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Lactobacillus rhamnosus GG: A Review of Clinical Use and Efficacy

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Abstract

Oral *Lactobacillus rhamnosus* GG (LGG) supplementation is generally recognised as a safe form of supplementation, which acts as an immunomodulator, an antimicrobial, and aids cell growth and proliferation. The aim of this review was to determine diseases where oral LGG supplementation has been indicated; and assess safety, colonisation, mechanisms of action and efficacy, and provide therapeutic recommendations. LGG following supplementation can successfully colonise the gut and other areas of the body owing to the expression of unique morphological features known as pili. Twenty-two disease areas were identified where LGG supplementation has been used, to determine effects. However, small study sizes, the use of multispecies probiotics and adjuvant therapies all meant that strong evidence for the use of LGG was lacking in several disease areas. Despite this, LGG was shown to be of benefit in the reduction of risk of developing attention-deficit hyperactivity disorder and gestational diabetes mellitus, in the prevention of allergies and dental caries, for improving immune reactions following vaccines, and for the management of diarrhoea associated with cancer treatments and antibiotic use.

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Introduction

Probiotics are defined as live microbes, which when administered in adequate amounts confer benefits to the host.[1] For this to occur, probiotics need to be safe, alive, of human origin and capable of surviving the pH of the gut.[2] Several different bacteria are used as probiotics, but species from the genera Lactobacillus and Bifidobacterium have been well researched and are believed to provide many health benefits.[3,4,5,6]

Amongst the most well-researched strains of probiotics is *Lactobacillus rhamnosus* GG (LGG). Its health benefits are thought to derive from its superior ability to colonise the gastrointestinal (GI) tract, outcompeting and producing antimicrobials to prevent pathogenic bacterial colonisation.[7] LGG may also promote GI barrier protection and healing through cell growth and proliferation,[8,9] and act as an immune effector both locally and systemically.[10] Based on the mechanisms of action, clinical trials in humans have been extensive, showing benefits in several disease areas.

This review paper aims to determine the disease areas where the use of LGG as an oral probiotic has been indicated, and review the clinical data on efficacy and safety with a view to making therapy recommendations. Data on the mechanistic actions of LGG will also be briefly reviewed. Randomised-controlled trials (RCTs) will dominate this review, as the beneficial effects of probiotics seem to be strain specific;[11] thus, pooling data in large meta-analyses and systematic reviews with different strains may result in misleading conclusions. Where LGG alone has been examined, these will be included.

Colonisation and adhesion

The success of probiotic supplementation relies upon the ability of the microbiota to colonise areas of the body, such as the GI tract. In comparison to other *Lactobacillus* strains, LGG has high adherence to human intestinal mucus glycoproteins[7] and, in adults, supplementation has indicated that it may survive for at least 1 week in the GI tract[12] (Figure 1). Amongst newborns and infants, a reduced intrinsic GI microbiome may ensure that LGG colonises the GI tract more readily, and it has been detected in faeces up to 2 weeks after administration, without affecting the establishment of a normal GI microbiome.[13]

Image 1: LGG has superior mucus adherence

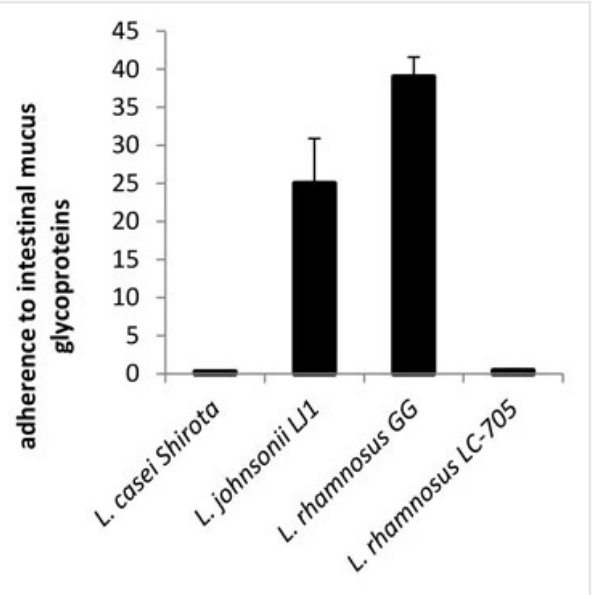

Figure 1: *Lactobacillus rhamnosus* GG (LGG) has superior adherence to mucus glycoproteins when compared with other probiotic strains, including a closely related strain of *L. rhamnosus* (LC705). In this study, radioactively labelled bacteria were allowed to adhere to isolated human intestinal mucus. The adhesion ratio (%) was determined by comparing radioactivity of bacteria added to the radioactivity of bound bacteria after washing.

Successful colonisation of LGG, compared with other *Lactobacillus* species, may be down to certain morphological features. Comparative genomic analysis with *Lactobacillus rhamnosus* LC705 has revealed the presence of a DNA sequence,

known as the *spaCBA* gene, resulting in pili-like appendages that run along the entirety of the LGG microbe, the inhibition of which lowers adhesion to the GI tract[14] (Figure 2). Differing conditions have been shown to promote or suppress the expression of the pili phenotype in other microbiota strains;[15] however, when exposed to different conditions such as low pH, the pili of LGG are still expressed.[16] Interestingly, whilst LGG still expresses pili under different conditions, when present in the oral and vaginal cavities the pili are absent,[16] which may have implications in diseases associated within these areas, such as urinary tract infections (UTIs) and dental caries.

Image 2: *Lactobacillus rhamnosus* GG-specific pili

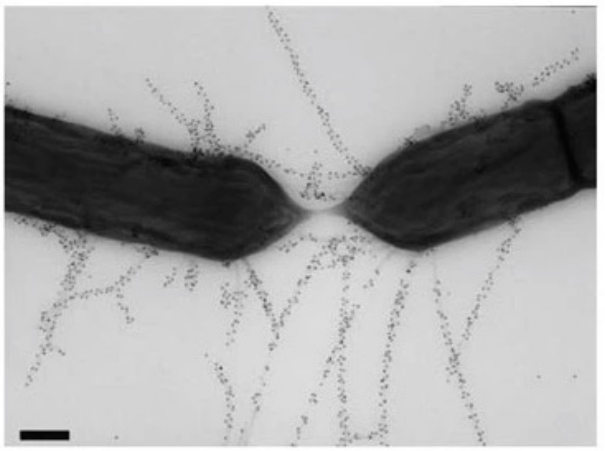

Figure 2: *Lactobacillus rhamnosus* GG (LGG)-specific pili, not present on other *Lactobacillus* spp., are involved in the mechanisms of adhesion to the intestinal mucosa. In addition, the pili facilitate a close interaction between the host and the bacteria or bacteria with each other. In this image, transmission electron microscopy reveals pili on LGG cells.

Certain probiotic strains have been shown to adhere to the GI tract, preventing mucolytic bacteria from digesting the protective layer of mucus, resulting in decreased vulnerability to intestinal permeability.[17] Although studies have shown limited effects of LGG on intestinal permeability in patients with chronic liver conditions,[18] investigations into efficacy in healthy patients is warranted.

General effects

Immunomodulation

The role of LGG in immunomodulation is controversial, with proteins isolated from it and its physico-chemical properties contributing to both inflammatory and anti-inflammatory actions. The pili on LGG have been implicated to have a role in immunomodulation. An *in vitro* study on LGG bred without the *spaCBA* gene, which encodes for the growth of the external pili, reported increased expression of the inflammatory cytokine interleukin (IL)-8, which was decreased with the wild-type strain.[19] Proteins secreted from LGG may also have an anti-inflammatory role in the immune response, and isolation of a novel soluble protein, HM0539, from LGG has been shown in colon tissue to suppress the TLR4/MyD88/NFκB inflammatory pathway.[20] However, overexpression of toll-like receptor (TLR)4 or myeloid differentiation primary response 88 (MyD88) did reverse this effect. The TLR4 signalling pathway may be responsible for upregulated inflammation in chronic and acute inflammatory disorders,[21,22] such as inflammatory bowel disease (IBD) and atherosclerosis,[23,24] indicating that LGG supplementation in highly inflammatory states may have a limited effect.

In contrast to its anti-inflammatory effects, effector substances such as lipoteichoic acid (LTA), found in the cell walls of certain gram-positive bacteria including LGG, may also be involved in the modulation of the immune response, with it displaying pro-inflammatory properties. Within the body, reporter cell lines are designed to monitor intracellular cell signalling pathways. LTA produced by a wild-type LGG strain has been shown to

activate the inflammatory NF-κB signalling pathway in reporter cells, a pathway that is significantly reduced when the LTA gene is removed.[10] Removal of the LTA gene resulted in a reduced capacity to activate TLR2/6-dependent NFκB signalling in reporter cells and reduced induction of IL-8 mRNA in CACO-2 cells from the human colon, acting both locally and systemically. However, the implications of this during certain disease states and the exact role of LGG in the inflammatory and anti-inflammatory pathways still needs to be elucidated.

Supplementation of LGG in individuals with inflammatory GI diseases has shown mixed results, and is discussed later in the review.[25,26,27]

Cell growth and proliferation

Proteins produced by LGG, known as Msp1 and Msp2, have been implicated in cell homeostasis through regulation of the protein kinase B (Akt) signalling pathway and inhibition of MAP kinases.[8,9] *In vitro* studies in animal and human colon tissue have shown that Msp1 and Msp2 promoted cell growth and attenuated GI permeability in hydrogen peroxide-damaged intestinal epithelium.[8] Msp2 has also been shown in intestinal epithelial cells, *in vivo* and *in vitro*, to inhibit cytokine-induced apoptosis,[28] indicating a role for LGG in the protection and recovery from intestinal permeability and injury.

Antimicrobial

In vitro, LGG has been shown to inhibit the growth and adherence of several pathogenic bacteria belonging to the *Salmonella, Shigella*, *Escherichia* and *Streptococcus* species.[7,29,30,31,32] In rabbit models, LGG has been shown to inhibit translocation of *Escherichia coli* in a dose-dependent manner.[33] In clinical trials, a decrease in the number of children colonised with vancomycin-resistant enterococci has been reported following LGG consumption for 21 days, with increased GI *Lactobacillus* counts observed in their stead.[34]

LGG DNA contains encodes for bacteriocins, which act like antibiotics, preventing the growth of closely related bacterial strains; however, the product of these genes has not been expressed under experimental conditions. Further experiments have reported that the antimicrobial action of LGG may be due to the production of microcin-type substances, which are small bacteriocins, mediated in part by lactic acid.[32,35,36]

LGG may also communicate with other gut microbiota via a process known as quorum sensing (QS), resulting in cooperation for nutrients and cellular adhesion against pathogenic bacteria.[37] The pili-like protrusions responsible for colonisation may ensure superior competitive inhibition by LGG during QS. However, further studies are required to determine the role of QS when the GI tract is faced with pathogenic bacteria.

Clinical uses

Cancer

It has been hypothesised that gut dysbiosis may promote colorectal cancer through the colonisation of pathogenic bacteria, which drives its development.[38] Furthermore, chemotherapy treatment may alter the composition of the gut microbiota,[39] indicating areas where probiotics may be of benefit. The use of LGG in a multispecies probiotic in combination with a prebiotic has been shown to alter several colorectal cancer biomarkers after 12 weeks.[40] In this trial, *Bifidobacterium* ($P = 0.008$), *Lactobacillus* ($P = 0.021$) and interferon-gamma (IFN-γ) were all increased, with *Clostridium perfringens* ($P = 0.022$) and DNA damage decreased amongst 37 patients with colon cancer and 43 polypectomised patients. However, administration of the synbiotic also prevented a rise in IL-2 inflammatory cytokines. In contrast to IL-2 suppression, a second RCT on a multispecies synbiotic containing LGG, *Bifidobacterium*

lactis and inulin reported increased IL-2 and IFN-γ in 34 patients with colon cancer who had undergone curative resection or polypectomy.[41] IFN-γ and IL-2 were both increased at 12 weeks compared with placebo ($P \leq 0.05$ both), but no other effects on immune factors were observed. Overall, it is difficult to conclude any specific effects of LGG from these trials as, when in combination, effects may be due to other species.

Failure of cancer treatments often occurs when severe side-effects result in a reduction or cessation of treatment.[42] Side-effects, such as diarrhoea, can occur in as many as 30–87% of patients, with severe and potentially life-threatening (grade 3–4) episodes occurring in 20–40% of patients.[43] Probiotics have been shown to be a safe and effective way to prevent chemotherapy-induced diarrhoea[39] and, as monotherapy, one of the most important effects of LGG may be for its use to reduce the frequency and severity of severe diarrhoea and GI symptoms during chemotherapy treatment. In one RCT of 150 patients with colorectal cancer given LGG twice daily [1–2 × 10^{10} colony-forming units (CFU)] for 24 weeks during 5-fluorouracil chemotherapy, patients had less grade 3 or 4 diarrhoea and fewer hospitalisations due to bowel toxicity compared with a fibre supplement (22% versus 37%, $P = 0.027$; and 8% versus 22%, $P = 0.021$, respectively), resulting in decreased chemotherapy dose adjustments (21% versus 47%, $P = 0.0008$).[44]

Perioperative administration of a multispecies probiotic containing LGG plus fructo-oligosaccharide has also been associated with reduced infection rate in postoperative patients with colorectal cancer. One trial in 91 patients undergoing surgery reported decreased infections at the incision site (2% versus 21.4%, $P = 0.002$), reduced intra-abdominal abscess ($P \leq 0.001$) and reduced incidence of pneumonia ($P \leq 0.001$), indicating a beneficial effect to complications associated with cancer treatment.[3]

Helicobacter pylori may be the strongest known risk factor for gastric cancer, and eradication may be an effective therapy in its prevention.[45] However, undesirable side-effects of eradication may include diarrhoea, pain, nausea and bloating, resulting in treatment cessation.[46] During *H. pylori* eradication, the supplementation of LGG (6 × 10^9 CFU twice daily) has been reported to increase eradication tolerability ($P = 0.04$) due to decreased side-effects, such as diarrhoea, nausea and taste disturbances [relative risk (RR) = 0.1, 95% confidence interval (CI): 0.1–0.9; RR = 0.3, 95% CI: 0.1–0.9; RR = 0.5, 95% CI: 0.2–0.9].[47] Although eradication rate remained unaffected, the supplementation of LGG in individuals undergoing *H. pylori* eradication may contribute to preventing the development of gastric cancer through increased treatment tolerability.

Results have not been as positive in other cancers, with one study in 40 patients undergoing head and neck cancer surgery reporting no impact of a multispecies synbiotic on postoperative outcomes, intestinal function or GI symptoms,[48] indicating that the beneficial effects of LGG in cancer may be localised and specific.

It is apparent that LGG monotherapy (6 × 10^9 CFU twice daily) has numerous benefits, including success in symptom management of *H. pylori* treatment and reducing the risk of developing gastric cancer. For individuals undergoing chemotherapy treatment for colon cancer, LGG (1–2 × 10^{10} CFU twice daily) may be more beneficial than fibre for the reduction of diarrhoea. In combination with other probiotics and prebiotics, LGG may reduce postoperative infections and improve immune function in patients with colon cancer, which is important during a time of reduced immune function.

Irritable bowel syndrome

Irritable bowel syndrome (IBS) is characterised by abdominal pain, flatulence and irregular bowel movements, and it is estimated that 10–20% of the worldwide

adult population suffers from this syndrome.[49] Treatments for IBS have limited success, and newer drugs such as 5-HT_4 agonists come with cardiovascular risks,[50] highlighting a need for treatments with limited side-effects.

In adults, it appears that several factors are involved in the pathophysiology of IBS, with mucosal large intestine low-grade inflammation and altered gut microbiota indicated.[51] As stated earlier, *in vitro* studies have highlighted that LGG may have both pro-inflammatory and anti-inflammatory properties, making its role as a supplement in inflammatory diseases uncertain.

