* were reduced. A similar but more extreme pattern was observed in children with CF who were taking AB.[346]

The authors themselves in a following randomized, double-blind, placebo-controlled trial in hospitalized children (6 mo to 5 y of age) administered LGG ($6 \times 10^9$ CFU/d) together with vitamins B and C and zinc or placebo, for 15 days, starting on the first day of hospitalization.[347] The incidence of GI and respiratory nosocomial infections after discharge was determined by follow-up telephone call at 7 days and after 3 months.

In total, 90 children completed the follow-up. Of 19/90 children with a nosocomial infection (20%), 4/45 children (9%) were in the treatment group and 15/45 (33%) in the placebo group ($P = 0.016$). Specifically, 2/45 (4%) children in the treatment group versus 11/45 (24%) children in the placebo group ($P = 0.007$) presented with diarrhea. The duration of hospitalization was significantly shorter in the treatment group ($3.9 \pm 1.7$ vs. $4.9 \pm 1.2$ d; $P = 0.003$). At the follow-up, a total of 11/45 (24.4%) children in the treatment group had at least 1 episode of infection compared with 22/45 (48.9%) in the placebo group ($P = 0.016$). These data confirm that LGG and micronutrients may reduce the incidence of nosocomial infections, supporting the hypothesis that this may represent a valid strategy to prevent nosocomial infections. In a different study was determined the prevalence of bacterial overgrowth before and after LGG administration in 20 patients with cystic fibrosis (mean age 10.33, range, 5 to 17 y).[348] The expired hydrogen test with a 2 g/kg of 20% dextrose overload was performed on 10 patients. After the test, *L. rhamnosus* LGG $10^{11}$ CFU was administered twice daily for 4 weeks. Fecal near infrared spectroscopy of water, fat, nitrogen, and sugar content in feces was performed before and after probiotics administration. Five patients (50%) showed bacterial overgrowth. A positive correlation was observed between the hydrogen test and steatorrhea (R = 0.57) and sugar in feces (R = 0.52). The fecal near infrared spectroscopy results pretreatment versus posttreatment were: fat $6.2 \pm 3.3$ versus $4.9 \pm 2.1$ g ($P < 0.05$), sugar $6.7 \pm 3.6$ versus $5 \pm 2.6$ g ($P < 0.05$) and nitrogen $0.87 \pm 0.27$ versus $0.91 \pm 0.14$ g (no significant), respectively. Thirteen patients (81.25%) had improved stool appearance and intestinal comfort and 9 (56.25%) decreased the number of daily stools.

Experiences with multistrains or single-strain (*Lactobacillus reuteri*) probiotics with significant improvement of clinical conditions have been also performed.[352,353] Two meta-analysis have been dedicated to the subject suggesting that probiotics may improve respiratory and GI outcomes in a stable CF clinic population with no reported evidence of harm. However, well-designed adequately powered RCTs assessing clinically meaningful outcomes are required to study this important issue.[354,355]

To confirm this issue a recent multicentre, randomized double-blind, clinical trial was conducted by the some authors who published in 2014 and 2016[346,347] positive data on LGG in children with CF. After 6 months of baseline assessment, enrolled children (2 to 16 y of age) received *Lactobacillus* GG ($6 \times 10^9$ CFU/d) or placebo for 12 months. In total, 95 patients were enrolled (51/95 female; median age of $103 \pm 50$ mo). In a multivariate Generalized Estimating Equation for Logistic Regression (GEE logistic analysis), the odds of experiencing at least 1 exacerbation was not significantly different between the 2 groups, also after adjusting for the presence of different microbial organisms and for the number of pulmonary exacerbations within 6 months before randomization (OR = 0.83, 95% CI = 0.38-1.82; $P = 0.643$). Similarly, LGG supplementation did not significantly affect the odds of hospitalizations (OR = 1.67, 95% CI = 0.75-3.72; $P = 0.211$). But to confirm the interest

for the argument, the Cochrane Oranization has published the protocol of an ongoing research on probiotic for people with cystic fibrosis.[356]

## *LACTOBACILLUS* GG AND ALLERGY

The prevalence of atopic diseases is increasing throughout the western world and atopic dermatitis it represent as a disease of early childhood. From the epidemiological point of view about 20% of all children develop symptoms of atopic dermatitis at some point in their lives and half of these within the first year of life with 95% experiencing onset below 5 years of age.[357–359] About 30% of all children with atopic dermatitis have food allergy, particulatlry cow's milk and egg but also soy, wheat, and fruits. A child with moderate to severe atopic dermatitis has a 50% risk of developing asthma, whereas the risk of developing hay fever is as much as 75%.[359]

According to the hygiene hypothesis[360] children growing up in a traditional farming environment and who therefore have been exposed to a variety of microflora in animal stabling and in unpasteurized cow's milk are also protected against development of allergic diseases.[361]

The immunologic background of the hygiene hypothesis is characterized by the infiltration of eosinophils and excessive IgE production due to T-helper type 2 (Th2) differentiation of naive T cells, with production of IL-4, IL-5, and IL-13 cytokines, as opposed to the Th1 differentiation, which is inhibited.[362]

Atopic dermatitis may be associated with aberrant barrier functions of the gut mucosa.

