Gut microbiota and probiotics in maternal and infant health[1–4]

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ABSTRACT

The interplay between both heredity and environmental factors seems to affect every stage of development from conception to the early postnatal period with potential long-term effects on child and adult health. During pregnancy, immune and metabolic functions of the fetus are dependent on the mother; moreover, the refinement of these functions seems to commence inside the uterus and to be diet sensitive. The microbiota inhabiting the intestinal tract develop an array of physiologic roles within the human body, which influences both metabolic and immune functions, particularly during early neonatal life and possibly even in utero. Transmission of bacteria from the mother to the neonate through direct contact with maternal microbiota during birth and through breast milk during lactation also seems to influence the infant's gut colonization, with potential health consequences. In this context, intentional modulation of microbiota composition through the use of probiotics during the perinatal and early postnatal period has been proposed as a possible dietary strategy to reduce risk of disease. Herein, studies are reviewed on the composition of the intestinal microbiota during pregnancy and clinical trials evaluating the effects of perinatal administration of probiotics on different clinical outcomes. *Am J Clin Nutr* 2011;94(suppl):2000S–5S.

INTRODUCTION

The gut microbiota constitutes a complex ecosystem involved in physiologic functions critical for human life (1, 2). The microbes inhabiting the human gut provide additional metabolic capacities to their host and regulate expression of genes involved in lipid and carbohydrate metabolism, which thereby influences the nutrient supply, energy balance, and body weight (1, 3). The gut microbiota is also a critical stimulus for the adequate maturation of the immune system, which contributes to reducing infections and aberrant immune responses (4). Exposure to microbes in early life, which largely occurs through the microbial colonization of the newborn intestine, has been related to susceptibility to infections and sensitization to environmental antigens in early and later life (5–7). These observations constitute the basis of the "hygiene hypothesis," according to which the lack of microbial exposure due to highly hygienic conditions found in the Western world prevents proper maturation of the immune system and predisposes individuals to allergies (8) and possibly to other immunologic diseases (9). This theory also fits in the programming concept, which refers to events or stimuli that during critical periods of development may "program" the long-term structure or function of an organism (10). Within this scenario, the administration of probiotics and prebiotics during the early postnatal period to intentionally modulate the microbiota composition has been proposed as a possible dietary strategy to reduce the risk of disease (6, 7, 11). The administration of probiotics during the perinatal period and lactation to favor infant gut colonization with potentially beneficial bacteria has also been proposed on the strength of the evidence that bacteria are transmitted from mother to neonate through direct contact with maternal microbiota during birth and through the supply of beast-milk bacteria during lactation (12, 13).

Observational and interventional studies suggest that diet and exposure to microbes during pregnancy may influence the metabolic and immunologic profiles of the pregnant uterus and the risk of disease developing in offspring later in life (14). Therefore, the possible roles the composition of the gut microbiota play in women's health during pregnancy and its possible influence on the maternal-fetal interactions in utero have also been investigated recently (15, 16). Herein, the current knowledge of gut microbiota in pregnant women and its possible influence on both maternal and infant health is reviewed.

GUT MICROBIA DURING PREGNANCY

Some studies have focused on the characterization of microbiota composition during pregnancy with a view to its possible influence on the mother's health and mother-fetal interactions influencing the infant's health later in life. In a recent observational study, the fecal microbiota of 50 pregnant women, classified as normal weight (n = 34) or overweight (n = 16), was analyzed, and the results were related to body weight, body weight gain, and serum biochemical variables at 24 wk of pregnancy (17). The numbers of *Bifidobacterium* and *Bacteroides* were low (0.7 logarithmic units), whereas the numbers of *Staphylococcus, Enterobacteriaceae*, and *Escherichia coli* were high (0.9–1.4 logarithmic units) in overweight compared with normal-weight pregnant women. In addition, *E. coli* numbers were higher (1 logarithmic unit) in women with excessive weight gain than in women with normal weight gain during pregnancy. Moreover, maternal *E. coli* numbers were

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positively correlated with infants' birth weight, which suggested the transfer of maternal features to the newborn (17). Another study was conducted that focused on the relation between the microbiota composition, body weight, and body weight gain during pregnancy; however, the associations were not so clearly established (18). In this case, the fecal microbiota of overweight women (n = 18) and normal-weight women (n = 36) at 10–15 wk of gestation and at 30–35 wk of gestation was followed up (18). The study reports differences in *Staphylococcus aureus* and *Bacteroides-Prevotella* group numbers, which were significantly higher in overweight than in normal-weight women; notwithstanding, the real differences were of <0.5 logarithmic units (6.50 compared with 6.15 log cells/g and 10.55 compared with 10.36 log cells/g). In addition, increased *Bacteroides* concentrations were correlated with excessive weight gain over pregnancy, but this was not confirmed by comparing the mean counts of this bacterial group in women with excessive and normal weight gain (18).

