Effect of *Bifidobacterium animalis* subsp *lactis* Supplementation in Preterm Infants: A Systematic Review of Randomized Controlled Trials

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ABSTRACT

Objective: To systematically evaluate and update evidence on the efficacy and safety of *Bifidobacterium animalis* subsp *lactis* CNCM I-3446 supplementation in preterm infants.

Materials and Methods: The Cochrane Library and MEDLINE databases and major pediatric conference proceedings were searched in December 2008 for randomized controlled trials (RCTs). The company that manufactures *B lactis* was contacted for unpublished data. The review was restricted to RCTs performed in preterm infants <37 weeks of gestation and/or with a birth weight <2500 g.

Results: Four RCTs involving 324 infants met the inclusion criteria. Compared with controls, *B lactis* supplementation has the potential to increase fecal bifidobacteria counts and to reduce Enterobacteriaceae and *Clostridium* spp counts. It also can reduce stool pH and fecal calprotectin concentrations, increase fecal immunoglobulin A and short-chain fatty acid concentrations, and decrease intestinal permeability. Compared with controls, *B lactis* supplementation had no effect on the risk of necrotizing enterocolitis stage ≥2 (3 RCTs, n = 293, risk ratio [RR] 0.53, 95% CI 0.16–1.83), risk of sepsis (2 RCTs, 397 cultures, RR 0.6, 95% CI 0.07–5.2), and use of antibiotics (2 RCTs, n = 255, RR 0.67, 95% CI 0.28–1.62). The power of these studies, however, does not allow for a definitive statement regarding a reduced risk of necrotizing enterocolitis. *B lactis* supplementation did have some effects on anthropometric parameters. No adverse events associated with *B lactis* supplementation were reported.

Conclusions: Evidence regarding the potential beneficial effects of *B lactis* supplementation in preterm infants is encouraging. Further studies to assess clinically relevant outcomes are needed.

Key Words: infant, premature, probiotics, RCT

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Presently, probiotics defined as ''live microorganisms which when administered in adequate amounts confer a health benefit on the host'' (1) are increasingly being used in the pediatric population; however, uncertainty exists regarding the use of probiotics in preterm infants. The rationale for such supplementation is based on data demonstrating differences in the establishment of the intestinal microbiota in preterm infants (2–4). Although possible consequences to health are not known, it has been speculated that abnormal patterns of colonization in preterm infants may contribute to the pathogenesis of necrotizing enterocolitis (NEC) and to the increased susceptibility to infections (5). It has also been suggested that enteral administration of probiotics to preterm newborns could prevent infections, prevent NEC, and reduce the use of antibiotics (5). Previously, 2 systematic reviews aimed at determining the effect of probiotics on the prevention of NEC in preterm infants were performed (6,7). Both found that the use of probiotics may reduce the risk of severe NEC and mortality in preterm infants. Critics of using a meta-analytical approach to assess the efficacy of probiotics argue that the beneficial effects of probiotics seem to be strain specific; thus, pooling data from different strains may result in misleading conclusions. Given these concerns, the present review was undertaken to update data on the efficacy and safety of using only 1 probiotic strain—*Bifidobacterium animalis* subsp *lactis* CNCM I-3446—in preterm infants. Hereafter, this probiotic strain is referred to as *B lactis*. The choice of the probiotic strain was determined by the fact that it is widely available in many countries and commonly used in formulas and foods for infants.

MATERIALS AND METHODS

Search Strategy

The guidelines from the Cochrane Collaboration for undertaking and reporting the results of this systematic review and meta-analysis were followed (8). The Cochrane Central Register of Controlled Trials (CENTRAL, the Cochrane Library, Issue 4, 2008), MEDLINE (1966–2008), and proceedings from the European and North American societies for pediatric gastroenterology, hepatology, and nutrition conferences were searched in December 2008 for randomized controlled trials (RCTs). The reference lists from identified studies and key review articles, including previously published meta-analyses assessing the effects of probiotics in preterm infants, were also searched. The Nestlé Nutrition Institute, representing the company that manufactures *B lactis*, was contacted to help identify unpublished data. No language restrictions were applied. The search strategy included the use of a validated filter for identifying RCTs, which was combined with a topic-specific strategy. In brief, the following search terms were used: neonate(s),

newborn(s), preterm(s), premature(s), probiotic(s), and bifidobacterium. The search strategy used both key words and MeSH terms.