Moreover, the composition of the gut microbiota may be different in individuals with atopic eczema from those without this condition, and such differences may precede the development of eczema. Epidemiological data show that allergic children have higher levels of *Clostridia*, and lower levels of *Bifidobacteria*. Nevertheless, *Bifidobacteria* and *Lactobacilli* are found more commonly in the composition of the intestinal microflora of nonallergic children. There is also growing evidence underlining the pivotal role of infant gut colonization in the development of the immune system. The possibility to modify gut colonization through probiotic supplementation in childhood could prevent atopic diseases. Studies on the treatment of atopic and food allergies have suggested that by restoring the permeability of the intestinal mucous membrane, by modulating the local immune response and by using probiotics (Table 32) that suitably alter the food antigens it is possible to reestablish the altered immune activity.[363,364] In one of the first studies on this topic, after a challenge in infants allergic to cow's milk proteins (CMP) fecal IgA levels were detected to be higher and TNF-α levels lower in LGG applied group compared with the placebo.[365] In following studies on this topic it has been shown that the intensity and the extension of the rash and subjective symptoms decreased significantly in children with an atopic eczema with diet containing *Lactobacillus*.[366,367] In another clinical study *Lactobacillus* GG was given prenatally and during the weaning period to mothers who had at least 1 first-degree relative (or partner) with atopic eczema, allergic rhinitis, or asthma, and postnatally for 6 months to their infants. Atopic eczema was diagnosed in 46 of 132 (35%) children aged 2 years, asthma in 6 of these children and allergic rhinitis in 1. The frequency of atopic eczema in the probiotic group was half that of the placebo group [15/64 (23%) vs. 31/68 (46%); RR = 0.51 (95% CI = 0.32-0.84)]. The number needed to treat was 4.5 (95% CI = 2.6-15.6).[368]

To investigate the interaction of *Lactobacillus* GG with skin and gut microbiota and humoral immunity 39 infants with AD were randomized for a 3-month period in a double-blind design to receive extensively hydrolyzed casein formula (EHCF) supplemented with (n = 19) or without (n = 20) LGG $5.0\times10^7$ CFU/g to achieve a daily intake of $3.4\times0^9$ CFU. Sampling (blood and fecal samples, cotton swab from the skin) was carried out at entry, 1 and 3 months thereafter. Ig-secreting cells were determined by enzyme-linked immunospot and the proportions of CD cells among peripheral blood leukocytes by flow cytometry. The major groups of gut and skin bacteria were characterized using PCR. The proportions of IgA-secreting and IgM-secreting cells decreased significantly in the treated group; the baseline-adjusted ratios for treated versus untreated at 1 month were 0.59 (95% CI = 0.36-0.99; $P = 0.044$) for IgA-secreting and 0.53 (95% CI = 0.29-0.96; $P = 0.036$) for IgM-secreting cells. The proportions of CD cells increased in the probiotic-treated infants but not in the untreated. There were no significant differences in bifidobacterial species composition of the gut between the study groups.[369] Moreover, LGG induced IFN-γ secretion in infants with cow's milk allergy (CMA) and in infants with IgE-associated dermatitis, but interestingly, not in infants with no CMA. Indeed LGG raises IFN-γ production of and may thus provide beneficial TH1 immunomodulatory signals This supports the view that the pattern of intestinal microflora may be aberrant in infants with an atopic predisposition, and the beneficial effects of probiotics are evident only in this.[370] Furthermore the addition of LGG to an EHCF significantly improved the recovery of the inflamed colonic mucosa in infants with blood in the stools and presumptive CMA colitis, as indicated indirectly by greater decreases in fecal calprotectin and in the number of infants with persistence of occult blood in stools after 1 month.[371] The supplementation of an EHCF with LGG accelerated the development of tolerance in infants to CMP. It is conceivable that the effect of LGG on acquisition of tolerance to CMP could be related to the immunoregulatory role played by LGG.[372] LGG can balance the generation of cytokines possibly involved in IgE-mediated or non–IgE-mediated CMA (ie, IL-4, IL-5, IL-10, IFN-γ, tumor growth factor (TGF)-β, and TNF-α.These effects were strain specific because studies conducted with other *Lactobacillus* species did not yield comparable results.[373]

An interesting question is whether the development of allergic diseases can be prevented in early infancy by modulating the intestinal microbiota with probiotic bacteria. In a double-blinded placebo-controlled study of 62 mother-

**TABLE 32.** Schematic Representation of the Potential Effects Mechanisms of Probiotics in Allergic Children

| Within Intestinal Lumen | At Mucosal Level | Beyond the Intestinal Mucosa |
|---|---|---|
| Modulation of microbiota | Modulation of gut permeability | Modulation of innate/adaptive immune system |
| Hydrolysis of antigenic peptides | Stimulation of cell growth and differentiation | Induction of oral tolerance |
| | — | Impact on the enteric nervous system |

infant pairs it is shown that administering *L. rhamnosus* GG at daily dose $2\times10^{10}$ CFU to the pregnant and lactating mother increased the immunoprotective potential of breast milk, as assessed by the amount of anti-inflammatory transforming growth factor β2 (TGF-β2) in the milk [2885 pg/mL (95% CI = 1624-4146) in mothers receiving probiotics versus 1340 pg/mL (95% CI = 978-1702) in mothers receiving placebo; $P = 0.018$] with risk significantly reduced of developing atopic eczema during the first 2 years of life in infants whose mothers received probiotics in comparison with that in infants whose mothers received placebo [15% and 47%, respectively; RR = 0.32 (95% CI = 0.12-0.85); $P = 0.0098$].[373] Both *Lactobacillus* GG (n = 72) and *Bifidobacterium lactis* BB-12 (n = 68) $1\times10^{10}$ CFU/d each from the first trimester of pregnancy to the end of exclusive breastfeeding had a protective effect against sensitization in infants with a high hereditary risk due to maternal sensitization (OR = 0.3, $P = 0.023$). The concentration of TGF-β2 tended to be higher in the colostrum of the mothers in the probiotic group as compared with those on placebo (probiotic/placebo ratio = 1.50; $P = 0.073$). A similar result was obtained in the subgroup of allergic mothers (probiotic/placebo ratio = 1.56; $P = 0.094$).[374,375]