Differences in the gut microbiota between healthy subjects and sufferers of IBS have been highlighted.[52,53] The administration of LGG may be able to promote colonisation and reinstate the composition of gut microbiota more associated with healthy individuals. Supplementation of a multispecies probiotic containing LGG (8–9 × 10^9 CFU) for 6 months in 42 patients with IBS resulted in a shift towards similar quantities of bacterial 16S rDNA to those of healthy controls; however, clinical outcomes were not reported in this study.[54] Although there was a shift in composition towards that of more healthy individuals, a gut microbiota composition that is characteristic of, or favourable for, sufferers of IBS has yet to be isolated and may be highly individual.[55] Regardless, the administration of probiotics containing LGG may be of benefit for symptom relief in individuals with IBS. In one double-blind RCT of 49 patients with IBS, the supplementation of a multispecies probiotic (5 × 10^9 CFU/day) containing LGG reported improvements to abdominal pain/discomfort and bloating after 4 weeks, which was attributed to alterations in the composition of the gut microbiota.[27] Faecal analysis highlighted that LGG, *B. lactis* and *Streptococcus thermophilus* had all significantly increased in the supplemented group, although several other strains were also included in the probiotic.

IBS can be divided into subtypes,[56] and improvements in symptoms may be highly dependent upon this, highlighting the importance of identifying the IBS type before commencing LGG supplementation. One 6-week unblinded RCT of 123 adults with IBS investigated the efficacy of LGG (6 × 10^9 CFU/day for 6 weeks) compared with a low-fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) diet on syndrome severity, assessed using the IBS symptom severity scoring system.[26] The results showed that LGG was as efficacious as the low-FODMAP diet, especially in the diarrhoeal (IBS-D) and mixed IBS (IBS-A) subtypes, with no improvement in those with the IBS constipation subtype. Quality of life (QoL) was also improved in those with IBS-D. Studies like this highlight the need to perform trials on dual therapy of diet changes and probiotics to further advance possible management strategies for IBS.

Children with IBS may spontaneously recover;[57] however, for those who have persistent symptoms, the use of LGG to effectively manage IBS may also be dependent on an accurate diagnosis. Improvements to the frequency and severity of abdominal pain when supplemented with LGG may be due to improvements in the gut barrier, and may be especially pronounced in children with IBS or functional pain.[4] In one 16-week RCT of 141 children with IBS or functional pain, LGG supplementation (6 × 10^9 CFU/day) reduced the frequency and severity of abdominal pain ($P \leq 0.01$ for both). Although it was concluded that this effect may not be unique to LGG, it is apparent that it may be of benefit. One systematic review of three RCTs with 290 children suffering from abdominal pain-related functional GI disorders concluded that a higher rate of children responded to treatment with LGG (6 × 10^9 CFU/day to 10^{10} CFU/day) compared with placebo if suffering from IBS, an effect that was not evident in children without IBS.[58] Frequency of pain was only reduced in the IBS subgroup; however, pain intensity was

reduced amongst the whole study population. This study did not perform a statistical test for publication bias, so this cannot be ruled out and conclusions should be made with caution.

A second systematic review of 11 RCTs examining various probiotic supplements in children with functional abdominal pain disorders (FAPD) highlighted that LGG reduces the frequency and severity of abdominal pain, but only in children with IBS.[25] One study of 4 weeks, which included IBS sufferers alongside those with dyspepsia and functional abdominal pain, concluded that children supplemented with 3×10^9 CFU of LGG twice daily were more likely to have improved pain frequency but not pain severity,[59] further supporting the need to identify IBS for LGG supplementation to be of benefit to symptoms.

In vitro studies have highlighted that LGG may have both pro-inflammatory and anti-inflammatory properties, and have a limited effect in inflammatory conditions. However, supplementation studies of the use of LGG to relieve symptoms of IBS in adults may be more positive, and dependent upon its use for the IBS-D and IBS-A subtypes, although this is based on data from studies using multispecies rather than LGG alone. Its use in children may be of benefit for the management of pain symptoms.

Diarrhoea

Diarrhoea is a leading cause of childhood mortality worldwide and is the second leading cause of childhood deaths.[60] The use of probiotics and LGG for the prevention and treatment of diarrhoea has been extensively researched, with varying success. One large RCT of 943 children with moderate-to-severe gastroenteritis reported that administration of a short 5-day course of LGG 1×10^{10} CFU twice daily was no more effective than placebo at decreasing the duration of all-cause diarrhoea (median LGG 49.7 hours versus 50.9 hours, $P = 0.26$).[61]

Despite negative outcomes in all-cause diarrhoea, the understanding of the mechanisms through which LGG interacts with the host may indicate specific types of diarrhoea where supplementation may have greater success. Antibiotic-associated diarrhoea (AAD) may result from dysbiosis of the host's gut bacteria.[62] LGG acts through several mechanisms to potentially prevent dysbiosis or restore normal bacterial flora resulting from antibiotic administration, such as competitive exclusion of pathogens,[63] modulation of the immune system,[64] and through outcompeting less acid-tolerant bacteria as LGG produces lactic acid.[65] The prevention of gut microbiota changes associated with antibiotic use has been shown in one RCT of 231 school-aged children.[65] In this trial, children given 10^6 CFU/ml LGG in 400 ml of milk long term reported changes in several gut bacteria, with especially increased abundance of the *Lactobacillus* spp. ($P < 0.0001$).

LGG for the reduction of risk of AAD has been extensively researched, and it may be important for the prevention of this disease and to provide new treatment options when antibiotics are prescribed. One meta-analysis of 12 RCTs and 1499 participants reported that, compared with placebo or no treatment, LGG was associated with a reduced risk of AAD (22.4% to 12.3%, RR = 0.49, 95% CI: 0.29–0.83), resulting in a number needed to treat (NNT) of 9.[66] LGG may also be one of the most effective probiotics for the prevention of AAD, and a meta-analysis of 32 RCTs has reported that LGG was superior to seven single or multispecies probiotics for the prevention of AAD (RR versus placebo = 0.30, 95% CI: 0.16–0.5).[67] Dosages of at least 2×10^9 CFU were recommended.

Although evidence exists for the use of LGG with AAD, other causes of diarrhoea have reported mixed results. As previously discussed, *in vitro* studies have indicated the ability of LGG to prevent the adherence and

viability of several gut pathogens.[36,68] Amongst these, *Clostridium* spp. have been shown to be inhibited *in vitro* through the production of a bactericide that resembles microcin.[32] However, *in vivo* studies in children have not been as positive. One meta-analysis of 20 RCTs concluded that the use of LGG reduced the risk of AAD from 23% to 9.6% (RR = 0.48, 95% CI: 0.26–0.89); however, it found no effect on the risk of *Clostridium difficile*-associated diarrhoea (RR = 0.95, 95% CI: 0.06–14.85).[69] It should be noted that the results on *C. difficile* were based on only one RCT, and this warrants more research.

Studies on children with rotavirus-associated diarrhoea, which is the leading cause of vaccine-preventable diarrhoea,[60] have been more positive. One meta-analysis of 19 RCTs concluded that, compared with control, the use of high-dose LGG (10^{10} CFU/day) in children with rotavirus-positive diarrhoea reduced the duration [mean difference (MD) −31.05 hours, 95% CI: −50.31, −11.80] and frequency of diarrhoea episodes (MD −1.08, 95% CI: −1.87, −0.28).[70] This trial also looked at children with acute diarrhoea caused by a mixture of rotavirus, bacterial pathogens and norovirus and, in contrast to Schnadower *et al*. (2018),[61] high-dose LGG reduced the duration of diarrhoea episodes (MD −15.83 hours, 95% CI: −20.68, −10.98), but only in those who had suffered from diarrhoea for less than 3 days at enrolment, indicating that earlier treatment at higher doses may have more success.

Dose-dependent effects of LGG supplementation on rotavirus-associated diarrhoea are also apparent. One open-label RCT in 23 children with rotavirus-associated diarrhoea showed no changes in faecal rotavirus concentrations when supplemented with low-dose LGG 2×10^8 CFU/day (36.1×10^5 particles/ml versus 73.5×10^5 particles/ml, $P = 0.895$), but at a high dose of 6×10^8 CFU/day concentrations were reduced (64.2×105 particles/ml versus 9.0×10^5 particles/ml, $P = 0.012$).[71] Although rotavirus shedding and not symptoms were assessed in this trial, it is indicative of disease severity. LGG may also aid recovery from rotavirus infection, with both intestinal permeability and immunoglobulin antibodies to rotavirus improved following LGG supplementation.[72]

Large, short-term trials for the treatment of all-cause diarrhoea have shown little improvements with the administration of LGG. Understanding the type of diarrhoea may result in a more targeted and successful approach. The use of LGG at a dose of at least 6×10^8 CFU during a course of antibiotics may prevent AAD, and early high-dose treatment during rotavirus-associated diarrhoea may decrease the duration of disease, the frequency of diarrhoea episodes and aid recovery.

Inflammatory bowel disease

IBD is an umbrella term for a number of different diseases, which includes Crohn's disease and ulcerative colitis.[73] As the name suggests, inflammation plays a major role in its development, and its aetiology is thought to be a combination of both genetic and lifestyle factors.[74,75,76] The gut microbiota may also play a major role in the pathogenesis of IBD, and in individuals with Crohn's disease, gut dysbiosis has been reported with an increased growth of *E. coli* and a reduction in the bacterial phyla Firmicutes, of which LGG is a member.[77,78]

As previously discussed, LGG may have pro-inflammatory properties, and limited effects in inflammatory GI diseases such as IBD. Remission of Crohn's disease, endoscopic recurrences and relapse times have all been shown to remain unaffected by LGG supplementation. One systematic review and meta-analysis of 41 studies, two of which were in LGG, reported no difference in endoscopic recurrences when supplemented with LGG, compared with placebo (0.93; 95% CI: 0.63, 1.38).[79] In a second meta-analysis of six RCTs, four of which were in LGG, the supplementation of LGG was concluded to increase the relapse

rate of individuals with Crohn's disease.[80] In this trial of 359 individuals, placebo showed a greater benefit on clinical relapse rates in adults (RR = 1.85, 95% CI: 1.00–3.41) and children (RR = 1.68, 95% CI: 1.07–2.64) compared with LGG, with little heterogeneity between the studies (P = 0.71, I2 = 0%).

Studies on the use of LGG in children have also not been efficacious. LGG in combination with standard Crohn's disease treatment has shown marginally shorter time periods between relapses. One RCT with a 2-year follow-up in 75 children who were in a period of inactive Crohn's disease reported a non-significant shorter time between the median time to relapse in those treated with LGG compared with those on placebo (9.8 months versus 11 months, P = 0.24).[81] There is a possibility that concomitant therapies may be masking the effect of LGG; however, in combination with the previously reviewed studies that have shown no effect of monotherapy, it would suggest that this is not the case.

When LGG is combined with other gut bacteria strains, anti-inflammatory actions have been observed; however, given the previous *in vitro* and *in vivo* research, it is likely that the extent of anti-inflammatory effects is due to the gut bacteria strain it has been combined with. One open-label parallel study reported an anti-inflammatory effect with a combination of LGG GR-1 strain and *Lactobacillus reuteri* in a yoghurt supplement given to 20 participants with Crohn's disease and ulcerative colitis.[82] In this study, LGG dosage of 2×10^7 CFU/ml and 1×10^3 CFU/ml and *L. reuteri* were associated with increased levels of $CD4^+$ $CD25^{high}$ T-cells (P = 0.007), which are involved in immune regulation, and decreased inflammatory cytokines, tumour necrosis factor-alpha (TNF-α) and IL-12 compared with healthy controls. However, the anti-inflammatory effects observed may be due to the presence of *L. reuteri*.

Given the *in vitro* evidence and the supplemental studies, there is very minimal evidence for the use of LGG in inactive Crohn's disease for endoscopic recurrences, and it may even be detrimental to overall relapse rates. Individuals with Crohn's disease have been reported to have antibacterial reactivity and a loss of tolerance for their own enteric flora[83] and, given the results above showing low colonisation of LGG in the guts of those with Crohn's disease, could indicate a need to increase supplemental doses above those already tested. However, this would raise safety concerns and, given the lack of dose–response trials, this is an area that requires more research.

Body weight

The relationship between body weight, diet and gut microbiota is complex, with each component influencing the other.[84] The involvement of the gut microbiota in the development of metabolic disorders is thought to involve nutrient and lipid metabolism, and hormone and immune modulation. In animal models, diets supplemented with LGG had hypercholesterolaemic effects and caused increased satiety, with increased peptide YY production;[85] however, mechanisms are still being debated.

The role of the two major phyla, Firmicutes, to which LGG belongs, and Bacteroidetes in obesity and weight loss has been extensively researched and remains controversial. Observations in obese children and overweight/obese women with metabolic syndrome have shown an increased Firmicutes to Bacteroidetes ratio, compared with their healthy counterparts.[86,87,88] Furthermore, a reduction in the Firmicutes to Bacteroidetes ratio has been observed in obese individuals following weight loss.[89]

The controversy over the role of Firmicutes and LGG occurs when looking at supplementation. Studies would suggest

that LGG has little effect on weight gain, as two trials of LGG with differing gut bacteria species have shown differing results. One RCT reported no significant effect of a combination supplement containing 6.5×10^9 CFU/day LGG and *B. lactis* on the prevention of excessive gestational weight gain in 230 obese pregnant women (probiotics versus placebo, RR = 1.14, 95% CI: 0.99–1.31).[90] However, in contrast, one RCT showed that supplementation with a multispecies probiotic of *Bifidobacterium animalis* and LGG at 1×10^9 CFU/day in 411 obese and overweight pregnant women resulted in lower maternal weight gain compared with placebo.[91] It could be concluded from these two studies that the presence of LGG is having no effect on weight loss or gain, and it is the other species of gut bacteria that may be exerting its effects.

Underlying pathophysiology during obesity may also remain unaffected by LGG supplementation. One RCT sub-study of 26 healthy adults reported that LGG supplementation of 6.2×10^7 CFU/day for 3 weeks in a milk-based fruit drink resulted in changes in serum global lipid profiles, with decreased lysophosphatidylcholines ($P \leq 0.05$), sphingomyelins ($P \leq 0.001$) and several glycophosphatidylcholines ($P \leq 0.05$), which may be involved in the pathophysiology of atherosclerosis.[92,93,94] It should also be noted that triglycerides were increased and when the trial adjusted their statistics to allow multiple hypothesis analysis, no changes were observed in global lipidomic profiles.

The effect of LGG in weight loss remains controversial. Observational studies have outlined a negative effect of the Firmicutes phyla on weight loss, but supplemental trials of specific strains are not straightforward. As effects may be species dependent, it cannot be discounted that positive trials of supplementing multispecies probiotics containing LGG were due to a symbiotic effect of the two strains or due to the *Bifidobacterium* strain present in the supplement. It should be noted that caloric intake and exercise were not measured in these trials, which could affect outcomes.

Liver disease

The gut–liver axis is now a well-recognised relationship that is thought to interact through the mesenteric portal vein.[95] The pathological progression of non-alcoholic fatty liver disease (NAFLD) development is thought to involve inflammation and lipotoxicity.[96] Thus, targeting the gut–liver axis to treat NAFLD may be promising for the treatment of this multi-factorial disease.

The use of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as clinical markers of liver damage is well established. However, an understanding of liver disease is not limited to liver function, but also considers the presence of small intestinal bacterial overgrowth (SIBO) and intestinal permeability, as endotoxaemia may contribute to reduced life expectancy in individuals with liver cirrhosis.[97] The use of probiotics to improve SIBO is thought to occur from successful colonisation of the small intestine, which prevents microbial translocation.[98] However, in one RCT of 53 patients with chronic liver disease, successful colonisation of LGG and improved SIBO did not translate into improved intestinal permeability and liver function after 4 weeks.[18] This study used a multispecies probiotic of six different strains, including LGG, and reported increased LGG in faeces ($P \leq 0.001$) and improved SIBO ($P \leq 0.05$), but only marginally improved intestinal permeability and no changes to liver chemistry. Short treatment duration and the study population could be responsible for observations.

In comparison, longer studies on LGG monotherapy in children have reported improved liver chemistry. Compared with placebo, 8 weeks of LGG supplementation (1.2×10^{10} CFU) in 20 children with NAFLD has been associated with decreased ALT ($P \leq 0.03$) and anti-peptidoglycan-polysaccharide

antibodies (P = 0.03),[99] which are polymers from the cell wall of bacteria that may contribute to inflammation in certain chronic inflammatory diseases.[100] Differences in dosages between the studies may account for conflicting results; however, the dosage was not disclosed in the previous study. The previous study may also have been too short to observe differences, or the use of a multispecies probiotic may be masking the effects of LGG. The small number of study participants in the monotherapy study may have also been giving a false-positive.