Studies and Participants

The review was restricted to RCTs carried out in preterm infants <37 weeks of gestation and/or with a birth weight <2500 g. Participants in the experimental groups received *B lactis* at any dosage regimen. Subjects in the control group received placebo or no intervention.

Outcome Measures

The nonclinical outcome measures were as follows: stool colony counts of bifidobacteria, lactobacilli, colonization with enteric pathogenic bacteria, and stool characteristics. The clinical outcome measures were as follows: anthropometric parameters, incidences of NEC stage 2 or greater, blood culture–proven sepsis, *B lactis*–positive blood cultures, use of antibiotics, overall mortality, time until full enteral feedings, and adverse events. In addition to these outcomes, a priori it was decided to extract other data reported by the investigators if clinically relevant to the present review.

Assessment of Risk of Bias in Included Trials

The following criteria for assessing the risk of bias in all of the studies that met the inclusion criteria were evaluated: generation of allocation sequences and allocation concealment; blinding of investigators, participants, outcome assessors, and data analysts; intention-to-treat analysis; and comprehensive follow-up (≥80%). In all of the cases, an answer of ''yes'' indicates a low risk of bias and an answer of ''no'' indicates a high risk of bias.

Data Extraction

Data extraction was performed using standard data-extraction forms by H.S. For dichotomous outcomes, the total number of participants and the number of participants who experienced the event were extracted. For continuous outcomes, the total number of participants and the means and SDs were extracted if provided by the authors. If not, we present data as reported by the authors of the original papers. If feasible, the data were entered into Review Manager (RevMan) (computer program, version 5.0, Copenhagen: the Nordic Cochrane Centre, the Cochrane Collaboration, 2007) for analysis.

Statistical Methods

The data were analyzed using RevMan. The binary measure for individual studies and pooled statistics is reported as the risk ratio (RR) between the experimental and control groups with 95% confidence intervals (95% CIs). The mean difference (MD) or weighted mean difference (WMD), as appropriate, between the treatment and control groups was selected to represent the difference in continuous outcomes (with 95% CI). The χ^2 test was used to assess heterogeneity, and the Higgins I^2 statistic was used to determine the percentage of total variation across the studies due to heterogeneity (9). A value of 0% indicates no observed heterogeneity, and larger values show increasing heterogeneity. If there was heterogeneity >50%, then results of both random effects and fixed effects models for the main analysis are presented. Although funnel plots to determine publication bias were planned, there were too few studies to warrant generation of funnel plots.

RESULTS

Table 1 summarizes the key characteristics of the 4 included RCTs that described 3 study populations. These studies involved 324 participants (169 in the *B lactis* group and 155 in the control group). All of the trials were full peer-reviewed publications (10–12a). Two trials obtained data from the same population but reported different outcomes (10,12). The Stratiki et al (11) article showed consistently n = 41 in the experimental group but reported the number of controls as n = 34 or n = 36 in different places. Because n = 34 was reported more often, this value has been used by us. Only 1 trial (12a) included a portion of preterm infants <27 weeks of gestation (of a total of 94 infants, 47 of them received *B lactis*). In the other trials, the average gestational age was 31 weeks (10–13). In all but 1 trial, *B lactis* was added to preterm formula; in the remaining trial, it was added to human milk as part of the human milk fortifier (12a). The durations of the interventions ranged from 3 to 6 weeks. The doses of the probiotic used ranged from 2×10^7 colony-forming units (CFU)/g of dry milk per day to 6×10^9 CFU/kg of body weight per day. For a number of clinical outcomes (eg, NEC), studies were not designed and powered sufficiently to demonstrate a difference if one actually exists.

Table 1 shows the results of the methodological quality assessment of the included studies. None of the trials reported an adequate method to conceal allocation. All of the trials were described as ''double blinded.'' An adequate description of the intention-to-treat analysis was provided in only 1 RCT (12a). Withdrawals and dropouts were described adequately in 2 studies (11,12a).