A later systematic review of the evidence included 11 RCTs for treatment (n = 1.115) and 4 for prevention (n = 1.429), mostly in infants (below 18 mo old) and children (up to the age of 13) with either moderate to severe atopic dermatitis, atopic eczema, suspected CMA, general atopic dermatitis, or atopic eczema/dermatitis syndrome. Three studies of *Lactobacillus* GG alone or with other probiotics given to pregnant women for 2 to 4 weeks before labor followed by treatment post-birth for up to 6 months with the same probiotics resulted in significantly lower rates of atopic dermatitis during the first 2 years of life compared with placebo.[376] In a prospective, double-blind, placebo-controlled clinical trial performed in Taiwan 191 pregnant women with atopic diseases were assigned to receive either probiotics (*Lactobacillus* GG, $1\times10^{10}$ CFU daily) or placebo from the second trimester of pregnancy (LGG group, n = 95; control group, n = 96). Symptoms of maternal allergic scores improved significantly in the LGG group ($P = 0.002$). Maternal allergic diseases improvement was more prominent in pregnant women with IgE > 100 kU/L ($P = 0.01$) and significantly associated with higher IL-12p70 levels ($P = 0.013$). No significant effects of prenatal and postnatal probiotics supplementation on sensitization, development of allergic diseases, and maternal IgE levels.[377] The aim of another meta-analysis[378] was to evaluate the effect of probiotic supplementation during pregnancy and early infancy in preventing atopic diseases. Seventeen studies, reporting data from 4755 children (2381 in the probiotic group and 2374 in the control group), were included in the meta-analysis. Infants treated with probiotics had a significantly lower RR for eczema (RR = 0.78, 95% CI = 0.69-0.89; $P = 0.0003$) compared with controls, especially those supplemented with a mixture of probiotics (RR = 0.54, 95% CI = 0.43–0.68; $P < 0.00001$). No significant difference in terms of prevention of asthma (RR = 0.99, 95% CI = 0.77-1.27; $P = 0.95$), wheezing (RR = 1.02, 95% CI = 0.89-1.17; $P = 0.76$) or rhinoconjunctivitis (RR = 0.91, 95% CI = 0.67-1.23; $P = 0.53$) was documented. The results of the present meta-analysis show that probiotic supplementation prevents infantile eczema, thus suggesting a new potential indication for probiotic use in pregnancy and infancy. Table 33 report the effect of LGG in the trials evaluated in the meta-analysis.

## MECHANISMS BEHIND THE EFFECTS

The effect of probiotics in the prevention and alleviation of allergy takes place with mechanism that are not yet fully understood.[364]

The gut microbiota influence the development of immune response and the balance of cell types (Th1/Th2) which in turn determines the development of oral tolerance. Th2 type immune cells produce IL-4, which is essential for B-cell differentiation into IgE-producing cells, and IL-5, which is important for the activity of eosinophil and lymphocytes. Intestinal permeability also is disturbed, allowing the absorption of antigenic macromolecules.[385]

Food antigens, like caseins, enhanced the mitogen-induced proliferation of lymphocytes of atopic children.[386] Caseins degraded by *Lactobacillus* GG also downregulated the IL-4 production of lymphocytes compared with the control. T-cell activation was suppressed in vitro by *Lactobacillus* GG-degraded caseins, production of IL-2 mRNA was suppressed and the production of IL-2 protein reduced. At the same time, the levels of IL-4 and IFN-γ were reduced. The mechanism was based on the inhibition of the translocation of protein kinase C (one of the markers of cell activation) in the peripheral blood mononuclear cells of healthy children.[367,387–391]

An EHCF containing LGG accelerated the development of tolerance acquisition in infants with CMA and reduced the incidence of other allergic manifestations due to the abundance of fecal butyrate-producing genera and to the increase concentration of fecal butyrate. Berni Canani et al[392] demonstrated that the use of EHCF+LGG induces stronger epigenetic regulation. These data were confirmed in a study in which EHCF administration before or after bovine β-lactoglobulin (BLG) induced sensitization significantly reduced acute allergic skin reaction, anaphylactic symptom score, body temperature decrease, intestinal permeability increase, IL-4, IL-5, IL-13, and

**TABLE 33.** Effect of *Lactobacillus* GG on Treatment of Atopic Dermatitis in Humans

| References | Probiotics | Outcome |
|---|---|---|
| Majamaa[366] | LGG | SCORAD improvement ($P = 0.008$) |
| Rosenfeldt[379] | *Lactobacillus rhamnosus*+*Lactobacillus reuteri* | Positive effect of probiotics seen in allergic subjects ($P = 0.04$) |
| Kirjavainen[380] | LGG | SCORAD decrease ($P = 0.02$) |
| Viljanen[381] | LGG | Positive effect seen only in IgE-sensitized infants ($P = 0.036$) |
| Brouwer[382] | LGG | No significant difference between probiotics and placebo |
| Fölster-Holst[383] | LGG | No significant difference between probiotics and placebo |
| Grüber[384] | LGG | No significant difference between probiotics and placebo |

SCORAD indicates scoring atopic dermatitis.

anti-BLG IgE production. EHCF increased expression of IFN-γ and IL-10. Many of these effects were significantly enhanced by LGG supplementation.[393] The data support dietary intervention with EHCF for CMA prevention and treatment through a favorable immunomodulatory action. The observed effects are significantly enhanced by LGG supplementation.