The research surrounding the use of LGG for the improvement of liver function and SIBO is still in its infancy. The use of LGG for the improvement of liver function in paediatric liver disease is promising at a dose of at least 1.2×10^{10} CFU/day. In adults, more large-scale RCTs are required, as there is yet no compelling evidence for LGG efficacy in NAFLD, despite promising results for improving SIBO when given in combination with other species. More research is also required in this area, especially with regards to those with severe disease, as the use of probiotics in immunocompromised individuals has raised some concerns.[18]

Insulin resistance and type 2 diabetes

Gut dysbiosis has been linked to insulin resistance (IR) and type 2 diabetes (T2D). In contrast to studies on individuals with obesity, individuals with T2D show compositional changes of the intestinal microbiota, which include decreased Firmicutes, resulting in an increased Bacteroidetes to Firmicutes ratio within the intestinal microbiota.[101] Despite this, studies on individuals with T2D have reported increased total *Lactobacillus* anaerobes, with pronounced levels of *L. reuteri* and *Lactobacillus plantarum*;[102] however, the role of native LGG in those with T2D is unclear.

As previously discussed, gut dysbiosis and the development of intestinal permeability may lead to endotoxaemia and systemic inflammation, and this may contribute to the pathogenesis of IR and T2D. The effects of probiotics in T2D may be through the production of glutathione, decreasing inflammation and oxidative stress. Supplementation of a multispecies probiotic containing LGG for 8 weeks in 54 individuals with T2D was shown in one trial to prevent a rise in fasting plasma glucose, decrease blood markers of inflammation (−777.57 ng/ml versus +878.72 ng/ml, P = 0.02) and increase the antioxidant glutathione compared with placebo (240.63 µmol/l versus −33.46 µmol/l, P = 0.03).[103] Measures of IR were increased in both groups; however, less so in the probiotic group (+2.38 versus +0.78, P = 0.03).

As with many studies in multispecies probiotics, it is important to understand the role of LGG to rule out influences from other species. Studies on streptozocin-induced diabetic rats reported improved glucose tolerance and IR after 4 weeks consumption of LGG.[104] However, animal studies on LGG dominate, and trials in humans with T2D are lacking. Amongst 200 healthy individuals, LGG supplementation of 1×10^9 CFU for 90 days was shown in one trial to help maintain glycaemic control.[105] Compared with placebo, where glycated haemoglobin (HbA1c) increased over the 90 days, the LGG-supplemented group reported sustained HbA1c levels (P = 0.005 between-group comparison), indicating possible attenuation of T2D development in healthy adults.

Results from the study above indicate that supplementation of LGG may be of benefit in slowing the development of T2D, an effect that was also observed in gestational diabetes.[106] In this trial of 256 pregnant women with normal glycaemic levels, those supplemented with multispecies LGG + *B. lactis* in combination with dietary advice reported improved blood glucose control during pregnancy and a reduced risk of elevated glucose concentration compared with placebo [odds ratio (OR) 0.31,

95% CI: 0.12, 0.78, $P = 0.028$]. These effects were sustained 12 months post-partum; however, dosages were not stated in the trial and the role of a single-strain LGG probiotic is unclear. Moreover, a potential effect of the dietary changes cannot be discounted. In contrast, a multispecies probiotic of LGG + *B. animalis* (1×10^9 CFU/day) discussed previously in this review failed to prevent gestational diabetes.[91] Taken in tandem, these studies would suggest that effects on glycaemic control in pregnancy could be dependent upon dietary changes.

The use of 1×10^9 CFU LGG as monotherapy or as part of a multispecies probiotic to slow the pathophysiological continuum from IR to T2D in healthy individuals may be of benefit. The studies on gestational diabetes mellitus (GDM) would suggest little effect of LGG, and that dietary changes were the driving factor.

Cystic fibrosis

Intestinal inflammation is a predominant feature in adults and children with cystic fibrosis (CF), with levels similar to individuals with IBD.[107,108] Improvements to intestinal inflammation in patients with CF have been reported following probiotics[109] and LGG supplementation as a monotherapy. The restoration of disrupted intestinal microbiota and improvements to intestinal inflammation in children with CF has been reported following supplementation with LGG.[108,110] In one RCT, supplementation of 6×10^9 CFU/day LGG resulted in reduced faecal calprotectin (CLP), which is indicative of intestinal inflammation, in the GI tract of 22 children with CF (184 ± 146 µg/g versus 52 ± 46 µg/g, $P \leq 0.01$).[110] Correlations between reduced microbial richness and intestinal inflammation were also observed in this trial ($r = 0.53$, $P = 0.018$).

Although inflammation may be improved, the effects of LGG supplementation on pulmonary exacerbations and hospital stays remain controversial. LGG supplementation of 6×10^9 CFU/day reduced pulmonary exacerbations and hospital admissions in one 6-month RCT of 43 children with CF; however, the duration of stay did not differ between the groups.[111] In a more recent, larger RCT in 95 children, LGG supplementation (6×10^9 CFU/day) failed to show any effect on hospitalisations (OR 1.67, 95% CI: 0.75–3.72, $P = 0.211$) or exacerbations (OR 0.83, 95% CI: 0.38–1.82, $P = 0.643$).[112] In contrast to the previous trial, this trial of 95 children with CF ran for 12 months. Study design and duration may account for differences, with parallel studies enabling comparisons between treatments at the same time amongst differing individuals, whereas crossover studies negate the effects of between-patient variability. In this instance, the crossover study could eliminate differences between the participants, such as severity of disease or dietary and lifestyle differences.

The data for the use of LGG in CF mainly revolve around studies in children. Supplementation with 6×10^9 CFU LGG may reduce intestinal inflammation and restore gut microbiota eubiosis; however, the exact effects on pulmonary exacerbations remain unclear.

Respiratory tract infections

Removal of the adenoids has been associated with increased long-term risk for respiratory tract infections (RTIs),[113] indicating their possible involvement in the body's immune defence against respiratory diseases. Although largely considered a member of the gut microbiota, the importance of LGG for the immune system may be apparent from its presence in large amounts in the adenoids of children, which has been shown to increase following 3 weeks of supplementation.[114]

However, its role in the prevention of RTIs and its ability to reduce symptoms and duration of illness remain controversial. The use of an LGG supplement (1×10^9 CFU/day) for 6 weeks in 59 adults artificially infected with human rhinovirus failed to affect viral load when compared with placebo.[115] Furthermore, one study failed to show benefits to severity of cold symptoms of a live LGG supplement (1×10^9 CFU/day)

compared with an inactivated form and placebo in 60 adults after 6 weeks.[116] Although this study did indicate a trend towards lower occurrence and severity of cold symptoms in the active LGG group, this was not significant.

Studies in younger adults and children have shown more beneficial outcomes of LGG supplementation in RTIs. Amongst 231 healthy college students, the use of LGG (1×10^9 CFU/day) in combination with *B. animalis* for 12 weeks was shown to improve the severity and duration of upper RTIs (URTIs), with a 2-day shorter average infection, leading to fewer school days missed compared with placebo ($P = 0.002$).[117] As this study was in combination with *B. animalis*, it is difficult to determine the exact effect of LGG; however, the combination therapy was shown to be of benefit.

Over-the-counter RTI medications in children under 6 years old are often avoided and discouraged due to concerns with safety and efficacy,[118] indicating a need for alternative therapies in this cohort. Children attending day care facilities are particularly susceptible to RTIs due to factors such as increased exposure to infections[119] and cessation of breastfeeding,[120] which contributes to a number of childcare and workdays lost.[121] Therefore, the use of probiotics may be of benefit. Strain-specific effects have been highlighted, with LGG reducing the duration of RTIs in children at day care, which other strains failed to do in a systematic review and meta-analysis of 15 RCTs with 5121 children (MD 0.78 days, 95% CI: −1.46 to 0.09).[122] Interestingly, this meta-analysis reported no effect on incidence, antibiotic use or days missed from day care, which differs to an earlier systematic review and meta-analysis of four RCTs in 1805 children, which reported reduced risk of URTIs (RR = 0.62, 95% CI: 0.50–0.78, NNT = 4) and antibiotic use (RR = 0.80, 95% CI: 0.71–0.91)[123] with LGG supplementation. Differences between the results may be due to a lack of recent data supporting LGG supplementation, or could be owing to differing trial designs and outcome measures.

In high-risk children, the supplementation of prebiotics and probiotics may reduce the risk of RTIs and rhinovirus infections. In one study of 94 preterm infants, supplementation with LGG (1×10^9 CFU/day for the first 30 days, and 2×10^9 CFU/day for a further 30 days) reduced the incidence of RTIs (RR = 0.50, 95% CI: 0.28–0.90, $P = 0.022$) and rhinovirus infections (RR = 0.49, 95% CI: 0.24–1.00, $P = 0.051$).[124] In a second study, LGG supplementation (1×10^9 CFU/day) in 742 hospitalised children reduced the risk of RTIs compared with placebo, but failed to impact hospitalisation duration.[125]

The apparent lack of efficacy of LGG in adults for the prevention of RTIs may be due to suboptimal dosages, as the trials above largely used dosages like those used in the studies on children. Dosage–response studies are warranted to investigate this. Studies on children have shown positive results for the use of LGG in reducing the duration of RTIs in those attending day care. In high-risk children, LGG may reduce the occurrence of rhinovirus infections when supplemented with at least 1×10^9 CFU/day for at least 2 months.

Otitis media

Usually considered an extension of an URTI in children, otitis media is a spectrum of diseases characterised by middle ear inflammation resulting in pain, irritability and fever.[126] As previously discussed, the presence of LGG in tonsil and adenoid tissue is indicative of its role in the immune system and RTIs,[114] but there are limited data on its role in otitis media. LGG has been detected in the middle ear following supplementation, but LGG may already be present in the middle ear of children with otitis media and, compared with placebo, LGG supplementation may have limited effects on children undergoing tympanostomy, which is a procedure to prevent fluid build-up in the middle ear.[127] In one study of 309 children prone to otitis, a multispecies probiotic containing LGG supplemented ($8–9 \times 10^9$ CFU/day) for 24 weeks failed to reduce pathogenic bacteria in the nasopharynx, or reduce

frequency and occurrence of acute otitis media.[128] In contrast, a multispecies probiotic containing LGG supplemented for 6 months reduced the presence of human bocavirus (HBoV), which is the primary pathogen in otitis media, in 269 children prone to otitis (6.4% versus 19.0%, OR 0.25, 95% CI: 0.07–0.94, P = 0.039); however, the effects on symptoms and otitis media were not discussed.[129]

The presence of LGG in the middle ear may be indicative of its role in infection prevention; however, there are limited data regarding the use of LGG for the management of otitis media in children and adults. Although studies are positive for the use of LGG as part of a multispecies probiotic to reduce the presence of the virus, more studies are warranted on LGG in isolation.

Anxiety and depression

Anxiety and disordered gut function often coexist, with symptoms of nausea and stomach pain reported by individuals with elevated anxiety.[130] The gut microbiota may communicate in a bi-directional manner with the brain along the gut–brain axis in a number of different ways, such as signalling through metabolites, through the enteric nervous system and the neural-immune system.[131] Under physiological conditions, neurotransmitters such as γ-aminobutyric acid (GABA) may be synthesised and released from *Lactobacillus*.[132] However, under pathophysiological conditions, inflammatory cytokines produced in the gut may also have the capacity to affect the brain and stimulate the release of cortisol, dysregulating the hypothalamic–pituitary–adrenal (HPA) axis leading to initiation of the stress response.[133] Furthermore, gut dysbiosis has been implicated in mental health disorders.[134] Therefore, modification of the gut microbiota using probiotics may hypothetically provide a novel treatment target for conditions such as anxiety and depression.

Studies on probiotics in anxiety and depression are extensive; however, they may not be translatable to the use of LGG in isolation. The inclusion of LGG in a multispecies probiotic supplemented for 6 weeks in 70 petrochemical workers showed improvements from baseline in general health (16.9 ± 1.8 versus 9.8 ± 1.9, P = 0.001) and depression and anxiety (18.9 ± 3.2 versus 9.4 ± 4.0, P = 0.006), and no effects were seen in the placebo group, or in any of the groups on the HPA axis, as measured by the General Health Questionnaire and the Depression Anxiety and Stress Scale.[135] This indicates that effects may be independent of the HPA axis, or that the short study period may have been insufficient for effects on symptoms to be observed.

The use of LGG as a monotherapy may have a beneficial effect on depression in individuals following the occurrence of myocardial infarction (MI). Supplementation with LGG (1.6×10^9 CFU/day) had beneficial effects on depression, oxidative stress and inflammation in individuals post-MI who had undergone percutaneous intervention (PCI).[136] This study of 44 individuals reported that, compared with placebo, 12 weeks of supplementation with LGG decreased symptoms of depression (−5.57 versus −0.51, P = 0.045) and increased QoL (23.6 versus 0.44, P = 0.023). Biomarkers for inflammation and oxidative stress were also decreased in the supplementation group compared with placebo. Low-grade inflammation may contribute to the development of depression,[137] and the mechanism of action of LGG in depression and inflammatory diseases may be through its immunomodulatory properties.

During pregnancy, physical and psychological changes can occur leading to stress and adverse outcomes in the baby.[138] Pregnancy with obesity may increase the risk for the development of depression and anxiety compared with entering pregnancy at a normal weight,[139,140,141] and as probiotics are considered safe during pregnancy,[142] they may hypothetically provide a treatment option. However, in practice, clinical trials on

a combination of LGG + *B. lactis* failed to improve the mental health of 230 obese pregnant women at 36 weeks of gestation.[143] There were no differences between depression scores, and anxiety and physical wellbeing worsened with time.

Studies on the use of LGG in isolation in depression and anxiety are limited, and although plausible mechanisms for its use exist, further studies are warranted in this cohort of individuals. The use of LGG (1.6×10^9 CFU/day) for 12 weeks for the development of depression and anxiety in individuals who are post-MI is promising.

Attention-deficit hyperactivity disorder and Asperger's syndrome

Attention-deficit hyperactivity disorder (ADHD) is a childhood neurodevelopmental disorder that is present in as many as 3% of children and predominantly in boys.[144] Symptoms such as inattention and hyperactivity are hallmark symptoms of ADHD, which are frequently observed in children with Asperger's syndrome (AS) alongside disordered emotional behaviour.[145] As previously discussed, LGG may affect emotional behaviour via the vagus nerve and regulation of GABA in the amygdala and hippocampus areas of the brain,[146] which may also be involved in the pathophysiology of mental health disorders.[147]

Animal studies have indicated a possible benefit to learning and memory following LGG supplementation;[148] however, in humans the life stage at which LGG supplementation occurs may be important for favourable outcomes. Pre-natal and post-natal factors have been implicated as risks for ADHD and AS, and intervention with LGG at both stages may impact development later in childhood. Compared with placebo, LGG supplementation (1×10^9 CFU/day) 4 weeks prior to and 6 months after birth reduced the risk of development of ADHD and AS in 75 children, 13 years later (17.1% versus 0%, $P = 0.008$).[149]

Studies on the use of LGG in ADHD and AS are limited; however, what does exist is promising for the use of LGG (1×10^9 CFU) during late pregnancy and infancy to reduce the risk of development. With such strong mechanistic links between neurodevelopmental disorders and the use of LGG, studies are warranted to further investigate possible clinical benefits.