Nonclinical Outcomes

Fecal Bifidobacteria

In a trial involving 75 infants, Stratiki et al (11) reported data related to the median counts of fecal bifidobacteria, both before supplementation with *B lactis* and 7 and 30 days later. Median counts of bifidobacteria were similar in the 2 groups at study entry. After 7 days of *B lactis* supplementation, median bifidobacteria counts were significantly higher in the probiotic group than in the control group ($P = 0.035$); at day 30, there was no statistically significant difference in median bifidobacteria counts between groups ($P = 0.075$). Mohan et al (10) reported that in a group of 69 preterm infants, the counts of bifidobacteria analyzed weekly were significantly higher in the probiotic group than in the placebo group ($P = 0.001$).

Enterobacteriaceae and Clostridia

In a trial involving 69 infants, Mohan et al (10) demonstrated that infants in the placebo group had higher numbers of Enterobacteriaceae and *Clostridium* spp than infants in the probiotic group ($P = 0.015$ and $P = 0.014$, respectively).

Other Bacteria

Mohan et al (10) reported that there were no significant differences between the probiotic and placebo groups in the numbers of *Staphylococcus* spp, *Streptococcus* spp, *Bacteroides* spp, and *Candida* spp. The investigators also reported data related to gut colonization by resistant bacterial strains in infants treated with or without antibiotics. There was no significant difference between the probiotic and placebo groups with regard to the number of neonates colonized with antibiotic-resistant bacterial strains, irrespective of the antibiotic treatment.

TABLE 1. Characteristics of included trials

Study ID	Design	Allocation concealment	Blinding	ITT	Description of withdrawals or dropouts	N (exp/cont)	Average gestational age	Duration of intervention	Intervention (dose)	Placebo
Mohan et al (10)	RCT	Unclear	Yes	Per protocol?	No	69 (37/32)	31 wk	21 d	2×10^9 CFU/g of powder (daily dose on day 1–3 was 1.6×10^9 CFU, then from day 4–4.8×10^9 CFU)	Formula-based placebo
Mohan et al (12) (same population as (10))	As above	As above	As above	As above	As above	As above	As above	As above	As above	As above
Stratiki et al (11)	RCT	Unclear	Yes	No	Yes	75 (41/34)	31 wk (27–37)	30 d	2×10^7 CFU/g of dry milk	Formula without probiotics
Mihatsch et al (12a)	RCT	Unclear	Yes	Yes	Yes	180 (91/89)	26 (23–29)	6 wk of life	Daily dose of 6×10^9 CFU/kg given in human milk fortifier	Placebo (human milk fortifier)
Total						324 (169/155)				

CFU = colony-forming units; ITT = intention-to-treat analysis; RCT = randomized controlled trial.

Intestinal Permeability

One study (11) provided results regarding intestinal permeability. The lactulose/mannitol (L/M) ratio declined in both groups from day 1 to day 7 of the study. At day 7, there was no significant difference between the groups ($P = 0.073$). On day 30, the L/M ratio was significantly lower in the probiotic group compared with the control group ($P = 0.003$).

Stool Parameters

Investigators in 1 RCT (12) involving 69 preterm infants reported on a number of stool parameters. In the probiotic group compared with in the placebo group, the fecal pH was significantly lower (5.68 ± 0.09 vs 6.38 ± 0.10; $P < 0.001$) as were fecal calprotectin levels ($P = 0.041$). Fecal immunoglobulin A (IgA) levels were significantly higher in the probiotic group compared with the placebo group ($P = 0.021$). The same study showed that the fecal concentration of acetate, which was the major short-chain fatty acid (SCFA) contributing 90% to the total SCFAs, was 42% higher in the probiotic group compared with the placebo group ($P < 0.001$). Fecal propionic and butyric acid concentrations were also higher in the probiotic group than in the placebo group ($P = 0.04$ and $P = 0.026$, respectively). The fecal lactate concentration was 38% higher in the probiotic group compared with the placebo group ($P = 0.011$).