Finally it is important to remember that the bacteria are transferred from a mother to her child at birth and that there are indications that the gut microbiota of atopic infants differs from the microbiota of healthy infants. At 3 weeks of age infants who later developed an atopic disease had a lower level of intestinal bifidobacteria than nonatopic ones.[394]

In infancy also asthma development is preceded by gut microbiota dysbiosis and metabolic dysfunction. In a recent study gut microbiota maturation over the first year of life in infants at high risk for asthma (HR) and whether it is modifiable by early-life *Lactobacillus* supplementation were evaluated, comparing stool samples collected from HR infants randomized to daily oral *L. rhamnosus* GG (HRLGG) or placebo (HRP) for 6 months, and healthy (HC) infants.[395]

Following 6 months of *Lactobacillus* supplementation, HRLGG subjects possessed a fecal metabolic milieu comprised of anti-inflammatory fatty acids known to promote immune tolerance in early infancy.[396] However, the metabolic profile observed in HRLGG infants at 6 months was largely unsustained at 12 months of age and paralleled diminished LGG levels following cessation of supplementation, but it promotes enrichment of fatty acid conjugating organisms, such as *Bifidobacteria*[397,398] capable of their production.

Moreover, *Lactobacillus* GG has been shown to enhance the growth of *bifidobacteria* in newborn babies[399] and in milk-hypersensitive adults.[400]

## GUT MICROBIOTA AND CANCER

The eubiosis contributes to the maintenance of intestinal homeostasis characteristic of gut microbiota at healthy state. The composition of gut microbiota may be influenced by various environmental factors such as diet, inflammation, stress, or host genetics promoving dysbiosis that may favor neoplastic progression through various carcinogenic activities (immunomodulation, toxins, metabolites, etc.), which ultimately affect epithelial cell DNA integrity and cellular transformation.[401]

Moreover, mucosal barrier integrity is compromised by dysbiosis, further enhancing bacterial uptake and activation of mucosal immune cells (releases of inflammatory mediators), thereby contributing to neoplastic progression.[402,403] It has been defined, for example, the mechanisms by which *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment activating host β-catenin-WNT signaling by the binding of its FadA adhesin to E-cadherin.[404] However, currently only *Helicobacter pylori* has been proved to be a human carcinogen causing gastric cancer.[405] The gut microbiota may cause cancer also at distant sites. The presence of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* in the oral microbiota is significantly associated with increased risk of pancreatic cancer (adjusted OR for presence versus absence of *P. gingivalis* = 1.60 95% CI = 1.15-2.22 and for presence versus absence of *A. actinomycetemcomitans* OR = 2.20 and 95% CI = 1.16-4.18),while the phylum *Fusobacteria* and its genus *Leptotrichia* were associated with decreased pancreatic cancer risk (OR per percent increase of relative abundance = 0.94 and 95% CI = 0.89-0.99; OR = 0.87 and 95% CI = 0.79-0.95, respectively).[406] Owing to its ability to modulate host metabolism, inflammation, and immunity, the microbiota is involved in the initiation and/or progression of various types of neoplasias of digestive tract[407–411]:

- Stomach cancer (*H. pylori*)
- Colorectal carcinoma (*E. coli*, Fusobacterium spp. and enterotoxigenic *Bacteroides fragilis*)
- Gallbladder carcinoma (*Salmonella enterica typhi*) and systemically in organs that are not normally associated with the gut microbiota
- Mucose-associated lymphoid tissue, ocular, and skin lymphoma
- Thymic lymphoma
- Hepatocellular carcinoma
- Mammary carcinoma
- Pancreatic cancer
- Prostate cancer
- Sarcoma
- Ovarian cancer

## DRUG AND RADIOTHERAPY-INDUCED TOXICITY

*L. acidophilus* and *B. bifidum* was shown to prevent intestinal toxicity in cancer patients treated with both radiotherapy and cisplatin.[412] The intestinal chemotoxicity of methotrexate is mediated in part by activation of TLR4 by either microbial products or endogenous damage-associated molecular patterns (DAMPs).[413,414]

Activation of TLR2 protects the mucosa against methotrexate-induced damage by increasing the expression of the ABC transporter multidrug resistance protein-1 which regulates the efflux of xenobiotics from intestinal epithelial cells.[415] In the fecal microbiota of patients with melanoma after treatment with anticytotoxic T-lymphocyte–associated antigen (CTLA4) the number of *Bacteroides* spp was increased at the expense of with *Prevotella* indicating that the therapy may in some patients modify the composition of the gut microbiota.[416] The modification of gut microbiota at epithelial surfaces with apoptosis in the intestinal crypts and breach of the intestinal barrier in patients and mice treated with RTX are considered the cause of the pathogenesis of oral mucositis, diarrhea, enteritis, colitis, and bone marrow failure.[417,418] *L. rhamnosus* GG have been shown in mice to protect the intestinal mucosa against chemotherapy or radiotherapy-induced toxicity by relocating cyclooxygenase 2 (COX2)-expressing cells from the villi to the base of the intestinal crypts and inducing ROS, which activate the cytoprotective NRF2 system.[419,420] Indeed, probiotics have been proved in some clinical studies to be beneficial in preventing radiation-induced enteropathy.[421,422] Administration of *L. brevis* CD2 lozenges during radiation and chemotherapy treatment of patients with head and neck cancer also decreased the incidence of therapy-induced mucositis and increased the treatment completion rate.[423,424]

## *LACTOBACILLUS* GG AND CANCER

An overview exploring the rationale of the use of *Lactobacillus* GG in cancer has been recently published[425]: a number of interesting data describing the effects of LGG on cancer cells proliferation and tumor invasion are given below:

### Immunomodulation

- In gastric carcinoma cells (HGC-27) exposed to LGG homogenate for 24 and 48 hours, a dose-dependent decrease of the polyamine profile, up to the 20% in the 48 hours was observed.[426]