Urinary tract infections

UTIs are a commonly occurring condition, which are ordinarily treated with the use of antibiotics.[150] However, research would suggest that this practise may be detrimental, due to the development of multi-drug-resistant bacteria.[151] *Escherichia coli* originating from the gut microbiota is thought to be the cause in the majority of cases, and in women it may colonise the vagina, transfer to the urethral opening and ascend to the bladder.[152]

Experiments in murine models have shown that the LGG-derived effector protein, HM0539, can competitively inhibit the adhesion of *E. coli* in the GI tract.[153] However, one pilot trial of 42 post-menopausal women indicated that although the GI tract is readily colonised by LGG, vaginal swabs show poor adhesion, with only 9.5% of women having colonisation in this area.[154] Possibly owing to this, clinical trials on the use of probiotics for the prevention of recurrent UTIs have shown mixed results, with vaginal colonies recurrently transferring to the urethral opening. The use of *Lactobacillus spp.* was shown in one systematic review and meta-analysis of nine clinical trials to reduce the risk of recurrent UTIs in females (RR = 0.684, 95% CI: 0.438–0.929, $P \leq 0.001$); however, different strains showed varying efficacy and LGG was not analysed.[152] When administered as a monotherapy, the regular consumption of cranberry juice, but not LGG, was determined to prevent *E. coli*-derived recurrent UTIs in one 12-month RCT of 150 women.[155] In this trial, 39% of women in the LGG group reported recurrent UTIs compared with 16% consuming cranberry juice and 20% of control. Poor colonisation of the periurethral area and consumption only

five times per week were determined to be the possible reasons for lack of efficacy. In contrast, a multispecies probiotic containing LGG in 181 children has been reported to be effective at reducing the risk of recurrent UTIs compared with placebo ($P = 0.02$); however, in individuals who did have a recurrent event, those on probiotics had a shorter duration to recurrence (3.5 months probiotic versus 6.5 months placebo, $P = 0.04$).[156] As this study looked at multispecies probiotics, it is difficult to determine the role of LGG monotherapy in this cohort.

Amongst individuals who have had a spinal cord injury, the risk of recurrent UTIs may be higher due to physiological alterations in the urogenital system.[157,158] The daily use of LGG in combination with *Bifidobacterium* BB12 (7×10^9 CFU) in a 6-month RCT failed to show efficacy in preventing UTIs in 207 people with spinal cord injury compared with placebo.[159] It would appear that only through intravesical administration does LGG improve symptoms of UTIs.[160]

There appears to be little benefit to women in the use of LGG for the prevention of recurrent UTIs possibly due to poor colonisation in the vaginal area in the absence of the pili that aid adhesion in the GI tract, and continual transfer to the urinary tract.[16] Amongst individuals with recurrent UTIs due to spinal cord injury, the use of LGG may only be of benefit to symptoms through intravesical administration. More studies are required in children to determine the role of LGG as a monotherapy, as its inclusion in a multispecies probiotic is promising for the prevention of UTIs.

Infant health

The gut microbiome begins to develop immediately after birth, and can be determined by mode of delivery and feeding.[161,162] Infants born vaginally are typically colonised with beneficial bacteria from the mother's vaginal canal, and those born through Caesarean-section (C-section), from the mother's skin.[163] Individuals born via C-section may have a higher risk of developing several metabolic and immune disorders later in life,[164] possibly due to a lack of *Escherichia-Shigella* and *Bacteroides* species, and lower bacterial richness and diversity.[165]

Colonisation of pathogenic bacteria early in life has been shown to contribute to poorer health outcomes later in life.[166] However, despite the ability of LGG to competitively inhibit pathogenic bacteria and act as an antimicrobial in adults, results have been mixed in children, with one RCT reporting no effect of 42 weeks of LGG supplementation on the colonisation of *Staphylococci* in 60 pre-term infants, despite rapid colonisation of LGG.[167] In addition, no effects were seen on growth rate or length of hospitalisation in this trial. The analysis of only one pathogenic bacteria may not be sufficient, and other strains may need to be analysed to understand the exact effects. In contrast, benefits to height and weight of babies at 12 months have been observed following *in utero* LGG supplementation.[168] In this RCT, 208 healthy pregnant women were given LGG (7×10^8 CFU/day) in combination with *B. lactis* (7×10^8 CFU/day), resulting in increased baby weight and height at 12 months compared with placebo. Furthermore, one 6-month RCT of 120 healthy infants fed with LGG (dosage not stated) in supplemented formula have reported better LGG colonisation (91% versus 76%, $P \leq 0.05$), which led to better growth compared with formula milk without LGG. Higher than normal defecation was reported in the LGG-supplemented group; however, this was not considered to be diarrhoea or detrimental to health.[169] Differing trial durations, stages of supplementation and follow-up times could be responsible for differences between the outcomes, with at least 6 months of treatment required to show benefits.

Observational studies have indicated that maternal nutrition and the *in utero* environment may also increase the risk of offspring having poor health outcomes,[170] indicating an area where probiotic supplementation may be of benefit. Better health outcomes of mothers and babies have been reported in one 2-year

follow-up of a RCT, concluding that pre-natal multispecies probiotic use may be a safe and cost-effective way of preventing metabolic disease in offspring.[171] In this study, the use of LGG and *B. lactis* (1×10^{10} CFU/day) in combination with dietary advice in 256 pregnant women from the first trimester to cessation of breastfeeding reduced the frequency of GDM compared with dietary advice alone ($P \leq 0.003$). In those who did develop GDM, smaller birth weight of babies was observed. With birth size being a risk factor for obesity in later life, LGG supplementation may have an impact *in utero* on later development of non-communicable diseases.

Supplementing LGG *in utero* or during infancy for improved outcomes at all stages of life is apparent. Although supplementation for improved growth rates in babies is controversial, the results would suggest that 6 months of supplementation either starting *in utero* or during infancy may be required at doses of at least 7×10^8 CFU/day, and potentially in combination with *B. lactis*. Supplementing LGG (1×10^9 CFU) in combination with *B. lactis* (1×10^9 CFU) during pregnancy may have benefits to both the mother and child in preventing the development of GDM and non-communicable diseases in later life.

Infantile colic

Although not life threatening, the impact of a child with colic extends to parental distress, anxiety and depression, and may be associated with the development of disorders such as allergic disease, migraine and GI disorders later in life.[172] Thus, the reduction in the time a child with colic spends crying may have huge neuropsychological implications. One recent RCT of 45 colicky breastfed infants showed that a high dose of LGG (5×10^9 CFU/day) in combination with elimination of cow's milk from the mother's diet reduced crying time and GI inflammation, with no adverse events reported even at this high dose.[173] However, a lower-dose LGG supplementation (1×10^9 CFU/day) for 6 months in an RCT of 184 infants was shown not to prevent colic based on symptoms or physician's diagnosis when compared with control.[174] This finding was supported in an earlier pilot study of 17 breastfed infants given LGG (4.5×10^9 CFU/day) in combination with behavioural support and cow's milk elimination by the mother.[172] LGG supplementation did not affect crying time or GI inflammation, but crying occurrences were decreased. Differing dosages used in the trials above may account for discrepancies between the results, with a higher dosage being more successful. Elimination of cow's milk may also account for discrepancies.

LGG when included as part of a multispecies regimen has shown more consistent success. When included as part of a nine-strain multispecies synbiotic, a recent RCT of 4 weeks in 17 breastfed infants reported efficacy, with a decrease in the number of crying days and average crying duration when compared with Simethicone, which is used to relieve gas and GI discomfort.[175] Although this trial was small, these findings were also supported in a larger, earlier RCT of 50 breastfed infants with higher treatment success and higher symptom resolution when given a seven-species synbiotic containing LGG (1×10^9 CFU/day).[176]

The use of high-dose LGG supplementation as monotherapy (5×10^9 CFU/day) in combination with cow's milk elimination has been shown to be efficacious in colic to reduce crying time and GI inflammation. Furthermore, when included as part of a multispecies synbiotic regimen, LGG may be of benefit to infants with colic to improve symptoms and crying. However, there are no studies to date showing efficacy of the use of LGG as a monotherapy.

Human immunodeficiency virus

As with colorectal cancer, the success of treatments for human immunodeficiency virus (HIV) is often dependent upon their tolerability. Diarrhoea is a common side-effect of anti-retroviral treatments[177] and, in addition, patients

who are immunocompromised may be at a higher risk of microbe-associated diarrhoea.[178] However, unlike patients with colorectal cancer, 17 patients infected with HIV who had suffered from diarrhoea for more than 1 month showed little improvement to diarrhoea or GI symptoms following LGG supplementation twice daily (1–5 × 10^{10} CFU) for 2 weeks compared with placebo.[179] There were no differences in faecal counts of LGG between the two treatments, indicating poor colonisation following supplementation.

Previous trials have shown lower faecal *Lactobacillus* cultures in patients infected with HIV compared with healthy individuals,[180] indicating a possible need for increased dosages. Further trials are warranted, as the use of LGG in other cohorts of patients for the prevention and treatment of diarrhoea has been of benefit.

Acne

The pathogenesis of acne involves several factors, including inflammation and alterations of insulin signalling.[181,182] Systemic supplementation of probiotics to improve the insulin signalling pathway has been discussed previously, and hypothetically LGG could be used to improve acne. Improvements to the expression of genes involved in the insulin signalling pathway of individuals with acne have been reported with supplementation of *L. rhamnosus* SP1 (3 × 10^9 CFU/day) for 12 weeks.[183] Subjects (n = 10) in the supplementary arm reported reductions in IGF-1 gene expression ($P \leq 0.001$) and increased FOXO1 gene expression ($P \leq 0.001$) from baseline, with no changes observed in the placebo arm (n = 10). This resulted in physician-rated improvements to skin appearance of acne in the *L. rhamnosus* SP1 group compared with the placebo group (OR 28.4, 95% CI: 2.2–411.1, $P \leq 0.05$). Although the study states that the strain used is also known as LGG, there is very little research to confirm this; however, based on the previous research on LGG and IR, it would appear it may have similar actions. It should also be noted that the study was only completed in Caucasian subjects, and translatability into the skin of other races is unknown. In addition, small sample sizes and the pilot nature of the study warrant further research.

Based on a single, small pilot study, the use of *L. rhamnosus* SP1 (3 × 10^9 CFU/day) for at least 12 weeks for the improvement of acne through modulation of the insulin signalling pathway is promising; however, more research is needed in larger higher-powered studies to confirm effects. Studies on the genetic sequencing of *L. rhamnosus* SP1 and its relationship to LGG or further research on LGG as the test probiotic are also warranted.

Allergy

Allergy development is thought to involve both genetic and environmental factors.[184,185] Dysbiosis and reduced diversity of the infant gut microbiome are thought to be included in the pathogenesis of allergic disease in children, due to factors such as antibiotic use *in utero* and birth by C-section.[186] However, effects may be ameliorated using probiotics. One RCT on the use of a multispecies probiotic containing LGG (5 × 10^9 CFU/day) during pregnancy, breastfeeding and infancy, reported altered effects of antibiotics and C-section birth on gut dysbiosis, increasing *Bifidobacteria* and reducing pathogenic *Proteobacteria* and *Clostridia*,[186] indicating that the use of probiotic supplementation during infancy may help to restore eubiosis.

Observational studies have indicated that the involvement of *Lactobacillus* species may be of particular importance in the development of allergies. The presence of LGG, *Lactobacillus casei* and *Lactobacillus paracasei* early in life is associated with lower prevalence of allergic disease in childhood, and there may be a lower presence of *Lactobacillus* in children with a genetic predisposition, due to one or more parent having allergic disease.[187,188]

The use of LGG supplementation to decrease the risk of allergy development has also been studied. Benefits to the prevalence of allergic

disease later in life were apparent in a follow-up of patients from four separate RCTs on 303 pre-term children given different strains of probiotic.[189] Children who were given LGG perinatally had a decreased prevalence of allergic disease compared with children given placebo at the 2-year follow-up (OR 0.62, 95% CI: 0.38–0.99, P = 0.047). Treatment durations from the four included trials ranged from 3 to 6 months, and dosages from 1×10^9 to 5×10^{10} CFU.

In children who have already developed an allergy such as cow's milk allergy (CMA), LGG supplementation may also be of benefit. One recent systematic review and meta-analysis of 10 studies on LGG (1.4×10^7–5×10^9 CFU/day) reported that supplementation may aid recovery from GI symptoms, promote tolerance to the allergen and improve faecal blood.[190] Evidence was rated as low-to-moderate quality, due to issues with blinding, concealment and unclear data; however, studies were RCTs. Tolerance acquisition following LGG supplementation in infants with IGE-mediated CMA may be due to its ability to influence gut microbiota structure, enabling colonisation of *Oscillospira*.[191] The modulation of epigenetic mechanisms involved in the immune system and pathogenesis of CMA may also occur following LGG supplementation, resulting in increased tolerance to cow's milk.[192]

Immunomodulation by LGG has also been observed in adults with birch pollen allergy and oral allergy syndrome.[193] This RCT of 38 patients received LGG (2×10^{10} CFU/day) for 5.5 months starting 2.5 months prior to allergy season resulting in increased allergen-specific immunoglobulin (Ig)A levels compared with baseline, effects that were not seen with placebo. This may be of benefit to symptoms, as IgA acts to prevent infections and maintain gut microbiota homeostasis, which if disrupted has been associated with an elevated risk of allergies in children.[184] A second RCT on the effects of LGG supplementation (3×10^8 CFU/day) for 3 months on allergy in 141 marathon runners reported no effect on the immune marker IgE or several other allergic inflammatory markers, compared with placebo.[194] Suboptimal dosages could be responsible for the lack of immunomodulatory effects in this trial, or the fact that the trial only looked at the inflammation-associated IgE and not the anti-inflammatory IgA. Although no symptom relief was observed in these trials, it is indicative of further immune effects in adults on IgA.

There is extensive clinical research on the efficacy and mechanisms behind the use of LGG to prevent allergic diseases and improve symptoms of CMA in children. Children at a high risk of developing allergic disease due to genetic predisposition, antibiotic use or C-section birth may benefit from at least 1 × 109–5×10^9 CFU/day for at least 3–6 months. Children with existing CMA may benefit from 1.4 × 107–5×10^9 CFU/day for at least 4 weeks and up to 3 years. Dosages of 2×10^{10} CFU LGG may be of benefit to adults with birch pollen allergy for immunomodulation and the promotion of IgA. However, further studies are warranted to determine the significance of immunomodulation, as without effects on symptoms, supplementation may be pointless.

Dermatitis and eczema

Atopic dermatitis (ADe) is the most common chronic skin condition, affecting up to 20% of children and 3% of adults worldwide.[195] Pathophysiology of ADe is not fully understood; however, dysbiosis may be involved, as individuals with ADe have lower diversity and levels of *Bifidobacterium* and *Actinobacteria*, and higher *Staphylococcus* than healthy subjects.[196,197] Furthermore, studies indicate that like other atopic diseases, gut dysbiosis may contribute to ADe development through immunomodulation.[197]

The effects of LGG supplementation on the immune system, as seen in patients with allergic disease, indicate a potential for its use in individuals with ADe. An early systematic review and meta-analysis of five RCTs with 889 subjects concluded that LGG was ineffective for the primary prevention of eczema in children, when given both prenatally and postnatally.[198] Dosages

ranged from 1×10^9 to 1.8×10^9 CFU per day, and the quality of data was good.

When looking at reduction of symptoms, recent RCTs not included in the above meta-analysis have shown differing results. Intrinsic microbiota at early infancy may affect outcomes, and infants with ADe who have higher levels of *Bifidobacterium dentium* have been shown to not respond to probiotic intervention, compared with those without disease.[199] One RCT of 67 children with ADe concluded that LGG as the supplement ComProbi (350 mg) in combination with corticosteroid use was effective at decreasing symptoms of ADe after 8 weeks compared with placebo and corticosteroids ($P = 0.014$), based on Scoring of Atopic Dermatitis (SCORAD).[200] However, it is difficult to determine that effects were due to LGG because corticosteroids were also being used. In a second RCT in 102 infants aged 3–12 months with ADe where corticosteroids were not used as the treatment, but were not precluded during the trial if individuals wanted to use them, no therapeutic effect of LGG based on SCORAD compared with placebo after 12 weeks was reported.[201] Results from these two trials would suggest that corticosteroids and not LGG may account for the favourable outcomes.

As part of a multispecies therapy, LGG has shown more consistent results. When combined, LGG and *B. animalis* were shown in one systematic review and meta-analysis of 21 RCTs on various multispecies combinations to reduce the risk of ADe compared with placebo when administered *in utero* and during infancy.[202] Furthermore, in a recent RCT of 290 children not included in the previous meta-analysis, the administration of LGG + *B. animalis* (1×10^9 CFU/day) in late infancy for 6 months prevented the development of eczema,[203] indicating that the use of LGG as part of a multispecies regime with *B. animalis* may be of benefit for the prevention of ADe and eczema.