Clinical Outcomes

Anthropometric Variables

The effect of *B lactis* administration on weight was studied in 2 trials (11,12) in a total of 144 infants. One trial (12) involving 69 preterm infants provided data on weight gain during the study period (21 days) (Fig. 1). Compared with controls, the use of *B lactis* was associated with a significant increase in weight in all of the infants (n = 69, 1882 ± 53 vs 1836 ± 71, MD 46 g, 95% CI 16.05–75.95), although this was reported as a nonsignificant difference by the authors; a significant increase in weight in infants treated with antibiotics (n = 46, 1574 ± 65 vs 1375 ± 74, MD 199 g, 95% CI 158–240; and a similar weight in infants treated without antibiotics (n = 23, 1900 ± 78 vs 1941 ± 79, MD −41 g, 95% CI −105 to 23.2). One RCT (11) provided data on weight gain in grams per day. The investigators reported that weight gain did not differ between the probiotic group and the control group (28.3 [range 12–38] vs 30 [range 10–40] g/day, respectively; $P = 0.14$). Also, length gain was similar in the probiotic and control groups (1.4 [range 0–3] vs 1.5 [range 0–3.5] cm/week, respectively; $P = 0.27$). Head growth was significantly greater in the probiotic group compared with the control group (1.1 [range 0.45–1.9] vs 0.9 [range 0–2] cm/week, respectively; $P = 0.001$).

Necrotizing Enterocolitis

The pooled results of 3 RCTs involving 293 preterm infants (10,11,12a) revealed no significant difference in the incidence of NEC stage 2 or greater between the probiotic and control groups (RR 0.47, 95% CI 0.15–1.45, fixed effects model). No heterogeneity was found ($\chi^2 = 1.94$, $P = 0.38$, $I^2 = 0\%$) (Fig. 2).

Culture-proven Sepsis

Two RCTs provided data regarding sepsis (11,12a) (Fig. 2). The pooled results showed no significant difference between the probiotic and the control groups in the incidence of culture-proven

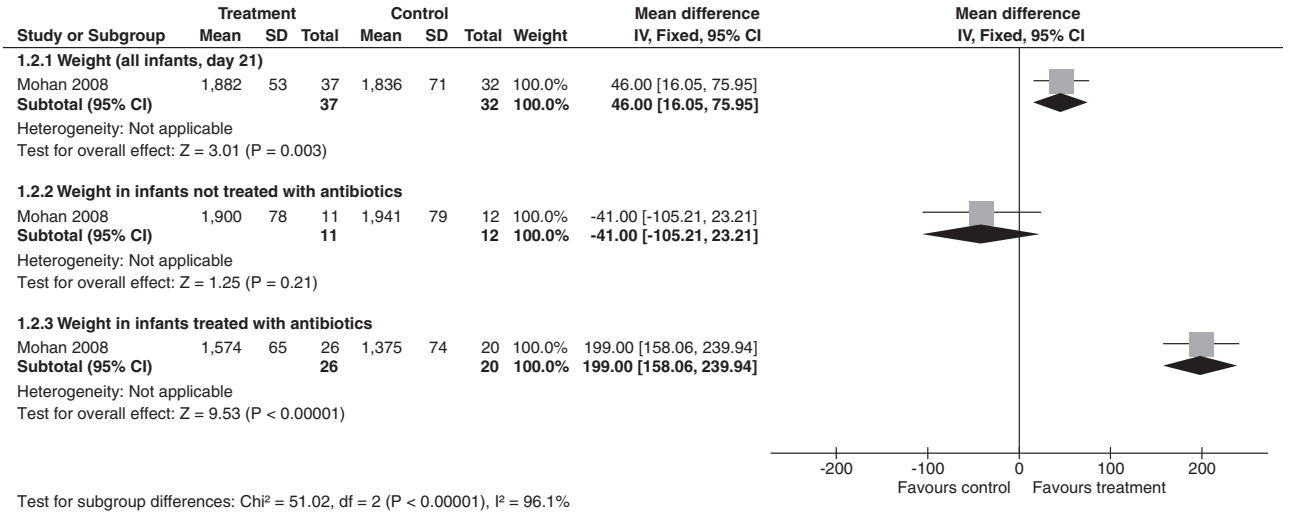

FIGURE 1. Weight parameters.

sepsis (397 cultures [multiple cultures were obtained from some patients]; RR 1.09, 95% CI 0.69–1.72, fixed effects model; and RR 0.6, 95% CI 0.07–5.2, random effects model). Some heterogeneity was detected between the trials ($\chi^2 = 2.48$, $P = 0.12$, $I^2 = 60\%$).

B Lactis-positive Cultures

Only RCT (12a) reported this outcome and found that none of the positive blood cultures grew *B lactis.*

Use of Antibiotics

This outcome was estimated in 2 RCTs (11,12a) (Fig. 2). The pooled results showed a significant difference between the probiotic and the control groups in the use of antibiotics (2 RCTs, n = 255, RR 0.83, 95% CI 0.72–0.96) in the fixed effects model. Significant heterogeneity between the trials was detected ($\chi^2 = 11.99$, $P = 0.0005$, $I^2 = 92\%$). The significant reduction in the use of antibiotics in the probiotic-supplemented group was lost in the random effects model (RR 0.67, 95% CI 0.28–1.62).