- LGG administered in combination with vitamin K1 to 3 different colon cancer cells (Caco-2, HT-29, and SW480) has shown a remarkable proapoptotic effect particularly on Caco-2 cells at 48 hours of treatment.[427]
- Metastatic colon cancer cells treated with cells free supernatants from LGG culture, achieved the gain of ZO-1 and the decrease of MMP-9 indicating an active role of the molecules released by LGG in reducing the infiltration property of tumor cells and the invasive and metastatic potential of colon cancer cells.[428]
- Neutrophils precultured with LGG can stimulate the dendritic cells maturation and the release of cytokines, like IL-12p70, which in turn activate the T cells–mediated immune response against the tumor environment.[429]
- LGG has proven to be effective in lowering both the *H. pylori*-induced IL-8 production and its adhesion on gastric adenocarcinoma cells.[193]
- LGG decrease flagellin-induced IL-8 production in Caco-2 cells.[186]
- Peripheral blood mononuclear cells incubated in vitro with LGG showed a higher secretion of cytokines, proteins, or peptides acting as mediators and regulators of the immune response.[430]

### Animal and Cells Colture Studies

- LGG has a protective role in male Fischer rats against colon cancer development by inhibiting or attenuating the mutagenic effects of dimethyl-hydrazine (DMH)[431]
- LGG induced apoptosis and reduced the expression of several angiogenetic and inflammatory proteins in rats with DMH-induced colon cancer[432]
- In human liver cancer cell line HepG2 treated with bacterial extracellular vesicles derived from *L. rhamnosus* GG the apoptotic index (bax/bcl2 expression ratio) was significantly increased leading to cancer cell death.[433]

### Anti-Inflammatory Effects During Anticancer Treatments

- LGG is effective in preventing radiation-induced and chemotherapy-induced toxicities.[434]
- LGG may induce bladder cancer regression in mice with lower inflammatory toxicity suggesting a protective role of toward inflammation.[435]

LGG administered as 1 to 2 capsules/d $10^{10}$ CFU for 24 weeks during anticancer treatment reduced by 15% the diarrhea episode of grades 3.[436]

## LGG AND POSTBIOTICS

Bioactive peptides[437] have been defined as specific protein fragments that have a positive impact on body functions or conditions and may influence health.[438] Currently, >1500 different bioactive peptides have been reported in a database named "Biopep."[439] In the field of probiotics the term postbiotic has recently emerged to denote that nonviable microbial cells, microbial fractions, or cell lysates that may offer physiological benefits to the host by providing additional bioactivity.[440] Postbiotics refers to soluble factors (products or metabolic byproducts), secreted by live bacteria, or released after bacterial lysis, such as enzymes, peptides, teichoic acids, peptidoglycan-derived muropeptides, polysaccharides, cell surface proteins, and organic acids. These postbiotics have drawn attention because of their clear chemical structure, safety dose parameters, long shelf life, and the content of various signaling molecules which may have anti-inflammatory, immunomodulatory, antiobesogenic, antihypertensive, hypocholesterolemic, antiproliferative, and antioxidant activities. These properties suggest that postbiotics may contribute, to the improvement of host health by improving specific physiological functions, even though the exact mechanisms have not been entirely elucidated.

In most cases, the effect of the administered probiotic is evident when the bacteria are still alive at the time they reach the small and large intestine, suggesting that it is dependent on the metabolic activity of the bacteria. Indeed, in some occasions it has been shown that the culture supernatant of these bacteria mediates the immunomodulatory effect conferred to the host. Recent work on relevant probiotic strains has also led to the isolation and characterization of certain probiotic-produced, soluble factors, called postbiotics, which were sufficient to elicit the desired response.[441–452]

Perhaps these small molecule products of the normal flora are at least partially responsible for the beneficial effects of the probiotics and could be used as a more controllable and safer therapeutic surrogate. Heat-killed probiotics may also function, in the broad sense, as postbiotics. Heat-killed microorganisms retain important bacterial structures that may exert biological activity in the host. The combinatorial effects of metabolites and other biological molecules together with live microorganisms may be more powerful. In this regard, the generation of probiotics with engineered changes in their metabolic pathways, aiming to enhance metabolite production to favor host health, is a formidable challenge and a potential therapy for inflammatory diseases. Nevertheless, with advances in the understanding of the microbiota-host metabolism axis, the use of postbiotic molecules has become a prominent strategy for treating many inflammatory diseases, as these molecules mimic the useful therapeutic effects of probiotics while avoiding the risk of administering live microorganisms to a host with an impaired immune system. For instance, metabolites are considered pivotal mediators of host-microbiota communication.

The postbiotics derived from LGG and their activities are reported in the Table 34.

## LGG AND SPORT

Moderate physical exercise is characterized by a minor number of infection in confront of a completely sedentary state. However, strenuous exercise may cause a depression of immune function that lasts 3 to 24 hours after exercise.[469]

Moreover, during intense exercise blood pools away from the GI tract to periphery muscles and organs that can cause gut mucosal barrier disruption, followed by an inflammatory response. In addition, it is possible an increase of stress hormones and of translocation of LPS tin the GI tract, which triggers immunity resulting in increased proinflammatory cytokines and intestinal permeability that in turn may be worsened by the increased production of ROS and by dysbiosis. Furthermore, GI tract responds to stress by releasing hormones such as γ-aminobutyric acid, neuropeptide Y (NPY), serotonin, and dopamine that have been purported to cause GI disturbances and anxiety.[470] Stress during intense training can influence the gut microbiota microbial through the release of stress hormones or sympathetic neurotransmitters that influence gut physiology and alter the habitat of the microbiota.[471] Moreover, it is important to remember that LGG can upregulate SERT mRNA and