The use of LGG for the primary prevention of ADe and eczema may be of benefit when used as part of a multispecies regime in combination with *B. animalis*, at a dose of 1×10^9 CFU/day, for at least 6 months. While there is yet no strong evidence for LGG alone, stratification of patients with ADe according to intrinsic microbiota may be of benefit for the improvement of symptoms following LGG use; however, more studies are required. The role of LGG in combination with corticosteroids also warrants more research.

Wounds

The role of skin microbiota in wound healing is well documented, with both skin barrier function and the immune response reported to be microbially mediated.[204] Topical application of probiotics for the treatment of burns has shown positive results;[205,206] however, oral probiotic supplementation lacks research. It has been hypothesised that the gut microbiota communicates with the skin microbiota in a bi-directional manner through the gut–skin axis, evidenced by cutaneous manifestations following GI disorders.[207] Oral LGG supplementation may have the potential to help treat certain skin diseases such as ADe and acne as documented above, therefore there may be potential for it to be of benefit to wound healing. The reduction of infections at incision sites in patients with cancer, detailed previously,[3] indicates a benefit of LGG supplementation as part of a multispecies probiotic to aid postoperative healing. However, research on the effects in 20 burn victims found only a modest, non-significant improvement in the time taken to complete wound healing, and no improvements to other clinical outcomes.[208]

There is no evidence for the use of LGG in combination with other probiotic strains for improvements to postoperative wounds. Research is lacking on monotherapy, and has found little effect on healing time in burn victims.

Dental caries

The presence of *Streptococcus* and *Lactobacillus* spp. in the oral cavity has been associated with the presence and onset of dental decay.[209] However, as previously discussed, LGG may have species-specific properties and produce an inhibitory microcin-like substance, which

has the ability to inhibit bacteria such as *Streptococcus*.[32] *In vitro* studies have indicated that the consumption of an LGG probiotic may also be able to colonise the oral cavity and inhibit *Streptococcus sobrinus*.[31,210] This may translate into a reduction in the risk of the development of caries. In one RCT, 594 children aged 1–6 years were given milk containing LGG ($5–10 \times 10^5$ CFU/ml) 5 days a week for 7 months, and showed a reduced risk of the development of dental caries (OR 0.56, 95% CI: 0.36–0.88, $P = 0.01$), based upon *Streptococcus* levels from dental plaque and saliva, and the presence of dental caries.[211]

Colonisation of the oral cavity may be affected by a lack of pili expression.[16] There is only one trial on the use of LGG for the prevention of dental caries, as detailed above,[211] and more studies are warranted given the mechanistic data. However, the trial that does exist was in many individuals over a relatively long period of time. It may therefore be of benefit to reduce the risk of dental caries in children and young adults. Dosages of at least $5–10 \times 10^5$ CFU may be needed in children.

Vaccine adjuvant

The recent COVID-19 pandemic and ability of SARS-CoV-2 to mutate has highlighted a need to improve immune response following vaccination. Orally ingested LGG may modulate the immune system in response to bacteria and viruses involved in the development of diseases. Research in mice given oral *Lactobacillus* has reported enhanced innate immune response following influenza virus challenge, with increased influenza-specific IgG antibodies and greater protection. RCTs have indicated that LGG may be a useful adjuvant for the immune response following influenza vaccine. One RCT in 42 healthy adults reported increased protection to the H3N2 influenza strain whilst supplementing LGG (1×10^{10} CFU) and inulin for 28 days following vaccination.[212] However, in the same study, no differences in seroprotection to the H1N1 or B influenza strains were observed.

Individuals with type 1 diabetes (T1D) are at increased risk of infections,[213] and influenza vaccine is recommended; however, whether influenza vaccines are truly successful in this cohort is still being debated.[214] Adjuvants to increase the immunogenicity of the influenza vaccine may be important, and use of LGG (1×10^9 CFU) 3 months pre- and post-influenza vaccination in 64 paediatric patients with T1D reduced the inflammatory immune response associated with T1D, decreasing IL-17, IFN-γ, IL-6 and TNF-α, without affecting the seroprotective antibodies, which are needed for effective vaccination.[215] However, although antibody-mediated immunity remained unaffected in this trial, the mediation of the inflammatory response may be important for individuals who suffer from autoimmune diseases such as T1D.

Studies on different types of vaccinations and studies on LGG as an adjuvant to the polio, rotavirus, Hib, diphtheria and tetanus vaccinations have been completed with varying success. One RCT of 66 healthy males reported that the use of LGG (1×10^{10} CFU), as an adjuvant to the polio vaccine, nearly doubled the increase of polio-specific IgG antibodies and significantly increased IgA antibodies, compared with placebo.[216] A second RCT of 98 pregnant women given LGG (5×10^9 CFU) resulted in more frequent occurrence of higher Hib antibody concentrations following vaccination with Hib, diphtheria and tetanus in the offspring; however, IgG remained unaffected. In contrast, LGG supplementation (1×10^{10} CFU) marginally but not significantly improved rotavirus antibodies following vaccination in 620 infants.[217] This may correspond to the findings above regarding LGG competitively inhibiting and acting as an antimicrobial against rotavirus, which could prevent the body from becoming infected and building an enhanced immune response when the body is faced with the live rotavirus as part of a vaccine.

The use of LGG supplementation (at least 1×10^9 CFU) as part of a vaccine adjuvant has been shown to be of benefit to the success of the response of biomarkers to vaccines, but only following influenza H3N2, polio and Hib. Further research needs to be performed with other vaccinations to determine effects.

Safety

Probiotics belonging to the genus *Lactobacillus* and *Bifidobacterium* are generally regarded as safe (GRAS) by the United States Food and Drugs Administration (FDA).[218] However, some studies on *Lactobacillus* have reported bacteraemia in specific populations, primarily amongst immunocompromised paediatric patients.[219] In adults, incidences of bacteraemia-associated endocarditis, primarily in those with a structural heart defect, have also been reported.[220] A recent systematic review has indicated that LGG may increase the risk of complications in patients who are immunocompromised, who have critical illnesses, structural heart disease or who have a central venous catheter.[69] In pregnancy and lactation, a recent meta-analysis and systematic review concluded that probiotics are safe for use during pregnancy and lactation. Data from the trials included in this review showed that adverse events in pregnancy and lactation following LGG supplementation were minor, and one systematic review and meta-analysis has concluded that probiotic use is safe during pregnancy and lactation;[142] however, it would still be recommended to consult with a doctor prior to commencement.

Drug–nutrient interactions are very few, with minor warnings whilst on anti-diabetes drugs due to potential hypoglycaemia and moderate interactions whilst taking antibiotic drugs, as LGG efficacy may be reduced.[221]

Conclusion

The unique morphological features of LGG may ensure that it has some use as an oral supplement in the reduction of risk of developing ADHD and GDM, in the prevention of allergies and dental caries, for improving immune reactions following vaccines, and for the management of diarrhoea associated with cancer treatments and antibiotic use. Three ways in which it may do this are through immunomodulation, cell growth and proliferation, and as an antimicrobial, aiding it to promote eubiosis. This results in LGG acting to prevent disease development, help manage symptoms and improve underlying pathology. The presence of pili on the exterior aid its colonisation of the GI tract, and its lack of efficacy in disease areas such as UTIs may be due to a lack of expression of these features in certain areas of the body. Effects may be systemic if there is a pathway through which LGG or its products can travel, like the gut–brain axis. However, effects may also be localised and specific, if a transmission pathway does not exist, as seen with its success only in specific cancer types, and diarrhoea treatment in colorectal cancer but not HIV.

There are, however, limitations of this study, and the inability to address genetic variation amongst LGG is apparent. Genetic variants have been found within the LGG species resulting in variations that do not have the *spaCBA* gene.[222] These variants may lack the ability to express the pili-like projections responsible for many of the physiological effects attributed to LGG. It is therefore difficult to exclude the possibility that positive or negative results were not attributable to within-strain differences. Until the research is performed, it is difficult for practitioners to determine which commercial products may have genetic variations. It may be that quality assurance legislation needs to be put in place; however, this has yet to be enacted. A second limitation is that this study could not account for individual intrinsic gut microbiota populations, which are highly personalised.[67] Therefore, although patients or disease areas may have been identified to benefit from LGG supplementation, differences between intrinsic gut microbiota may affect efficacy. Amongst the studies, issues with small sample sizes, contradictory results and the fact that the large majority of the research involved the use of multispecies supplements, and the use of other treatments and therapies amongst some of the research, means that conclusions need to be interpreted with caution.

Appendix

Cancer

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Armuzzi *et al.*[47] Italy RCT		LGG 6 × 10^9 CFU twice daily versus placebo, 14 days	60 healthy patients undergoing anti-*Helicobacter pylori* regimen	Side-effect profile Tolerability	LGG aided eradication, tolerability and overall side-effects Diarrhoea, nausea and taste disturbance all reduced by LGG (RR = 0.1, 95% CI: 0.1–0.9; RR = 0.3, 95% CI: 0.1–0.9; RR = 0.5, 95% CI: 0.2–0.9) Treatment tolerability higher in LGG (P = 0.04) No benefit to eradication rate	Not stated
Flesch *et al.*[3] Brazil RCT	To determine the effect of perioperative use of multispecies placebo + oligosaccharide in patients with colorectal cancer	LGG + *Lactobacillus acidophilus* + *Lactobacillus casei* + *Bifidobacterium* (all 1 × 10^8 –1 × 10^9 CFU/day) + fructo-oligosaccharide (6 g) versus placebo, 5 days pre-operative and 14 days postoperative	91 patients undergoing surgery for colorectal cancer	Infection occurring within 30 days of surgery	Perioperative administration of synbiotics reduced the occurrence of postoperative infections in patients with colorectal cancer Infection at incision site in one patient in synbiotic group and nine in the placebo group No infections in synbiotic group versus 7 in control group (P = 0.001)	Not stated
Lages *et al.*[48] Brazil RCT	To determine the postoperative outcomes of head and neck cancer surgical patients with multispecies probiotic + fructo-oligosaccharides	LGG + *Lactobacillus paracasei* + *Lactobacillus acidophilus* + *Bifidobacterium lactis* (all 6 × 10^9 CFU) + 6 g fructo-oligosaccharides versus placebo (duration not stated)	40 postoperative head and neck cancer patients	Intestinal function and permeability, number of total stool episodes, stool consistency and adverse GI symptoms	Synbiotics did not impact on postoperative outcomes or intestinal function of head and neck cancer surgery patients Postoperative complications similar in other groups (P > 0.05) Inflammatory markers similar in both groups ($P \geq 0.05$) Total daily stools similar ($P \geq 0.05$) and GI symptoms similar ($P \geq 0.05$)	Method to test intestinal permeability not optimal, as antibiotic use and ageing may impact its sensitivity Small sample size
Rafter *et al.*[40] Ireland RCT	To determine whether multispecies probiotic + prebiotic can reduce the risk of colon cancer	LGG + *B. lactis* Bb12 + inulin (SYN1 brand), 12 g sachet per day, 12 weeks	37 patients with colon cancer and 43 polypectomised patients	Not stated	Probiotics may alter several colorectal cancer biomarkers Probiotic changed *Bifidobacterium, Lactobacillus* and *Clostridium perfringens* Decreased level of DNA damage in polyp patients Increased IL-2 secretion prevented in polyp patients but not cancer Increased IFN-γ in patients with cancer but not polyp group	Limited biopsies
Roller *et al.*[41] Germany RCT	To determine the effect of daily intake of multispecies probiotic + prebiotic on immune function in patients with colon cancer	LGG and *B. lactis* (1 × 10^{10} CFU/day) + 10 g inulin versus placebo, 12 weeks compared with baseline	34 patients with colon cancer who had undergone curative resection and 40 polypectomised patients	Phagocytic and respiratory burst activity of neutrophils and monocytes, lytic activity of NKCs, transforming growth factor, prostaglandin E2 and inflammatory markers	Supplementation with multispecies probiotic had modest effects on the immune system of the two study groups IL-2 significantly increased in the cancer group (P < 0.05) between 0 weeks or 6 weeks and 12 weeks IFN-γ increased at 12 weeks ($P \leq 0.05$) No other immune factors affected	Limited biopsies

CFU, colony-forming units; CI, confidence interval; GI, gastrointestinal; IFN-γ, interferon-gamma; IL, interleukin; LGG, *Lactobacillus rhamnosus* GG; NKCs, natural killer cells; RCT, randomised-controlled trial; RR, relative risk.

Irritable bowel syndrome

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Francavilla *et al.*[4] Italy RCT	To determine whether LGG relieves symptoms in children with recurrent abdominal pain	16 weeks (8 weeks treatment, 8 weeks follow-up) 6×10^9 CFU/day	141 children with IBS or functional pain	Change in abdominal pain according to VAS score	LGG but not placebo reduced frequency and severity of abdominal pain from baseline Effects may be due to improvement of gut barrier	Effect may not be unique to LGG Did not assess gut microbiota at baseline or end Cannot exclude possibility that effect is short-lived
Horvath *et al.*[58] Poland Meta-analysis	To assess the effect of LGG for treating abdominal pain-related functional GI disorders in children compared with no treatment or placebo		3 RCTs, 290 children with abdominal pain-related functional GI disorders	Study 1: change in abdominal pain score Study 2: VAS Study 3: Faces pain scale	Beneficial effect of LGG in IBS Intensity and frequency of pain significantly reduced	Did not perform a statistical test for publication bias
Lyra *et al.*[54] Finland RCT	To determine if a multispecies probiotic can affect IBS-associated microbiota alterations	6 months LGG + *Lactobacillus rhamnosus* Lc705, *Propionibacterium freudenreichii* spp. *shermanii* JS and *Bifidobacterium* Bb99 $8–9 \times 10^9$ CFU/day	42 patients with IBS	Changes in faecal microbial composition	Multispecies probiotic altered IBS-associated microbiota quantities of the bacterial 16S rDNA phylotypes, to those reflective of IBS-free subjects, particularly *Clostridium thermosuccinogenes*	Not stated
Pedersen *et al.*[26] Denmark Unblinded RCT	Investigate the effects of a low-FODMAP diet versus LGG in IBS	6 weeks, 6×10^9 CFU/day (Dicoflor 60 capsules)	123 males and females with IBS	Disease severity of IBS using IBS-SSS questionnaire	Both treatments efficacious for IBS, especially in the IBS-D and IBS-A subtypes	Lack of blinding Not placebo controlled Diet adherence not evaluated
Wegh *et al.*[25] Netherlands Systematic review	Investigate the effects of probiotics on FAPD and functional constipation in children		17 studies with 1321 children (3 on LGG)		LGG reduces frequency and severity of abdominal pain, but only in children with IBS	Majority of studies have unclear or high risk of bias Many studies did not compare the results from baseline, only between groups High heterogeneity between groups Only studies in English included Crossover studies included Studies only had a 2-week washout period
Yoon *et al.*[27] Korea RCT	Investigate the efficacy of a multispecies probiotic on IBS symptoms and gut microbiota alterations	4 weeks multispecies, 5×10^9 CFU/day LGG + *Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium lactis, Lactobacillus acidophilus* and *Streptococcus thermophilus*	49 patients with IBS	Proportion of patients who experience IBS symptom relief based on answers to two questions	Multispecies probiotic supplementation is effective at relieving symptoms of abdominal pain, bloating and discomfort in individuals with IBS, and caused a change to the gut microbiota	Faecal analysis not in whole study population Faecal microflora analysis only reflects bacterial composition in the intestinal lumen Validated measurement of symptom improvement was not used Did not look at gender or IBS subtypes

CFU, colony-forming units; FAPD, functional abdominal pain disorders; FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides and polyols; GI, gastrointestinal; IBS, irritable bowel syndrome; IBS-A, IBS-mixed subtype; IBS-D, IBS-diarrhoeal subtype; IBS-SSS, IBS-Severity Scoring System; LGG, *Lactobacillus rhamnosus* GG; RCT, randomised-controlled trial; VAS, Visual Analogue Scale.