Nosocomial Infections

One RCT (12a) revealed an increase in the total number of nosocomial infections (defined as periods of elevated C-reactive protein >10 mg/dL) in preterm infants receiving *B lactis*, although the difference between groups was of a borderline statistical significance (n = 180, RR 1.36, 95% CI 1.03–1.79, random effects model). The present study also revealed a similar rate of the incidence density of nosocomial infections (defined as the number of nosocomial infections/total number of patient days) during the first 6 weeks of life in the probiotic and placebo groups (0.021 vs 0.016, $P > 0.9$).

Time Until Full Enteral Feedings (Day)

In the study by Stratiki et al (11), there was no significant difference in the time until full enteral feedings in the probiotic group compared with the control group (10 [range 0–52] vs 10 [range 0–30] days; $P = 0.615$). Also, Mihatsch et al (12a) found no significant difference between the groups in the time until feeding 150 mL/kg (17.9 ± 6.8 days in the *B lactis* group vs 18 ± 7.4 days in the placebo group; MD −0.1, 95% CI −2.2 to 2).

Adverse Events

B lactis was well tolerated, and no adverse events associated with this supplementation were reported in any of the trials.

DISCUSSION

The objective of this review was to provide some resolution to the uncertainty regarding the use of *B lactis* in preterm infants. The use of *B lactis* resulted in significantly higher stool colony counts of bifidobacteria (although transient in 1 study), as assessed by appropriate microbiological analyses. It is generally accepted that the gut flora is of great importance to gastrointestinal physiology and appears to modulate the health and well-being of the host organism (14,15). The lower incidence of gastrointestinal and other infections found in breast-fed infants (16–18) may, in part, be related to their gut flora; that is, a predominance of *Bifidobacterium* and *Lactobacillus* is found in their feces, which in term infants may contribute to up to 90% of the total flora (19). Therefore, the establishment of a gut microbiota, closer to that of breast-fed term infants, in preterm infants after supplementation with *B lactis* may be considered in light of the present hypothesis that aberrant gut microbiota may influence the development of NEC (5).

The use of *B lactis* reduced growth of Enterobacteriaceae and *Clostridium* spp (10). This may contribute to resistance against infections; however, interventional studies with clinically relevant outcomes are needed to confirm this finding.

B lactis supplementation stimulates the production of SCFAs, primarily acetic acid and lactic acid; these SCFAs are measurable products of bacterial fermentation and play a role in normal colonic functions (12). One of the SCFAs that was increased by the administration of *B lactis* was butyric acid. Butyrate is able to beneficially affect oxidative stress in the healthy human colon (20); it is thought to be an important energy source for intestinal epithelial cells and plays a role in the maintenance of colonic homeostasis (21). Overall, however, whether the increase in SCFA concentrations per se is of benefit in preterm infants is presently not well established. The same applies to other stool parameters such as the reduced fecal pH values seen in preterm infants who have received