**TABLE 34.** LGG Postbiotics Activities

| |
|---|
| LGG-CM induces both soluble factors Hsp25 and Hsp72 in a time-dependent and concentration-dependent manner. These effects are mediated by a low–molecular-weight peptide that is acid and heat-stable[453] |
| LGG conditional media produced 7 peptides showing various degrees of antibacterial activity with different inhibition activities on *E. coli* growth. Peptide NPSRQERR has inhibitory activities on gram-negative and gram-positive microorganisms. Peptides NPSRQERR, PDENK and VHTAPK showed inhibitory activities on several antibiotics-resistant bacteria Peptides NPSRQERR, VHTAPK, and PDENK were found to inhibit growth of kanamycin-resistant *E. coli* SM10 δpir and tetracycline-resistant *E. coli* TOPO10. Moreover, growth of methicillin-resistant *Staphylococcus aureus* was also found to be inhibited by peptides NPSEQERR, VHTAPK, and PDENK.[454–456] |
| Peptides and proteins producted by LGG have a role on antimicrobial activity (peptides NPSRQERR and PDENK, on growth promotion (protein p40), on the reduction of the injuries caused by TNF-α and attenuation of the TER decrease induced by hydrogen peroxide (proten p75) and on decrease of IL-8 production in epithelial cells (p40 and p75 supenatant)[457–460] |
| LGG adhesins, molecules conferring stress tolerance and nutritional versatility, antimicrobial products against competing microbes, and factors promoting resistance against the host immune system have been isolated from LGG supernatants[461] |
| LGG supernatants significantly reduced the LPS-induced morphofunctional alterations of muscle cells, ie, cell shortening and inhibition of contractile response, protecting human SMCs from LPS-induced myogenic damage[462,463] |
| LGG exopolysaccharide metabolite influences in liquid from cell cultures of lymphocytes the increase of TGF-β1 and IL-4 and the decrease in IFN-γ concentration[464] |
| LGG heat-killed preparations of the probiotic accelerates intestinal barrier maturation and induces claudin 3 expression[465] |
| p40 LGG protein ameliorates intestinal injury and colitis, reduces apoptosis, and preserves barrier function by transactivation of the EGF receptor in intestinal epithelial cell[466] |
| LGG produces 23 peptides enhancing bacterial binding (> 4-fold increase as compared with no-peptide control) on a cellulose membrane. Remarkably, one of the identified peptides, QRCVNLQA, induced aggregation of lactic acid bacteria and promoted bacteria-mucosa interaction[467] |
| Preincubation of human colonic carcinoma cell line Caco-2 and neonatal rats with and without LCS and then exposed to *E. coli* K1 inhibit adhesion, invasion and translocation of *E. coli* K1 to Caco-2 monolayer as well as alleviate bacterial intestinal colonization, translocation, dissemination and systemic infection in neonatal rats. Furthermore the preincubation with LCS could promote the maturation of neonatal intestinal defense and thereby, enhance the resistance of neonatal rats to oral *E. coli* K1 infection.[468] |

*E. coli* indicates *Escherichia coli*; IL, interleukin; IFN, interferon; LCS, LGG culture supernatant; LPS, lipopolysaccharides; SMC, smooth muscle cells; TGF, tumor growth factor; TNF, tumor necrosis factor.

SERT-P levels in intestinal epithelial cells and in mice intestinal tissues[48,49] and can induce ROS generation in intestinal epithelia in vitro and in vivo. LGG products activate ROS signaling in a FPR-dependent manner and define a mechanism by which cellular ROS influences the ERK pathway through a redox-sensitive regulatory circuit. It is now accepted that consuming probiotics may modify the gut microbiota's population and structure and may influence immune function as well as intestinal epithelium cell proliferation, function, and protection in individuals who follows exercise[471] (Table 35). Any way differences in fecal microbiota between athletes and sedentary controls show even greater separation at the metagenomic and metabolomic than at compositional levels and provide added insight into the diet-exercise-gut microbiota paradigm. Further studies are necessary to confirm these interesting data. The influence of LGG on serotonin and ROS[48,49] could be considered suggesting the possibility to its use during sport performances.

## LGG IN THE ELDERLY

Aging is defined as deterioration of physiological functions accompanied by the development of age coupled with decline in the functionality of the immune system and chronic low-grade inflammation, which is usually referred to as inflammaging.[463–466]

Aging itself has a relatively insignificant influence on the GI tract, but due to a decrease in adaptive capabilities of the GI tract, elderly people do not recover easily from disease. A reduction in time for gastric evacuation results in a higher satiation and higher risk of an unbalanced diet in elderly people. There are many theories that GI microbiota actively participates in the processes of an organism's resistance to diseases, and the fact is that the balance of intestinal microbiota is influenced by unfavorable environmental factors and stressful conditions, including psychological ones. As inflammaging is thought to contribute to many diseases associated with ageing, a new study showing for the first time that gut bacteria from old mice induce age-related chronic inflammation when transplanted into young mice highlights that the gut microbiota plays a role in this process.[467] Gut dysbiosis and inflammaging are interlinked, possibly through a relationship sustained by complex homeostatic mechanisms. This suggests that direct manipulation of the gut microbiota may offer direct means to improve adaptive immune response and reduce inflammatory secretions, therefore compensating immunosenescence. However, it would be most valuable that future research work should consolidate these effects, but also that they would include longer term studies to record improvements of clinical manifestations.

The following Table 36 summarize the studies conducted with probiotics in the elderly.

## LGG Suggested Dosage

It is difficult to define the quantity of live probiotic bacteria to prescribe in different indications.