Diarrhoea

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Agamennone *et al.*[67]	To produce a guide on the use of probiotics to prevent AAD		32 RCTs		Results indicate that seven single or multispecies favouring the treatment group, with LGG being the most effective (RR = 0.30 versus placebo, 95% CI: 0.16–0.5) Dosage recommendations of at least 2×10^9 CFU may be needed	Not stated
Fang *et al.*[71] Taiwan Open-label RCT	To assess whether there is a dose-dependent effect of LGG on the reduction of faecal rotavirus shedding in children	0 CFU/day in control 2×10^8 CFU/day low dose 6×10^8 CFU/day high dose	23 children with acute rotaviral gastroenteritis	Not stated	Low-dose group had no change in faecal rotavirus concentrations (36.1×10^5 particles/ml versus 73.5×10^5 particles/ml, $P = 0.895$); however, the high-dose group did (64.2×10^5 particles/ml versus 9.0×10^5 particles/ml, $P = 0.012$) It appears there is a dose-dependent effect of LGG on faecal rotavirus shedding in children	Not stated
Korpela *et al.*[65] India RCT	To determine the effect of long-term LGG consumption on pre-school children's antibiotic use Also assessed its effect on gut microbiota	400 ml milk containing 10^6 CFU/ml LGG for 7 months	231 school-aged children	First antibiotic purchase	Long-term LGG may prevent specific bacterial infections for up to 3 years, and may prevent some of the gut microbiota changes associated with antibiotic use Increased abundance of the *Lactobacillus* spp. ($P < 0.0001$)	Not stated
Li *et al.*[70] China Systematic review and meta-analysis	Evaluate the efficacy of LGG in children with acute diarrhoea		19 RCTs	Development of persistent diarrhoea, including duration	High-dose LGG reduced duration and frequency of diarrhoea episodes Results pronounced in those who were treated early and who presented with rotavirus-positive diarrhoea Reduced duration (MD −31.05 hours, 95% CI: 50.31, −11.80) and frequency of episodes (MD −1.08, 95% CI: −1.87, −0.28)	Limitations amongst the studies included limited pathogen identification, small sample sizes, varying dosages and limited blinding
Schnadower *et al.*[61] USA RCT	Determine the effectiveness of a 5-day course of LGG compared with placebo in children with acute gastroenteritis	5 days, 1×10^{10} CFU twice daily versus placebo	943 children aged 3 months to 4 years with acute gastroenteritis	Presence of moderate-to-severe gastroenteritis	Administration of LGG to preschool children with acute gastroenteritis did not result in a smaller number of moderate-to-severe gastroenteritis cases, and did not show benefit to duration or frequency of vomiting or diarrhoea compared with children receiving placebo	Possible inaccurate recall by participants Potential for LGG preparation to be inadequately stored
Szajewska *et al.*[69] Poland Meta-analysis	To provide recommendations on the use of probiotics and prebiotics for the prevention of AAD in children		20 RCTs	Diarrhoea/AAD and *Clostridium difficile*-associated diarrhoea	Recommended using LGG or *Saccharomyces boulardii* for preventing AAD For *C. difficile*-associated diarrhoea then LGG *not* recommended AAD risk reduction (RR = 0.48, 95% CI: 0.26–0.89)	The authors question the validity of pooling different strains of probiotic, when they all have differing effects
Szajewska & Kołodziej[66] Poland Meta-analysis	To determine the efficacy of LGG to prevent AAD in children and adults		12 RCTs, 1499 participants	Incidence of diarrhoea or AAD	Treatment with LGG compared with placebo or no additional treatment reduced the risk of ADD from 22.4% to 12.3% (RR = 0.49, 95% CI: 0.29–0.83, NNT = 9)	Definition of AAD varied amongst studies Unclear risk of bias

AAD, antibiotic-associated diarrhoea; CFU, colony-forming units; CI, confidence interval; LGG, *Lactobacillus rhamnosus* GG; MD, mean difference; NNT, number needed to treat; RCT, randomised-controlled trial; RR, relative risk.

Inflammatory bowel disease

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Bousvaros *et al.*[81] USA RCT	To determine if addition of LGG to standard therapy prolonged remission in children with Crohn's disease	2-year follow-up, LGG 10^{10} CFU/day + 295 mg inulin versus placebo	75 children aged 5-21 years	Time to clinical relapse	Median time to relapse 9.8 months in LGG versus 11.0 months in placebo group ($P = 0.24$) LGG did not prolong remission in children with Crohn's disease	Concomitant therapies could be masking effects of LGG
Jonkers *et al.*[79] Netherlands Systematic review and meta-analysis	Assess the use of probiotics in IBD management		41 RCTs, two in LGG		No difference in LGG supplementation and placebo for endoscopic recurrences in inactive Crohn's disease, even though there was an OR of 0.93 (95% CI: 0.63, 1.38) High drop-out rates amongst studies	
Lorea Baroja *et al.*[82] Canada Open-labelled study	Assess whether a combination of LGG GR-1 strain and *Lactobacillus reuteri* in a yoghurt supplement was able to promote an anti-inflammatory state in individuals with Crohn's disease	125 g probiotic yoghurt per day for 30 days LGG dosage 2 × 10^7 CFU/ml and *L. reuteri* 1 × 10^3 CFU/ml	20 participants with Crohn's disease and ulcerative colitis, 20 healthy controls	Changes in the prevalence of inflammatory markers *T*reg cells ($CD4^+$ $CD25^{high}$), TNF-α and IL-12	Amongst patients with IBD, increased $CD4^+$ $CD25^{high}$ *T*-cells ($P = 0.007$) This correlated with a decrease in the percentage of TNF-α and IL-12 Probiotic yoghurt intake was associated with an anti-inflammatory effect	Not stated
Shen *et al.*[80] China Meta-analysis	Assess the effect and adverse events of *Lactobacilli* strains compared with placebo as maintenance therapy in Crohn's disease		6 RCTs, 4 trials in LGG 359 individuals	Clinical relapse rates	LGG may increase the relapse rate of those with Crohn's disease Significant benefit of placebo (RR = 1.68; 95% CI: 1.07–2.64)	Different measures of relapse rates amongst the studies Different study durations

CFU, colony-forming units; CI, confidence interval; IBD, inflammatory bowel disease; IL, interleukin; LGG, *Lactobacillus rhamnosus* GG; OR, odds ratio; RCT, randomised-controlled trial; RR, relative risk; TNF-α, tumour necrosis factor-alpha.

Body weight

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Callaway *et al.*[91] RCT Australia	To determine whether multispecies probiotic in overweight and obese women prevents GDM	Probiotic LGG + *Bifidobacterium animalis* 1 × 10^9 CFU/day versus placebo	411 pregnant overweight and obese women	Frequency of GDM at 28 weeks gestation Secondary outcomes: gestational weight gain, preeclampsia, hypertension, Caesarean delivery, and gestation age of delivery	Probiotics did not prevent GDM (18.4% probiotic versus 12.3% placebo, $P = 0.10$), but did prevent excessive weight gain during gestation in overweight and obese pregnant women (32.5% probiotic versus 46% placebo, $P = 0.01$)	Oral glucose tolerance test not completed at start of trial Changes to trial design meant some women only taking probiotics for 1–4 weeks
Kekkonen *et al.*[92] RCT sub-study	To determine the effect of 3-week LGG supplementation on serum lipid profiles and inflammatory markers	250 ml milk-based fruit drink with LGG 6.2 × 10^7 CFU/ml for 3 weeks	26 healthy adults	Not stated	LGG supplementation may lead to a change in serum global lipid profiles Decreased LysoGPCho ($P \leq 0.05$), sphingomyelins ($P \leq 0.001$) and glycerophosphatidylcholines ($P \leq 0.05$)	When allowing for multiple hypothesis testing, no changes in global lipidomic profiles
Okesene-Gafa *et al.*[90] RCT New Zealand	To determine a culturally tailored dietary intervention and/or daily probiotic in obese pregnant women reduces gestational weight gain and birthweight	Dietary intervention versus routine dietary advice + probiotic containing LGG and *Bifidobacterium lactis* BB12 6.5 × 10^9 CFU/day until birth	230 obese pregnant women and their babies	Proportion of women with excessive gestational weight gain Birth weight	Neither treatment had a significant effect Total maternal weight gain was lower with dietary intervention than probiotic and routine dietary advice (9.7 kg versus 11.4 kg, adjusted MD −1.76, 95% CI: 3.55–0.03)	Not stated

CFU, colony-forming units; CI, confidence interval; GDM, gestational diabetes mellitus; LGG, *Lactobacillus rhamnosus* GG; MD, mean difference; RCT, randomised-controlled trial.

Liver disease

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Kwak *et al.*[18] Korea RCT	To determine the efficacy of probiotics to improve SIBO and gut permeability in liver disease	Multispecies containing 5×10^9 CFU *Bifidobacterium bifidum, Bifidobacterium lactis, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus rhamnosus* and *Streptococcus thermophilus* versus placebo, once daily for 4 weeks	53 patients with chronic liver disease	Changes in the composition of faecal bacteria, SIBO, intestinal permeability and clinical symptoms	LGG increased in faeces of probiotic group ($P \leq 0.001$) SIBO significantly disappeared in probiotic group compared with placebo ($P \leq 0.05$) Intestinal permeability improved but not significantly Liver chemistry remained unaffected Short-term probiotics effective in alleviating SIBO but not liver function in patients with chronic liver disease	Hydrogen breath test not jejunal aspiration used to test for SIBO Study participants had only mild disease as administration of probiotics in immunocompromised individuals is not recommended
Vajro *et al.*[99] Italy Double-blind, pilot study	To evaluate the effects of short-term probiotic treatment in children with NAFLD	LGG 1.2×10^{10} CFU/day for 8 weeks	20 children with NAFLD	Not stated	Compared with placebo, LGG was associated with a decrease in ALT ($P = 0.03$) and in anti-peptidoglycan-polysaccharide antibodies ($P = 0.03$) LGG should be considered as a therapy for children with NAFLD who do not comply with lifestyle interventions	Not stated

ALT, alanine aminotransferase; CFU, colony-forming units; LGG, *Lactobacillus rhamnosus* GG; NAFLD, non-alcoholic fatty liver disease; RCT, randomised-controlled trial; SIBO, small intestinal bacterial overgrowth.

Insulin resistance and type 2 diabetes

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Asemi *et al.*[103] Iran RCT	To determine the effect of multispecies probiotic on metabolic profiles	*Lactobacillus acidophilus* (2×10^9 CFU), *Lactobacillus casei* (7×10^9 CFU), LGG (1.5×10^9 CFU), *Lactobacillus bulgaricus* (2×10^8 CFU), *Bifidobacterium breve* (2×10^{10} CFU), *Bifidobacterium longum* (7×10^9 CFU), *Streptococcus thermophilus* (1.5×10^9 CFU) versus placebo, 8 weeks	54 diabetic patients	Anthropometrics Plasma glucose HbA1c levels HOMA-IR blood lipid concentrations Antioxidants	Multispecies probiotic for 8 weeks in patients with diabetes prevented a rise in fasting plasma glucose, and decreased serum hs-CRP and increased GSH Measures of IR were increased in both groups, but less so in the probiotic group ($P = 0.03$)	Not stated
Laitinen *et al.*[106] Finland RCT	To determine whether supplementation of multispecies probiotic with dietary counselling affects glucose metabolism in normoglycaemic pregnant women	LGG + *Bifidobacterium lactis* + dietary advice versus placebo during pregnancy and 12 months post-partum Dosage not stated	256 normoglycaemic pregnant women	Glucose metabolism through plasma glucose concentration and HbA1c, serum insulin and HOMA and QUICKI	In normoglycaemic pregnant women, diet + probiotics may improve blood glucose control Blood glucose at lowest in diet + probiotic group during pregnancy and 12 months post-partum ($P \leq 0.025$ for both) Better glucose tolerance in diet + probiotic group through HOMA-IR ($P = 0.028$), insulin concentration ($P = 0.032$) and QUICKI ($P = 0.028$) Reduced risk of elevated glucose concentration compared with placebo (OR 0.31, 95% CI: 0.12, 0.78, $P = 0.028$)	Not stated
Sanborn *et al.*[105] USA RCT sub-analysis	To determine whether probiotic supplementation improves glycaemic control in healthy individuals	LGG 1×10^{10} CFU versus placebo, 90 days	200 healthy middle-aged and older adults	HbA1c	LGG may help maintain glycaemic control in healthy adults HbA1c increased in placebo but maintained in the LGG group (between-group difference $P = 0.005$)	Not stated

CFU, colony-forming units; CI, confidence interval; GSH, glutathione; HbA1c, glycated haemoglobin; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; hs-CRP, high-sensitivity C-reactive protein; IR, insulin resistance; LGG, *Lactobacillus rhamnosus* GG; OR, odds ratio; QUICKI, Quantitative Insulin-Sensitivity Check Index; RCT, randomised-controlled trial.

Cystic fibrosis

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Bruzzese *et al.*[110] Italy RCT	To determine the gut microbiota composition of children with CF, and whether correlations between microbial balance and inflammation exist Then to determine whether LGG restores intestinal flora and decreases inflammation	6×10^9 CFU versus placebo daily for 1 month	22 children with CF	Intestinal inflammation Bacterial composition	CF restored gut microbiota reducing intestinal inflammation and pulmonary exacerbations LGG reduced faecal CLP (184 ± 146 mg/g versus 52 ± 46 mg/g; $P \leq 0.01$) Correlation between reduced microbial richness and intestinal inflammation (r = 0.53; $P = 0.018$)	Not stated
Bruzzese *et al.*[112] Germany RCT	To investigate the effects of LGG on clinical outcomes of children with CF	LGG 6×10^9 CFU/day versus placebo, 12 months	95 children with CF	Proportion of subjects with at least one pulmonary exacerbation over the 12-month study period	LGG had no effect on respiratory and nutritional outcomes in children with CF Odds of experiencing at least one exacerbation were not significantly different from placebo (OR 0.83, 95% CI: 0.38–1.82, $P = 0.643$) The odds of hospitalisations also remained unaffected (OR 1.67, 95% CI: 0.75–3.72, $P = 0.211$)	Not stated
Bruzzese *et al.*[108] Germany Prospective study	To assess the incidence of intestinal inflammation in children with CF, and whether probiotics decrease it	LGG 5×10^9 CFU/day	75 children (30 with CF, 30 with IBD and 15 healthy controls)		Intestinal inflammation is a feature of CF as indicated by increased CLP (versus control, $P \leq 0.01$) similar to levels of children with IBD ($P \geq 0.05$) Intestinal microflora play a major role in this LGG reduced inflammation (210 ± 42 to 140 ± 43 mg/g, $P = 0.01$)	Not stated
Bruzzese *et al.*[111] Italy Prospective RCT crossover	To determine the effect of LGG on pulmonary exacerbations in children with CF	LGG 6×10^9 CFU/day for 6 months and then shifted to dissolved oral rehydration solution for 6 months Or dissolved oral rehydration solution for 6 months and then LGG for 6 months	43 children with CF	Incidence and severity of pulmonary exacerbations Number and duration of hospital admissions Route of antibiotic administration (indication of severity of episode) FEV_1 Body weight Serum immunoglobulin concentrations	LGG reduced pulmonary exacerbations and hospital admissions in children with CF Pulmonary exacerbations reduced (group A, median difference 1, CI 95%: 0.1–2, $P = 0.035$; Group B, median difference 1, 95% CI: 0–2, $P = 0.02$) Rate of hospital admissions (LGG = 16, ORS = 32) Significant differences only in period one (MD 1, 95% CI: 0.1–1, $P = 0.01$) Mean duration of hospital stay did not differ between the two groups	Not stated

CF, cystic fibrosis; CFU, colony-forming units; CI, confidence interval; CLP, calprotectin; FEV_1, forced expiratory volume; IBD, inflammatory bowel disease; LGG, *Lactobacillus rhamnosus* GG; MD, mean difference; OR, odds ratio; RCT, randomised-controlled trial.