In most human and animal studies, increases in the abundance of *Bacteroidetes* phylum or *Bacteroides* subgroups have been associated with a lean phenotype and with weight loss under dietary intervention (19–22), with some exceptions (23, 24) as reported in pregnant women by Santacruz et al (17). Increased numbers of *Bifidobacterium* were also found in the feces of children maintaining normal weight, whereas increased numbers of *S. aureus* were found in the feces of those becoming overweight during infancy (25), following the same trend as that reported in the first study in pregnant women (17).

Features of the fecal microbiota of women have also been associated with serum biochemical variables of relevance to the nutritional and health status during pregnancy (eg, cholesterol, folic acid, ferritin, and reduced transferrin) and with possible consequences on fetal health programming (17). However, there is no direct evidence of the roles and mechanisms of action of each bacterial group in the regulation of these variables. In animals following a high-fat diet, obesity has also been associated with increases in numbers of intestinal Gram-negative bacteria and increases in intestinal permeability and plasma lipopolysaccharide concentrations. In particular, lipopolysaccharide was identified as an inflammatory factor causative of chronic metabolic disorders such as diabetes (26, 27). Increased serum lipopolysaccharides have also been associated with increased BMI in patients with cardiovascular disease (28) and following high-fat diets (29). In light of this evidence, one can speculate that enterobacteria and *E. coli* could play a similar adverse role in pregnant women; nevertheless, direct evidence of this assumption should be provided.

Overall, the mother's intestinal microbiota, body weight, and metabolic biomarkers seem to be linked, which could contribute to fetal health programming in utero and to the inoculation of the newborn intestine with an aberrant or healthy microbiota after birth, with consequences on later health, which deserve further investigation.

EFFECTS OF PROBIOTIC INTAKE DURING THE PERINATAL PERIOD IN HUMANS

The clinical trials carried out to investigate the different outcomes of oral administration of probiotic bacteria to the pregnant women alone or to both pregnant women and their infants are summarized in **Table 1**. A pilot study including 6 women, who were taking *L. rhamnosus* GG during late pregnancy but discontinued its consumption at the time of delivery, was carried out to evaluate the influence of probiotic intake on their children, who did not received the probiotic after birth (12). Despite the limited number of subjects studied, the results showed that temporary colonization of the infant's gut with *L. rhamnosus* GG was possible by giving the probiotic to the pregnant mother before delivery and that this colonization was stable for up to 6 mo (12). Further studies showed that the administration of *L. rhamnosus* GG to mothers (n = 29), 4 wk before and 3 wk after delivery, induced specific changes in the transfer and initial establishment of bifidobacteria in neonates compared with those receiving placebo (n = 20) (30). Infants whose mothers received *L. rhamnosus* GG had a higher prevalence of *B. breve* and a lower prevalence of *B. adolescentis* than did those in the placebo group at 5 d of age. The rationale behind the influence of *L. rhamnosus* GG on *Bifidobacterium* species composition was not provided. In the aforementioned study, the prevalence of *B. adolescentis* in the mother before delivery was also correlated with its presence in infant samples at 1 and 5 mo, and similar effects were detected for *Bifidobacterium catenulatum* and *Bifidobacterium longum* at 1 mo, although these effects were only significant in the placebo group. Altogether, these results suggest that bacteria are transferred from mother to newborn. However, *L. rhamnosus* GG consumption also increased the bifidobacterial diversity in infants at 3 wk and reduced the similarity of *Bifidobacterium* microbiota between mother and infant (30). This partly contradicts the evidence of fecal microbiota transference from mother to newborn or suggests that the intake of probiotics alters the transfer process identified in the placebo group.

The administration of probiotics during pregnancy is also under consideration because of the positive effects some strains exert on certain clinical conditions. The effectiveness of probiotics in preventing preterm labor and birth has been the focus of recent studies, because in the presence of maternal infection the risk of this outcome reaches values of 30–50% (31). It has been suggested that specific probiotics could exert beneficial effects on such applications because of their ability to displace and inhibit pathogens and to interfere with the inflammatory cascade that leads to preterm labor and delivery. The 2 randomized controlled trials, reported in 2006, assessing the prevention of preterm birth by administration of probiotics in pregnant women and women planning pregnancy were reviewed recently (31). One study, using orally administered fermented milk as a probiotic, enrolled women after 34 wk of pregnancy, whereas the other study enrolled women with bacterial vaginosis in early pregnancy and administered commercially available yogurt vaginally. The results showed an 81% reduction in the risk of genital infection after the probiotics were administered. However, these are the only prespecified clinical data available; insufficient data are available to assess the actual effect on preterm birth and its complications.