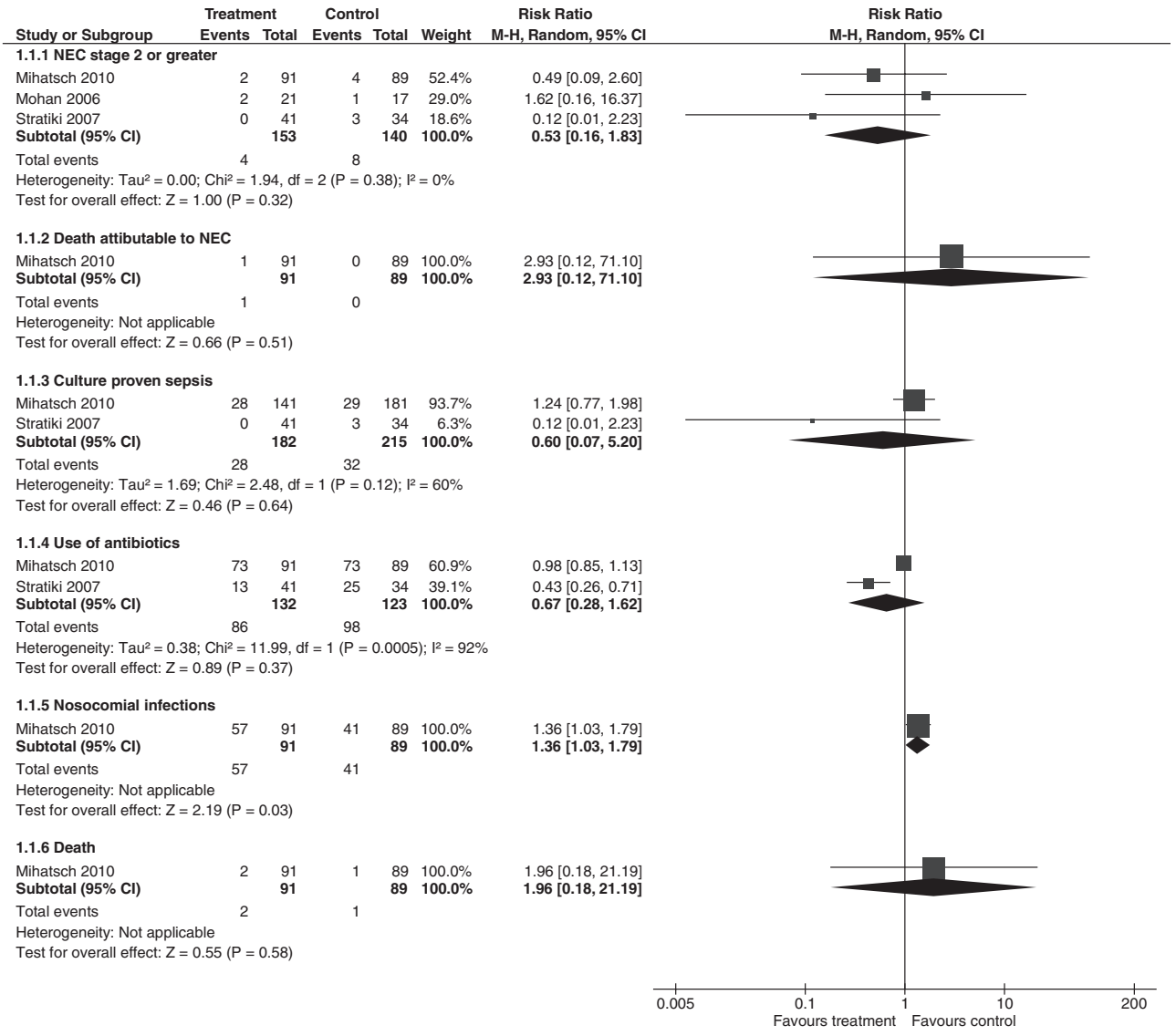

FIGURE 2. Clinical outcomes.

B lactis. One trial showed that *B lactis* supplementation reduced the fecal calprotectin level (12) known to be a useful marker of gastrointestinal mucosal inflammation in neonates (22). It also showed that *B lactis* supplementation increased fecal IgA secretion (12). Considering that IgA is an antibody that plays a critical role in mucosal immunity, *B lactis* supplementation may have an effect on the development of the immune system in prematurely born infants.

B lactis decreased intestinal permeability, as measured by the double sugar (L/M) absorption test (11). This test has been widely used in pediatrics, and it is a well-established means of assessing the permeability of the intestinal barrier to exogenous molecules (23). It is known that the L/M ratio (although not directly related to gestational age) is higher in preterm infants than in term infants at birth; however, within 2 weeks of life, it decreases to values similar to those found in full-term newborns (24,25). It is thought that the higher intestinal permeability observed in preterm infants (and also in full-term infants when compared to subsequent months) may have disadvantageous effects. For example, it may cause the increased uptake of antigens, potentially leading to the development of inflammation and systemic hypersensitivity. Thus, the favorable effect of *B lactis* supplementation on intestinal permeability may indeed prove beneficial, although clinical evidence to support this notion is lacking.