Respiratory tract infections

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Liu *et al.*[123] China Systematic review and meta-analysis	To review the effectiveness of LGG for the prevention of respiratory infections in children		4 RCTs, 1805 children	Incidence of respiratory infections	LGG may reduce the incidence of otitis media, URTIs and antibiotic use in children LGG was associated with reduced otitis media (RR = 0.76, 95% CI: 0.64–0.91, NNT = 17), reduced risk of URTIs (RR = 0.62, 95% CI: 0.50–0.78, NNT = 4) and antibiotic use (RR = 0.80, 95% CI: 0.71–0.91) Risk of overall respiratory infections was only reduced in those older than 1 year (RR = 0.73, 95% CI: 0.57–0.92, NNT = 8) No difference in the incidence of lower respiratory infections	Not stated
Hojsak *et al.*[125] Croatia RCT	To determine the role of LGG in preventing nosocomial GI infections and RTIs in children	LGG 1×10^9 CFU/day in 100 ml of a fermented milk product versus placebo Duration not stated	742 hospitalised children	GI tract infections Upper and lower RTIs	LGG can decrease risk for nosocomial GI infections and RTIs in paediatric facilities Reduced risk of RTIs compared with placebo (RR = 0.38, 95% CI: 0.18–0.85, NNT = 30) No difference in hospitalisation duration	Infants prone to severe nosocomial infections were excluded The study period in most cases was short Cause of nosocomial infection was often unknown
Tapiovaara *et al.*[115] Finland Randomised control pilot study	To determine whether beneficial effects of LGG in RTIs are due to a reduced viral load	LGG 1×10^9 CFU/day versus placebo, 6 weeks	59 adults given human rhinovirus	Viral load	The use of LGG did not affect viral load in individuals with human rhinovirus Viral load LGG versus placebo ($P = 0.57$)	Samples collected 5 days after given human rhinovirus Validated symptom survey not used
Kumpu *et al.*[116] Finland RCT	To determine whether inactivated LGG would demonstrate similar effects to live LGG in humans with induced rhinovirus infection	LGG 1×10^9 CFU in 100 ml fruit juice, 6 weeks	60 individuals induced with the human rhinovirus	Occurrence, duration and severity of cold symptoms	Live LGG may be more effective in reducing rhinovirus infection than the inactivated form, but differences were not significant Occurrence and severity of cold symptoms was lowest in the LGG live group, but this was not statistically significant due to the pilot-scale of study ($P = 0.45$)	Not stated
Laursen & Hojsak[122] Denmark Systematic review and meta-analysis	To evaluate strain-specific effects of probiotics on RTIs in children at day care		15 RCTs with 5121 children in day care	Number of children with RTIs	Of the probiotics analysed, LGG significantly reduced the duration of RTIs (MD −0.78 days, 95% CI: −1.46, −0.09), but no effect on incidence, antibiotic use or days missed from day care	Studies included differed in methodological quality Only included studies in English
Laursen *et al.*[121] RCT Denmark (ComProbi study)	To determine the effects of multispecies probiotic on absence from childcare due to respiratory and GI infections in healthy infants	LGG + *Bifidobacterium animalis* 1×10^9 CFU, 6 months	290 infants who attend childcare	Number of days absent from childcare because of respiratory or GI infections	A multispecies probiotic for 6 months did not affect the number of days absent from childcare in healthy infants ($P = 0.19$)	Data on infant illness recorded using questionnaires

Swanljung *et al.*[114] Finland RCT	To determine whether 3-week supplementation of LGG would lead to the presence of the probiotic in adenoid tissue	8–9 × 10^9 CFU LGG in 150 ml commercial dairy product versus placebo, 3 weeks	40 children aged 1–5 years about to undergo adenotomy	Presence of LGG in adenoid tissue Secondary outcome rhinovirus and enterovirus in adenoid tissue	After 3 weeks supplementation, more LGG identified in the adenoids of children on probiotics (P = 0.07); however, its effect on the occurrence of rhinovirus or enterovirus was not apparent, as no significant differences between the groups (P = 0.67) A large amount of LGG was found in the adaenoids of the placebo group No differences in symptoms	Small study size Diaries used so reporting methods not standardised Limited diary data supplied
Luoto *et al.*[124] Finland RCT	To determine whether early prebiotic or probiotic supplementation reduced the risk of virus-associated RTIs in the first year of life in pre-term infants	LGG 1 × 10^9 CFU for first 30 days and 2 × 10^9 CFU for final 30 days versus placebo	94 preterm infants	Incidence of viral RTIs	Prebiotics and probiotics may reduce the risk of RTIs and rhinovirus infections Lower incidence of RTIs in infants receiving prebiotics (RR = 0.24, 95% CI: 0.12–0.49, $P \leq 0.001$) and probiotics (RR = 0.50, 95% CI: 0.28–0.90, P = 0.022) Rhinovirus episodes also reduced in prebiotics (RR = 0.31, 95% CI: 0.14–0.66, P = 0.003) and probiotics (RR = 0.49, 95% CI: 0.24–1.00, P = 0.051) compared with placebo	Studying preterm infants may mean results are not generalisable to full-term and older infants

CFU, colony-forming units; CI, confidence interval; GI, gastrointestinal; LGG, *Lactobacillus rhamnosus* GG; MD, mean difference; NNT, number needed to treat; RCT, randomised-controlled trial; RR, relative risk; RTI, respiratory tract infection; URTI, upper respiratory tract infection.

Otitis media

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Lehtoranta *et al.*[129] Finland RCT	To determine the prevalence and persistence of HBoV, and whether multispecies probiotics reduce occurrence	LGG + *Lactobacillus rhamnosus* Lc705 + *Bifidobacterium breve* 99 and *Propionibacterium freudenreichii* JS (dosage not stated) versus placebo, 6 months	269 otitis-prone children	Not stated	Probiotic treatment may reduce the presence of HBoV in children A high load of HBoV was detected in 152 children Probiotic supplementation decreased the number of HBoV-positive samples (6.4% versus 19.0%, OR 0.25, 95% CI: 0.07–0.94, P = 0.039) HBoV has been associated with lower RTIs	Not stated
Tapiovaara *et al.*[127] Finland RCT	To determine the effect of 3-week oral administration of LGG on MEE in children with otitis media	8–9 × 10^9 CFU/day versus placebo, 3 weeks	40 children undergoing tympanostomy	LGG findings in MEE	LGG was detected in the middle ear of children with otitis media, but did not affect the occurrence of bacteria or viruses LGG was detectable in 4 of the children in the LGG group and 1 in the placebo group, but differences were not significant (P = 1.0) Pathogenic bacteria present in 12 of the samples in the LGG group and 3 of the samples in the placebo group (P = 0.65) The most prominent species of bacteria was *Haemophilus influenzae*	Small study size PCR-assay used may not be optimised to detect bacteria in MEE
Hatakka *et al.*[128] Finland RCT	To determine the effect of multispecies probiotic	LGG + *L. rhamnosus* Lc705, *B. breve* 99 and *P. freudenreichii* JS 8–9 × 10^9 CFU/day versus placebo, 24 weeks	309 otitis-prone children	Occurrence and duration of acute otitis media episodes	Probiotic treatment did not reduce the occurrence (probiotic versus placebo, 72% versus 65%, OR 1.48, 95% CI: 0.87–2.52, P = n.s.), reoccurrence (18% versus 17%, OR 1.04, 95% CI: 0.55–1.96, P = n.s.) or duration (5.6 versus 6.0 days, P = n.s.) of acute otitis media episodes	Not stated

CFU, colony-forming units; CI, confidence interval; HBoV, human bocavirus; LGG, *Lactobacillus rhamnosus* GG; MEE, middle ear effusion; OR, odds ratio; PCR, polymerase chain reaction; RCT, randomised-controlled trial; RTI, respiratory tract infection.

Anxiety and depression

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Dawe *et al.*[143] New Zealand RCT	A secondary analysis to determine whether probiotics would improve maternal mental health	LGG + *Bifidobacterium lactis* 6.5×10^9 CFU/day versus placebo Duration not stated	230 women at 36 weeks of pregnancy	Depression Anxiety Functional health and wellbeing	Probiotics did not improve the mental health of pregnant women at 36 weeks gestation No difference between depression scores ($P \geq 0.05$) Anxiety and physical wellbeing worsened over time, and mental wellbeing did not differ at 36 weeks	Probiotic strain used may not be optimal Dosage may not be optimal Adherence to treatment was via self-reporting not capsule count Small sample size
Mohammadi *et al.*[135] Iran RCT	To determine the effects of multispecies probiotic and probiotic yoghurt on mental health and hypothalamic–pituitary axis	Yoghurt contained 1×10^7 CFU *Lactobacillus acidophilus* + *B. lactis* Multispecies probiotic contained *Lactobacillus casei* 3×10^3 CFU, *L. acidophilus* 3×10^7 CFU, LGG 7×10^9 CFU, *Lactobacillus bulgaricus* 5×10^8 CFU, *Bifidobacterium breve* 2×10^{10} CFU, *Bifidobacterium longum* 1×10^9 CFU, *Streptococcus thermophilus* 3×10^8 CFU/g, 6 weeks	70 petrochemical workers	GHQ DASS scores	Improvements within probiotic yoghurt group in GHQ (18.0 ± 1.5 versus 13.5 ± 1.9, $P = 0.007$) and DASS (23.3 ± 3.7 versus 13.0 ± 3.7, $P = 0.02$) Improvements within the probiotic capsule group in GHQ (16.9 ± 1.8 versus 9.8 ± 1.9, $P = 0.001$) and DASS (18.9 ± 3.2 versus 9.4 ± 4.0, $P = 0.006$) No improvements in conventional yoghurt group for GHQ ($P = 0.05$) or DASS ($P = 0.08$)	Short supplementation period Did not assess short-chain fatty acid production
Moludi *et al.*[136] Iran RCT	To determine the effects of probiotics on symptoms of depression, measures of QoL, oxidative stress and inflammation in individuals who had recently had a MI	Secondary analysis LGG 1.6×10^9 CFU/day versus placebo, 12 weeks	44 adults with recent MI and PCI	Depression, QoL, inflammation and oxidative stress	Probiotics had beneficial effects on depression and markers of oxidative stress and inflammation in individuals post-MI with a PCI Compared with placebo, Beck Depression Inventory score decreased (−5.57 versus −0.51, $P = 0.045$) and QoL increased (23.6 versus 0.44, $P = 0.023$) Total antioxidant capacity increased in the probiotic group (93.7 versus 27.54 mmol/l, $P = 0.009$) and malondialdehyde (−40.7 versus −4.2, $P = 0.033$) and hs-CRP (−1.74 versus 0.67 mg/l, $P = 0.04$) decreased, with levels stronger than placebo	Small sample size Short supplementation duration Sample was predominantly male

CFU, colony-forming units; DASS, Depression, Anxiety, and Stress Scale scores; GHQ, General Health Questionnaire; hs-CRP, high-sensitivity C-reactive protein; LGG, *Lactobacillus rhamnosus* GG; MI, myocardial infarction; PCI, percutaneous intervention; QoL, quality of life; RCT, randomised-controlled trial.

Attention-deficit hyperactivity disorder and Asperger's syndrome

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Partty *et al.*[149] Finland RCT	To determine the involvement of the gut–brain axis in the incidence of ADHD and AS in a cohort followed until 13 years old	LGG 1×10^9 CFU/day versus placebo to pregnant women 4 weeks before expected delivery, and then 6 months post-delivery to the infant Follow-up for 13 years	75 mothers and children	Clinical diagnosis of ADHD and AS	LGG supplementation in early life may reduce the risk of developing ADHD or AS By age 13 years, 6 children developed ADHD or AS or both, all of which were in the placebo group ($P = 0.008$) At 6 months old, numbers of *Bifidobacterium* were less in children with neuropsychiatric disorder than those without ($P = 0.03$) At 18 months old, *Bacteroides* and *Lactobacillus-Enterococcus* group were less in children with neuropsychiatric disorder ($P = 0.008$ and $P = 0.01$, respectively)	Not stated

ADHD, attention-deficit hyperactivity disorder; AS, Asperger's syndrome; CFU, colony-forming units; LGG, *Lactobacillus rhamnosus* GG; RCT, randomised-controlled trial.

Urinary tract infections

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Colodner *et al.*[154] Israel Pilot study	To determine the vaginal colonisation in post-menopausal women by LGG	100 ml yoghurt daily containing 1×10^9 CFU LGG or 200 ml yoghurt daily containing 1×10^9 CFU LGG, 1 month	42 post-menopausal women	Colonisation count in vaginal and rectal swabs	LGG has a low vaginal adhesion rate and is not a good probiotic for UTIs The vaginas of only 4 women (9.5%) were colonised with LGG, but 33 women (78.6%) had positive rectal swabs indicating GI colonisation	Not stated
Kontiokari *et al.*[155] Finland RCT	To determine whether recurrent UTIs can be prevented with cranberry–lingonberry juice or with LGG	50 ml cranberry–lingonberry juice daily for 6 months versus 100 ml LGG drink (4×10^{10} CFU) 5 times per week for 1 year versus no intervention	150 women with UTIs caused by *Escherichia coli*	First recurrence of UTI	Regular consumption of cranberry juice but not LGG prevents the recurrence of UTIs Rate of first UTI recurrence differed between the groups ($P = 0.048$) Recurrent UTIs in 16% of women in cranberry group, 39% of women in LGG group and 36% of women in control Difference between cranberry juice and control 20% reduction in absolute risk (95% CI: 3–5%, $P = 0.023$, NNT = 5.95)	Not stated
Ng *et al.*[152] Singapore Systematic review and meta-analysis	To determine whether *Lactobacillus* spp. can prevent recurrent UTIs in females		9 clinical trials with 726 patients	Prophylactic efficacy and incidence of adverse events	The use of *Lactobacillus* spp. reduced the risk of recurrent UTIs (RR = 0.684, 95% CI: 0.438–0.929, $P \leq 0.001$) However, different strains showed varying efficacy	Inter-study variability and short treatment durations
Sadeghi-Bojd *et al.*[156] Iran RCT	To determine the efficacy of multispecies probiotic for the prevention of recurrent UTIs in children	LGG 1×10^9 CFU + *Lactobacillus acidophilus* 15×10^9 CFU + *Bifidobacterium bifidum* 4×10^9 CFU + *Bifidobacterium lactis* 15×10^9 CFU	181 children with normal urinary tracts given LGG + *L. acidophilus, B. bifidum, B. lactis* versus placebo, 18 months	Composite cure at 18 months	Multispecies probiotic more effective at reducing the risk of recurrent UTIs Composite cure in probiotic 96.7% versus 83.3% placebo ($P = 0.02$) Time to first recurrent event was 3.5 months in probiotic group and 6.5 months in placebo group ($P = 0.04$)	Patients from a limited selection pool Did not include uncircumcised boys Did not test to see if supplementation reduced GI colonisation by pathogenic bacteria
Toh *et al.*[159] Australia RCT		Four arms: (i) *Lactobacillus reuteri* RC-14–*Lactobacillus rhamnosus* GR-1 (5.4×10^9 CFU) + LGG–*Bifidobacterium animalis* BB-12 (7×10^9 CFU); (ii) RC-14–GR1 (conc. as above) + placebo; (iii) LGG–BB-12 (conc. as above) + placebo; (iv) placebo + placebo, 6 months	207 individuals with spinal cord injury	Occurrence of first symptomatic UTI	No effect of either probiotic combination for preventing UTIs in people with spinal cord injury RC-14–GR-1 had a similar risk of UTI to placebo (HR 0.67, 95% CI: 0.39–1.18), and those on LGG–BB-12 also had a similar risk to those on placebo (HR 1.29, 95% CI: 0.74–2.25, $P = 0.37$)	Did not recruit the target number of 372 participants No trial follow-up
Tractenberg *et al.*[160] USA Prospective 3-stage study	To determine the efficacy of intravesical LGG on urinary symptoms in individuals with spinal cord injury	Self-administration of a catheter with LGG + saline (2×10^{10} CFU live organisms)	96 adults and 7 children with spinal cord injury	Change in USQNB-IC	Intravesical administration of LGG improved symptoms of UTIs compared with individuals who did not administer the probiotic ($P \leq 0.05$)	No randomisation

CFU, colony-forming units; CI, confidence interval; GI, gastrointestinal; HR, hazard ratio; LGG, *Lactobacillus rhamnosus* GG; NNT, number needed to treat; RCT, randomised-controlled trial; RR, relative risk; USQNB-IC, Urinary Symptom Questionnaire for Neurogenic Bladder-IC; UTI, urinary tract infection.