The use of probiotic bacteria during pregnancy has also been proposed as a means of modulating immune development in the fetus, thereby reducing the risk of immune aberrancies and improving the host's defenses. In this context, the effects of the consumption of milk fermented with the strain *Lactobacillus casei* DN11401 by pregnant women (n = 54), during the 6 wk before delivery and the 6 wk of lactation, were determined and compared with those of a placebo group (n = 39) (32). Mothers taking the probiotic showed a significant increase in natural killer cells in peripheral blood samples and a nonsignificant increase in T and

TABLE 1
Effects of the perinatal administration of probiotics in humans[1]

Probiotic/prebiotic	Administration regimen	Outcome	Reference
Lactobacillus rhamnosus GG	Women at late pregnancy but not after delivery	Probiotic colonization of the infant's gut	12
L. rhamnosus GG	Women 4 wk before and 3 wk after delivery	Changes in bifidobacteria transfer and establishment in the neonates	30
Fermented milk and yogurt bacteria	Women at 34 wk of pregnancy orally or vaginal application from first trimester onward	Reduction of genital infection risk	31
Lactobacillus casei DN11401	Women 6 wk before delivery and during 6 wk of lactation	Natural killer cell increase in mother's peripheral blood and TNF-α decrease in breast milk; decrease in gastrointestinal episodes in infants	32
L. rhamnosus GG and LC705, *Bifidobacterium breve* Bb99, *Propionibacterium freudenreichii* subsp. *shermanii*, and galactooligosaccharides	Women carrying fetus at allergy risk during the last month of pregnancy and by their infants until the age of 6 mo plus a prebiotic	Increased resistance to respiratory infections in children for 2 y; tended to reduce IgE-associated diseases and prevented atopic eczema at 2 y and at 5 y; only in cesarean-delivered children; increase in fecal lactobacilli and bifidobacteria	11, 33, 34
L. rhamnosus GG	Women at family risk of atopic eczema for 4 wk before delivery and postnatally for 6 mo	Reduction of atopic eczema risk for up to 7 y; increase in TGF-β2 in mother's milk	35, 36
L. rhamnosus GG and *Bifidobacterium lactis* Bb2	Women carrying fetus at allergy risk from the first trimester of pregnancy until the end of exclusive breastfeeding	Modest increase in TGF-β2 only in colostrum; reduced allergen sensitization in infants	37
L. rhamnosus GG	Women carrying fetus at allergy risk for 36 wk before delivery	No effect on fetal antigen-specific immune responses evaluated in cord blood cells	15
L. rhamnosus GG	Women at risk of atopic diseases from 4 to 6 wk before delivery and postnatally for 6 mo	No effect on incidence of atopic dermatitis	38
Lactobacillus reuteri ATCC 55730	Women from gestational week 36 and by infants until 12 mo	Less IgE-associated eczema during the second year of life; no effect on cumulative incidence of eczema	39
L. rhamnosus HN001 or *Bifidobacterium animalis* subsp *lactis* HN019	Women from 35 wk gestation until 6 mo if breastfeeding; infants from birth to 2 y	Only infants in *L. rhamnosus* group had a significantly reduced risk of eczema	40
Bifidobacterium bifidum W23, *B. animalis* subsp. *lactis* W52, and *Lactococcus lactis* W58	Women 6 wk before delivery and infants for 12 mo	Parent-reported eczema was significantly lower during the first 3 mo of life but not later	41
Dietary recommendations, *L. rhamnosus* GG, and *B. lactis*	Women from first trimester of pregnancy onward	Highest and lowest intakes of specific nutrients associated with higher blood pressure in children at 6 mo but not with probiotic intake	42
L. rhamnosus GG, *B. lactis* Bb12, and dietary counseling	Women from first trimester of pregnancy onward	Reduced blood glucose concentrations and increased glucose tolerance during pregnancy and over the 12 mo postpartum	16
L. rhamnosus GG	Women 4 wk before expected delivery and for 6 mo postnatally	Childhood growth patterns and the development of overweight for 10 y not significant; a trend only to moderate the initial phase of weight gain and reduce the birth weight	43

[1] TNF-α, tumor necrosis factor-α; TGF-β2, transforming growth factor-β2; IgE, immunoglobulin E.