The optimal dose is likely to depend on the strain and targeted health effect.[494] However, within specific strain or combination of strains, very few trials have attempted to reveal a dose-effect relationship to specific health effects.[495]

Lacking specific studies on dose-response, some parts of the statement from the AFSSA (*Agencie Francaise de Sécurité Sanitaire*) can be consider[496]: "The ingested dose of probiotics is an important factor in obtaining high concentrations in the various sections of the gastrointestinal tract. (…) It was often stated that the concentrations of probiotics must be anyway $\geq 106$ CFU/mL in the small intestine and $\geq 108$ CFU/g in the colon, but the scientific bases of this statement are relatively weak. (…) The concentrations to be reached in the colon were proposed since they corresponded to less than 1/1000 of the autochthonous flora." In a study in

**TABLE 35.** Probiotics and Sport

| References | N | Exercise | Duration | Results/Conclusions |
|---|---|---|---|---|
| Clancy[472] | 27 | Prospective study. A total of 18 healthy athletes and 9 fatigued athletes were included in the study, supplemented with *L. acidophilus*, $2\times10^{10}$ CFU/d | 4 wk, | Fatigued athletes had significantly less secretion of IFN-γ from blood CD4+ T cells. After *L. acidophilus* there was a significant increase in secretion of whole-blood IFN |
| Cox[473] | 20 | RCT. Distance runners (i) supplementation with *Lactobacillus fermentum* $1.26\times10^{10}$ CFU/d (ii) placebo capsules | 4 wk | *L. fermentum* elicited greater change in the whole-blood culture of IFN-γ compared with placebo, and significantly reduced (50%) the no. days of respiratory illness and its severity |
| Gill[474] | 8 | RCT. Endurance trained males: (i) *L. casei* ($1\times10^{11}$ CFU/d) (ii) placebo | 1 wk | No changes with *L. casei* in resting circulatory endotoxin concentration or plasma cytokine profile compared with placebo. Increased levels for IL-6, TNF-α, IL-10, and IL-8 in response to exertional-heat stress |
| Gleeson[475] | 66 | RCT highly active individuals: (i) *L. salivarius*; $2.0\times10^{10}$ CFU/d (ii) placebo. | 16 wk | The no. URTI episodes was significantly higher in the placebo group than in the probiotic group |
| Gleeson[476] | 84 | RCT endurance: (i) *L. casei Shirota* $6.5\times10^{9}$ CFU/d (ii) placebo | 16 wk | The no. URTI episodes was significantly higher in the placebo group than in the probiotic group |
| Haywood[477] | 30 | RCT. Rugby: (i) *L. gasseri*, $2.6\times10^{12}$ *B. bifidum* $0.2\times10^{12}$ *B. longum* $0.2\times10^{12}$ CFU/d (ii) placebo | 4 wk | 14/30 probiotics group never experienced a single URTI or GI episode vs. 6/30 on the placebo |
| Kekkonen[478] | 141 | RCT. Marathon runners: (i) *Lactobacillus rhamnosus* GG ($4.0\times10^{10}$ CFU/d) (ii) placebo | 14 wk | The no. healthy days was 79.0 in the probiotic group and 73.4 in the placebo group. The duration of GI episodes in the probiotic group was 2.9 vs. 4.3 d in the placebo group |
| Lamprecht[453] | 23 | RCT Trained men: (i) multispecies probiotic group ($1\times10^{10}$ CFU/d, EcologicPerformance or OMNi-BiOTiCPOWER, n = 11) or (ii) placebo group (n = 12) | 14 wk | Probiotic decreased zonulin in feces (~25%) and reduced TNF concentration by ~25% at rest and postexercise, and exercise-induced protein oxidation by ~8% and IL-6 production |
| Martarelli[454] | 24 | Controlled trial, no placebo. Active individuals random: (i) 1:1 *L. rhamnosus* IMC 501 and *L. paracasei* IMC 502; $\sim10\times10^{9}$ CFU/d) (ii) control group | 4 wk | Probiotics increased plasma antioxidant levels (~9%), thus neutralizing ROS and exerted strong antioxidant activity |
| Salarkia[455] | 46 | RCT Endurance swimmers girls: (i) 400 mL of probiotic yogurt (ii) ordinary yogurt daily | 8 wk | Consumption probiotic reduced the no. episodes of respiratory |
| Shing[456] | 10 | RCT Male runners: (i) 45 billion cells/d of Lactobacillus, Bifidobacterium and Streptococcus strains (ii) placebo | 4 wk | 4 wk of supplementation with a multistrain probiotic increased running time to fatigue in high temperatures |
| Valimaki[457] | 127 | RCT Runners: (i) LGG $3\times10^{10}$ CFU/d (ii) placebo | 3 mo before marathon | No changes in serum antioxidant potential before marathon, but during run serum antioxidant potential raised by 16% in both groups |
| West[458] | 241M, 224 F | RCT: (i) *B. animalis subsp. lactis* $2.0\times10^{9}$ CFU/d; (ii) *L. acidophilus B. animalis sub. lactis* 5×10 CFU/d (iii) placebo | Preparation marathon | The risk of an upper respiratory illness episode was significantly lower in the Bl-04 group compared with placebo |
| West[459] | 99 | RCT Cyclists (64 males, 35 females): (i) *L. fermentum* $1\times10^{9}$ CFU/d (ii) placebo | 11 wk | The load (duration×severity) of respiratory symptoms was less by a factor of 0.31 in males taking the probiotic compared with placebo but increased by a factor of 2.2 in females |
| Moreira[460] | 141 | Marathon runners randomized to consume 2 bottle LGG drink contained LGG $3.0\times10^{8}$ CFU/mL or placebo the pollen season before the marathon | 3 mo | In all runners, the marathon run induced a significant eosinopenia, but serum ECP did not change. The responses to the marathon run were similar in the LGG and placebo groups |
| Jager[461] | 33 | RCT. Highly trained individuals: (i) a multispecies probiotic (*B.bifidum*, *B. lactis*, *Enterococcus faecium*, *L. acidophilus*, *L. brevis*, *L. lactis*) $1\times10^{10}$ CFU/d (n = 17) (ii) Placebo (n = 16) | 12 wk | URTI symptoms was increased 2.2-fold in placebo group compared with probiotics group (PLA 0.79, PRO 0.35; $P = 0.02$) |
| Strasser[462] | 25M,5F | RCT. Subjects randomly received: *L. acidophilus* 10 billion CFU 10 *B. bifidum* 9.5 billion CFU *B. animalis sub. lactis* 0.5 bil. CFU FOS 400 mg, lipoic acid, 600 mg/d Or placebo | 12 wk before triathlon | Multistrain pro/prebiotic use reduced endotoxin unit levels |

*B. bifidum* indicates *Bifidobacterium bifidum*; *B. longum*, *Bifidobacterium longum*; ECP, eosinophil cationic protein; GI, gastrointestinal; IFN, interferon; IL, interleukin; *L. acidophilus*, *Lactobacillus acidophilus*; *L. casei*, *Lactobacillus casei*; *L. gasseri*, *Lactobacillus gasseri*; PLA, placebo; PRO, probiotics; RCT, randomized controlled trial; URTI, upper respiratory tract infection.