Infant health

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Chrzanowska-Liszewska *et al.*[167] Poland RCT	To determine the colonisation of LGG and its impact on growth and length of hospital stay in pre-term infants	LGG 6 × 10^9 CFU/day versus placebo, 42 days	60 pre-term infants (before 32 weeks)	Difference in the amount of Bifidogenic microflora and *Escherichia coli*	Although LGG rapidly colonised the gut of preterm formula-fed infants, this did not decrease the number of pathogenic bacteria or affect growth or hospital stay duration LGG higher in supplemented group than placebo at days 7 ($P = 0.041$) and 21 ($P = 0.024$) *Staphylococci* higher in supplemented group at days 7 ($P = 0.001$) and 42 ($P = 0.011$) No difference to weight gain (95% CI: −1.68, 305, $P = 0.567$) or mean hospital duration (95% CI: −13.43, 5.71, $P = 0.421$)	Lack of follow-up No precise CFU count for organisms analysed
Lundelin *et al.*[189] Finland Follow-up study of 4 RCTs	To determine the clinical benefit and safety of probiotics during the perinatal period Follow-up of 4 previous RCTs	Included trials were 3–6 months duration, and dosages ranged from 1 × 10^9–1 × 10^{10} CFU, 2-year follow-up	303 children pre-term or increased allergy risk	Not stated	Children given LGG had a decreased prevalence of allergic disease compared with placebo (OR 0.62, 95% CI: 0.38–0.99, $P = 0.047$) No difference in prevalence of asthma (OR 0.55, 95% CI: 0.24–1.25, $P = 0.15$), non-communicable diseases or growth	Follow-up completed unblinded
Luoto *et al.*[171] Finland Follow-up of RCT	To determine the safety and efficacy of multispecies probiotic containing LGG on pregnancy outcome, and foetal and infant growth	Diet + LGG (1 × 10^{10} CFU/day) + *Bifidobacterium lactis* (1 × 10^{10} CFU/day) versus diet + placebo from first trimester to cessation of breastfeeding	256 pregnant women 191 completed the 24-month follow-up	Pregnancy outcome and infant growth	The use of probiotics in pregnancy could be safe and cost-effective to prevent future metabolic disease Probiotics + diet reduced the frequency of gestational diabetes ($P \leq 0.003$)	Not stated
Vendt *et al.*[169] Estonia RCT	To determine the effect of LGG-enriched formula on growth and faecal microflora in the first 6 months of healthy infants	LGG dosage not stated, 6 months	120 healthy infants	Not stated	Infants fed with LGG-supplemented formula grew better than those with regular formula Length and weight higher in supplemented versus control (0.44 ± 0.37 versus 0.07 ± 0.06, $P \leq 0.01$ and 0.44 ± 0.19 versus 0.07 ± 0.06, $P \leq 0.005$) More frequent colonisation amongst supplemented formula group (91% versus 76%, $P \leq 0.05$) More frequent defecation in LGG group (9.1 ± 2.6 versus 8.0 ± 2.8, $P \leq 0.05$)	Not stated
Mantaring *et al.*[168] Philippines RCT	To determine the effect of probiotics during pregnancy and early lactation on infant diarrhoea	LGG 7 × 10^8 CFU + *B. lactis* 7 × 10^8 CFU per day versus control	208 healthy pregnant women in third trimester	Incidence of infant diarrhoea until age 12 months	Maternal supplementation showed beneficial effects on infant weight and length gain; however, did not affect incidence of infant diarrhoea Weight and height increased compared with placebo (8.97 kg versus 8.61 kg, $P = 0.001$ and 74.2 cm versus 73.4 cm, $P = 0.031$)	Limited generalisation Diet and exercise not considered

CFU, colony-forming units; CI, confidence interval; LGG, *Lactobacillus rhamnosus* GG; OR, odds ratio; RCT, randomised-controlled trial.

Infantile colic

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Cabana *et al.*[174] USA Secondary analysis of RCT	To determine whether LGG supplementation prevents infant colic	LGG 1 × 10^9 CFU/day versus control, 6 months	184 infants	Likelihood of diagnosis of colic before 4 months old	Early LGG supplementation does not prevent infant colic No difference between two groups in infants with colic based on symptoms (control 5.4% versus LGG 9.8%, P = 0.19) or physician diagnosis (control 3.2% versus LGG 7.6%, P = 0.26) or combination of both (6.5% versus LGG 13.0%, P = 0.13)	Parent report of symptoms and crying length High rate of breastfeeding in sample may mask effects of probiotic Samples were not racially or socially diverse
Kianifar *et al.*[176] Australia RCT	To determine efficacy of multispecies probiotic and prebiotic to reduce crying time	1 × 10^9 CFU *Lactobacillus casei* + LGG + *Streptococcus thermophilus* + *Bifidobacterium breve* + *Lactobacillus acidophilus* + *Bifidobacterium infantis* + *Lactobacillus bulgaricus* + fructo-oligosaccharide versus placebo, 30 days	50 breastfed infants	Treatment success	Synbiotic significantly improved colic symptoms compared with placebo At day 7 and day 30, treatment success was higher in synbiotic compared with placebo (day 7, 82.6% versus 35.7%, $P \leq 0.005$; day 30, 87% versus 46%, $P \leq 0.01$) Symptom resolution higher in synbiotic group at day 7 (39% versus 7%, $P \leq 0.03$) but not day 30 (56% versus 36%, P = 0.24)	Stool samples not evaluated at baseline or after intervention Small sample size Non-validated outcome measure, no measure of compliance
Partty *et al.*[172] Finland RCT	To determine the efficacy of LGG to reduce daily crying of infants with colic	LGG 4.5 × 10^9 CFU/day versus placebo, 4 weeks	17 healthy breastfed infants under 6 weeks old	Difference in daily average crying time between LGG and placebo	LGG in combination with behavioural support and cow's milk elimination was not efficacious for the reduction of crying time in infants with colic Daily crying time comparable between the groups (173 minutes probiotic versus 174 minutes placebo, P = 0.99) However, occurrence of crying decreased in the probiotic group compared with placebo (68% versus 49%; 95% CI: 32–66, P = 0.05)	Not stated
Savino *et al.*[173] Italy RCT	To determine the efficacy of LGG together with maternal avoidance of cow's milk in treating infantile colic	LGG 5 × 10^9 CFU/day versus placebo, 28 days	45 colicky breastfed infants	Faecal CLP, crying and fussing	LGG in combination with elimination of cow's milk from maternal diet reduced crying time (104 minutes versus 242 minutes, $P \leq 0.001$) and faecal CLP (P = 0.026), and increased total gut bacteria (P = 0.04) and Lactobacillus (P = 0.048)	Possible false-positive with the use of PCR test Small sample size

CFU, colony-forming units; CI, confidence interval; CLP, calprotectin; LGG, *Lactobacillus rhamnosus* GG; PCR, polymerase chain reaction; RCT, randomised-controlled trial.

Human immunodeficiency virus

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Salminen *et al.*[179] RCT Finland	To determine the efficacy and safety of LGG for GI symptoms in patients with HIV on anti-retroviral therapy	LGG 1–5 × 10^{10} CFU twice daily versus placebo, 2 weeks	17 HIV-infected patients with diarrhoea for more than 1 month	GI symptoms Safety parameters Faecal microbiology	LGG supplementation was well tolerated, but showed no benefits to diarrhoea or GI symptoms in HIV-infected patients No differences between faecal counts of LGG between supplemented and placebo No adverse events reported	Not stated

CFU, colony-forming units; GI, gastrointestinal; HIV, human immunodeficiency virus; LGG, *Lactobacillus rhamnosus* GG; RCT, randomised-controlled trial.

Allergy

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Tan *et al.*[190] China Systematic review and meta-analysis	To determine the effects of LGG in children with CMA	LGG dosages ranged from 1.4×10^7 CFU to 5×10^9 CFU/day, with treatment durations from 4 weeks to 3 years	10 studies 853 children		LGG may have moderate-quality evidence to promote tolerance and aid recovery from GI symptoms in children with CMA Higher tolerability rates favouring LGG over controls were observed (RR = 2.22, 95% CI: 1.86–2.66; I2 = 0.00; moderate-quality evidence) No significant differences in SCORAD values (MD 1.41, 95% CI: −4.99, 7.82, $P = 0.67$; very low-quality evidence), and LGG may have improved faecal occult blood (RR = 0.36, 95% CI: 0.14–0.92, $P = 0.03$; low-quality evidence) No adverse events reported	Limited number of studies
Korpela *et al.*[186] Finland RCT	To determine whether multispecies probiotic could ameliorate antibiotic use or Caesarean birth on infant gut microbiota	LGG (5×10^9 CFU) + *Bifidobacterium breve* Bb99 (2×10^8 CFU) + *Propionibacterium freudenreichii* spp. *shermanii* JS (2×10^9 CFU) + *Lactobacillus rhamnosus* Lc705 (5×10^9 CFU) versus placebo	199 breastfed or formula-fed infants		LGG supplementation may ameliorate changes in the gut microbiota due to antibiotic use or Caesarean birth	Not stated
Piirainen *et al.*[193] Finland RCT	To determine the effects of LGG on oral immune response of adults with birch pollen allergy	LGG (2×10^{10} CFU/day) versus placebo	38 birch pollen allergy sufferers	Not stated	rBet v1 (0.319 versus −0.136, $P = 0.02$) and Mal d1 (0.097 versus −0.117, $P = 0.02$) specific IgA levels increased compared with placebo	Not stated
Moreira *et al.*[194] Finland RCT	To determine the effect of LGG supplementation on allergic inflammatory markers in marathon runners with asthma and allergy	LGG (3×10^8 CFU/day) versus placebo	141 marathon runners with allergies	ECP, total IgE levels and Phadiatop test	Compared with placebo, LGG supplementation did not prevent an increase in allergic markers during birch pollen season	Not stated

CFU, colony-forming units; CI, confidence interval; CMA, cow's milk allergy; ECP, eosinophil cationic protein; GI, gastrointestinal; LGG, *Lactobacillus rhamnosus* GG; MD, mean difference; RCT, randomised-controlled trial; RR, relative risk; SCORAD, Scoring of Atopic Dermatitis.

Dermatitis and eczema

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Szajewska & Horvath[198] Poland Systematic review and meta-analysis	To determine the efficacy of LGG prenatally/postnatally for the primary prevention of eczema	LGG dosages ranged from 1×10^9 CFU to 1.8×10^{10} CFU	5 RCTs with 889 subjects	Eczema	LGG was ineffective in reducing eczema, and guidelines should be revised to reflect this (1 RCT: RR = 0.88, 95% CI: 0.63, 1.22, $P = 0.69$, $I^2 = 0\%$) No reduction of risk for eczema when LGG administered during pregnancy (3 RCTs, RR = 0.93, 95% CI: 0.49, 1.76, $I^2 = 72\%$) No reduction of risk when LGG administered to infants (1 RCT: RR = 0.93, 95% CI: 0.59, 1.45)	Different trials used different definitions of eczema

Schmidt *et al.*[203] Denmark RCT	To determine the effect of multispecies probiotic in late infancy and early childhood on the development of allergic diseases	LGG + *Bifidobacterium animalis* spp. lactis versus placebo, 6 months	290 participants starting prior to attending day care	Incidence of allergic disease	Probiotics administered in late infancy may prevent the development of eczema Incidence of eczema was 4.2% in probiotic group and 5% in eczema group ($P = 0.036$)	Study set from a previous trial of high-income families
Tan-Lim *et al.*[202] Philippines Systematic review and meta-analysis	To determine the effectiveness of multispecies probiotics in prevention of ADe in children	LGG + *B. animalis*	21 RCTs, 5406 children with ADe		Specific probiotics reduce the risk of dermatitis in children when administered *in utero,* during infancy or both Reduced risk of ADe (RR = 0.50, 95% CI: 0.27–0.94) compared with placebo LGG had less adverse events compared with placebo (RR = 0.70, 95% CI: 0.32–1.52) In infants, reduced risk of ADe (RR = −0.46, 95% CI: 0.22–0.97) All based on low-quality evidence	When ranking evidence, quality not considered
Wu *et al.*[200] Taiwan RCT	To determine the efficacy and safety of LGG in children aged 4–48 months with ADe	LGG (ComProbi brand containing 350 mg) versus control, 8 weeks	67 children aged 4–48 months with ADe[3] 15 on SCORAD	Mean change from baseline in SCORAD at 8 weeks	LGG was effective to decrease symptoms of ADe compared with placebo ($P \leq 0.05$)	Lack of laboratory assessment Patients could use topical steroids Unethical to withhold corticosteroid treatment Lack of follow-up

Ade, atopic dermatitis; CFU, colony-forming units; CI, confidence interval; LGG, *Lactobacillus rhamnosus* GG; RCT, randomised-controlled trial; RR, relative risk; SCORAD, Scoring of Atopic Dermatitis.

Wounds

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Mayes *et al.*[208] USA RCT	To determine the efficacy and safety of LGG supplementation in acutely burned, paediatric patients	LGG 1.5×10^{10} CFU/day versus placebo within 10 days of burn until wound closure	20 acutely burned paediatric patients	Not stated	No difference between infection days, length of hospitalisation or antibiotic use Time required to complete wound healing shortened with LGG but not significant	

CFU, colony-forming units; LGG, *Lactobacillus rhamnosus* GG; RCT, randomised-controlled trial.

Dental caries

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Nase *et al.*[211] Finland RCT	To determine whether milk containing LGG had an effect on caries and caries risk in children	LGG ($5–10 \times 10^5$ CFU/ml) versus control 5 days per week for 7 months	594 children	Not stated	LGG reduced the risk of caries (OR 0.56, $P = 0.01$), an effect that was pronounced in 3–4-year-olds	Not stated

CFU, colony-forming units; LGG, *Lactobacillus rhamnosus* GG; OR, odds ratio; RCT, randomised-controlled trial.

Vaccine adjuvant

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Bianchini *et al.*[215] Italy RCT	To determine whether LGG can modify immune response in children and adolescents with T1D leading to an increased immune response to the influenza vaccine	LGG 1×10^9 CFU/drop, 5 drops twice per day versus placebo Three months prior and post vaccination	64 paediatric patients with T1D	Seroconversion rate	Combination of vaccine and LGG reduced the inflammatory response without dampening seroprotective antibodies IL-17 significantly lower in LGG ($P = 0.01$)	Small study size
De Vrese *et al.*[216] Germany RCT	To determine whether and how probiotics affect the immune response following polio vaccine	LGG 1×10^{10} CFU or *Lactobacillus acidophilus* CRL431 1×10^{10} CFU/serving in milk versus placebo, 5 weeks	66 healthy males	Not stated	Probiotics induce an immune response that may provide enhanced protection from viruses LGG or CRL431 nearly doubled the increase in polio-specific IgG ($P < 0.01$) IgA titre increases after vaccination ($P \leq 0.036$)	Not stated
Lazarus *et al.*[217] India RCT	To determine the effect of probiotics and/or zinc supplementation on the immune response to rotavirus vaccination	4 arms: LGG (1×10^{10} CFU) + zinc sulphate; 5 mg probiotic + placebo; zinc + placebo; placebo + placebo Duration not stated	620 infants given rotavirus at 6 and 10 weeks old	Seroconversion to rotavirus at 14 weeks old	Zinc supplementation did not improve immunogenicity of rotavirus vaccine, and probiotic supplementation only marginally increased seroconversion No changes to seroconversion in zinc arm and only modest improvement among infants receiving probiotic ($P = 0.066$)	Absence of immune correlate of protection for rotavirus vaccine
Davidson *et al.*[212] USA RCT	To determine the effects of LGG as an immune adjuvant to increase rates of seroconversion after influenza vaccine	LGG 1×10^{10} CFU + inulin twice daily versus placebo twice daily, 28 days	42 healthy adults	Protective HAI assay	LGG may be an important adjuvant to improve immunogenicity following influenza vaccine No LGG well tolerated No differences in seroprotection of H1N1 and B influenza strains Increased protective titre with LGG following H3N2 strain vaccine (OR 1.84, 95% CI: 1.04–3.22, $P = 0.048$)	Small sample size Subjects previously vaccinated were included

CFU, colony-forming units; CI, confidence interval; HAI, haemagglutinin inhibition; IL, interleukin; LGG, *Lactobacillus rhamnosus* GG; OR, odds ratio; RCT, randomised-controlled trial; T1D, type 1 diabetes.

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