On the basis of the results of 3 studies, there was no significant difference in the incidence of NEC stage 2 or greater between the groups. The power of these studies does not allow for a definitive statement regarding a reduced risk of NEC with *B lactis* supplementation. Given the positive results with other probiotics as documented in 2 recent meta-analyses (6,7), a large RCT to investigate the efficacy of *B lactis* supplementation in the prevention of severe stage NEC is warranted. Interestingly, the overall risk reduction rate in the development of NEC with the use of different probiotic strains, at different dosages and with different postnatal starting dates, is around 60% (6,7); this percentage is close to what is found as the risk reduction rate when using *B lactis* supplementation. The mechanism by which bifidobacteria exert their action in preventing the development of NEC in preterm infants is unclear. Possible mechanisms include an increased barrier to the

translocation of bacteria across the mucosa, exclusion of pathogens, modification of the host response, acidification of intestinal content, and enhancement of enteral nutrition.

No effect of *B lactis* supplementation on the risk of sepsis was observed. *B lactis* supplementation also did not reduce the use of antibiotics. The lack of an effect on the risk of sepsis is in line with the results of 2 recent meta-analyses that evaluated the efficacy of various probiotic supplementation regimens; in both, the risk of sepsis did not differ significantly between the probiotic and control groups (6,7).

The effect on growth is an important part of the safety evaluation of any product used in infants (26). *B lactis* supplementation results in weight gain and length gain similar to what are found in nonsupplemented infants. The head circumference gain may be greater in *B lactis*–supplemented infants compared with nonsupplemented infants. The mechanism as to how *B lactis* supplementation influences head circumference growth, without having an influence on weight or length gain, however, is poorly understood.

The optimal dose of *B lactis*, as with other probiotics, is largely unknown because no dose-response studies have been performed. On the basis of the available data, the dose of *B lactis* for preterm infants should not be less than $1.6 \times 10^9\,\mathrm{CFU} \cdot \mathrm{kg}^{-1} \cdot \mathrm{day}^{-1}$. No information on safety is available for doses higher than $6.0 \times 10^9\,\mathrm{CFU} \cdot \mathrm{kg}^{-1} \cdot \mathrm{day}^{-1}$.

The safety profile of *B lactis* seems to be good. In the included trials, no adverse events associated with *B lactis* supplementation were reported; however, the included studies were underpowered for addressing adverse events. The safety issue is important, as based on the available literature; there is concern that the use of probiotics in at-risk populations may result in harmful events. Most complications have occurred in immunocompromised subjects or in patients with other life-threatening illnesses, who were managed in intensive care units and treated with probiotics (27); however, *B lactis* or any other *Bifidobacterium* spp was not involved in any of the reported cases. In this context, particularly relevant is the absence of blood cultures positive for *B lactis*, as documented in 1 of the included trials. Although there are no data to suggest that any categories of preterm infants should avoid use of *B lactis*, it is noteworthy that data related to infants of very low birth weights (<1000 g) are limited.

Limitations

There are several limitations to this review that we acknowledge. The number of trials, as well as the sample size in some trials, were small. The methodological quality and the quality of reporting results were variable and sometimes poor. Potential limitations include unclear allocation concealment and no intention-to-treat analysis. The findings are, therefore, likely to be affected to a varying degree by selection, attrition, and/or performance biases.

Conclusions and Further Research

Evidence related to the use of *B lactis* in preterm infants is encouraging, even if not yet fully convincing. *B lactis* supplementation has the potential to increase the total number of bifidobacteria in feces and to reduce enterobacteria and clostridia. It can also reduce stool pH and fecal calprotectin levels, increase the concentrations of fecal IgA and SCFA, and decrease intestinal permeability. Presently, the safety profile of *B lactis* is good. Although there is no well-documented evidence of clinical benefits associated with *B lactis* supplementation in preterm infants, a reduction in the risk of developing NEC is likely. Further data are particularly required to determine the effect of *B lactis* supplementation on the risk of NEC as the primary outcome measure. Additional validated clinical outcome measures (eg, growth parameters, all-cause mortality, NEC-related mortality, adverse effects) assessing the effects of *B lactis* supplementation in preterm infants should be used in well-designed and carefully conducted RCTs, with relevant inclusion/exclusion criteria and adequate sample sizes. Such trials should also define the optimal dose and intake durations. In addition, biomarkers of protection and inflammation should be identified. The evidence suggests that it is safe to supply preterm infants with *B lactis* under medical supervision; however, data related to the use of *B lactis* in infants with birth weights <1000 g are still too limited to allow any such conclusion for this subgroup of preterm infants.

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