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**TABLE 36.** Effects of Probiotics in Old Subjects

| Strain | Product | Age | Effect | References |
|---|---|---|---|---|
| LGG | $10^8$ CFU/d | Retired nurses | Glycocholic acid hydrolase and tryptic activities were significantly decreased | Ling et al[468] |
| *B. lactis* | Dehydrated sachets | Median 69 | > T-helper cells (CD4), activated T lymphocytes (CD25) | Gill et al[479] |
| *L. casei* (Shirota) | Dairy product | 61 ± 7.3 | Improved mood. No improvement defecation | Benton et al[480] |
| *B. lactis* | Skim milk | > 60 | > *bifido*, *lactobacilli*, *enterococci* < *enterobacteria* | Ahmed et al[481] |
| *L. delbrueckii subsp bulgaricus* | Capsules | > 85 | > NK cells > antimicrobial peptide β-defensins < proinflammatory IL-B | Moro-Garcia et al[482] |
| *B. longum* | Tube feeding | 81.7 ± 8.1 | > *bifido* > IgA after influenza vaccination | Akatsu et al[483] |
| *Lactobacillus plantarum* | Capsules | 65-85 | > response to influenza vaccination (> IgA and IgG) | Bosch et al[484] |
| *B. fantis*, *B. longum LGG*, *L. casei* | Fermented milk | 65-76 | > NK activity, > *Bifidobateria*, > IFN-γ, > IL-6 production | You and Yaqoub 2012[485] |
| *B. longum* | Tube feeding | 65-102 | Regularized bowel movements | Kondo et al[486] |
| *B. longum*, *L. helveticus* | Biscuits | 71-88 | < opportunistic pathogens | Rampelli et al[487] |
| *B. bifidum*, *B. lactis* | Capsules+inulin | > 62 | > *Bifidocteria*, *Lactobacilli* | Bartosch et al[488] |
| *B. longum*, *B. animalis* | Fermented oat meal | 84 ± 3 | > *Bifidocteria* < TNF-α and IL-10 | Ouwehand et al[489] |
| *B. longum*46, *B. longum*2C | Fermented oat | 84 ± 8 | > *Bifidocteria* | Lahtinen et al[490] |
| LGG+FOS | Yoghurt LGG, FOS | 76-90 | > LGG in feces, No increase *Bifidobacteria* | Granata et al[491] |
| LGG | $1\times10^{10}$ CFU/d | 66-80 | Safe and well tolerated in healthy adults | Hibberd et al[492] |
| LGG | LGG $10^{10}$ CFU twice daily ×28 d | 65-80 | LGG may promote interactions between key constituents of the microbiota and the host epithelium | Eloe-Fadrosh et al[493] |

*B. bifidum* indicates *Bifidobacterium bifidum*; *B. lactis*, *Bifidobacterium lactis*; *B. longum*, *Bifidobacterium longum*; IL, interleukin; IFN, interferon; *L. casei*, *Lactobacillus casei*; TNF, tumor necrosis factor.

7 volunteers on dose-response colonization of feces after oral administration of LGG no *Lactobacilli* were detected in the fecal samples before LGG administration. When LGG was given orally at dose levels of $10^6$ to $10^8$ it could not be recovered from the feces. The limit of detection was $10^3$ CFU/g feces. When a dose level of $10^9$ was given, 2 of 7 volunteers were occasionally colonized by *Lactobacillus* GG at a low level of $10^3$ to $10^4$ CFU/g feces. With a LGG dose of $10^{10}$ CFU/d all volunteers were colonized. During the study period the mean level of *Lactobacillus* GG in fecal samples was $10^5$ to $10^6$ CFU/g feces. Similarly, with a daily dose of $10^{10}$ bacteria all volunteers were colonized. The mean level of LGG found in feces was between $10^6$ and $10^7$ CFU/g. It appears that the colonizing dose of LGG is $10^{10}$ to $10^{11}$ CFU/d.[497] Data of a meta-analysis[75] (Table 5) evidenziate the different outcomes of high ($>10^{10}$ CFU/d) versus low doses ($<10^{10}$ CFU/d) of LGG in acute gastroenteritis in children with the greater efficacy of higher dose. The greater efficacy of a dose exceeding $10^{10}$ CFU/d of *L. rhamnosus* GG was confirmed in another meta-analysis on acute gastroenteritis in children.[85]

The suggested dosage of LGG is reported in Table 37.

**TABLE 37.** Suggested Dosage of LGG

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|---|
| The suggested LGG amount for intestinal colonization is $\geq 5\times10^9$ CFU/d |
| LGG should be administered in high doses, usually 5-$10\times10^9$ CFU/d for children and 10-$20\times10^9$ CFU/d for adults, for ≥ 5-7 d |
| In case of antibiotic therapy LGG should be administered during the treatment and for 1-3 wk longer than the duration of antibiotic treatment |
| LGG should be taken with food because to avoid a too low pH |
| Microencapsulation enhance the viability during processing and for the targeted delivery in gastrointestinal tract |

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