B lymphocytes. Maternal milk also showed a decrease in the proinflammatory cytokine tumor necrosis factor-α. Breastfed children of the mothers who consumed *L. casei* also registered fewer total gastrointestinal symptoms, including oral candidiasis, regurgitation, diarrhea, colic, and constipation during the 2–6-mo period (29.4 compared with 54.1). The safety and effects of a mixture of 4 probiotic bacterial strains (*L. rhamnosus* GG and LC705, *Bifidobacterium breve* Bb99, and *Propionibacterium freudenreichii* subsp. *shermanii*) has also been evaluated in pregnant women carrying children at high risk of allergic diseases and in their infants together with a prebiotic galactooligosaccharide ($n = 461$ in the synbiotic group and 464 in the placebo group) for 24 mo. Pregnant women consumed a probiotic preparation or a placebo for 2–4 wk before delivery, and their infants received the same probiotics plus galactooligosaccharides for 6 mo. No differences in growth, infant colic, morbidity, or other adverse health effects were found between the 2 groups of children. A slightly higher percentage of children in the placebo group (28%) than in the probiotic group (23%) were prescribed antibiotics [odds ratio (OR): 0.74; 95% CI: 0.55, 1.00; $P = 0.49$] during the intervention period (6 mo). Also, the total number of respiratory infections occurred less frequently in the synbiotic group (3.7 compared with

4.2 mean infections; OR: 0.87; 95% CI: 0.79, 0.97) throughout the follow-up period (6–24 mo) (33).

Administration of the probiotic *L. rhamnosus* GG to both pregnant mothers and their infants was shown to reduce [42.6% compared with 66.1%; relative risk (RR): 0.64; 95% CI: 0.45, 0.92] the risk of developing atopic eczema during the first 7 y of life in a Finish population of children (n = 116) who completed the follow-up study (35). *L. rhamnosus* GG was given prenatally to mothers who had at least one first-degree relative with atopic eczema, allergic rhinitis, or asthma for 4 wk before expected delivery and to their children, postnatally, for 6 mo. *L. rhamnosus* GG was effective in preventing early atopic disease in children at high risk as determined by considering chronic recurring atopic eczema as the primary endpoint. A subgroup analysis of the cohort found that probiotic administration to the pregnant and lactating mother increased the amount of antiinflammatory cytokine transforming growth factor-β2 in the mother's milk, which was suggested to increase its immunoprotective potential and to be associated with a reduction in the risk of atopic eczema during the first 2 y of life (15% compared with 47%; RR: 0.32; 95% CI: 0.12, 0.85) (36). In addition, Huurre et al (37) provided dietary counseling and probiotic supplementation (*L. rhamnosus* GG and *B. lactis* Bb2) to pregnant women at risk of developing atopy and evaluated the effects on their children. Children of atopic mothers, specifically when exclusively breastfed for 2.5 or 6 mo, had a higher risk of sensitization at the age of 12 mo; however, this risk could be reduced by the use of probiotics during pregnancy and lactation (OR: 0.34; 95% CI: 0.13, 0.88; P = 0.023). The preventive effects were considered to be the result of a beneficial change in breast-milk composition characterized by a modest increase in transforming growth factor-β2 concentration (37); however, this increase was not statistically significant and was only detected in the colostrum but disappeared after 1 mo. Boyle et al (15) investigated whether *L. rhamnosus* GG influenced fetal immune responses when administered to pregnant women for 36 wk before delivery. The effects of stimulation of cord blood mononuclear cells from women who received the probiotic or placebo with heat-killed *L. rhamnosus* GG and ovalbumin were evaluated; no effects of the treatment on CD4(+) T cell proliferation, forkhead box P3 expression, dendritic cell phenotype, or cytokine secretion were observed (15). The effects of the administration of the same probiotic strains and prebiotic used on the study by Kukkonen et al (33) on allergic disease prevention were also evaluated. Probiotic treatment compared with placebo showed no effect on the cumulative incidence of allergic diseases, but prevented atopic eczema (OR: 0.66; 95% CI: 0.46, 0.95) at 2 y (11). Lactobacilli and bifidobacteria more frequently colonized the intestine of supplemented infants, which suggested an inverse association between atopic diseases and gut colonization by probiotics (11). Notwithstanding, in the 891 infants (88%) who were followed up for 5 y, frequencies of allergic and immunoglobulin E (IgE)–associated allergic disease and sensitization were similar in the probiotic and placebo groups (34). No significant differences in the frequencies of eczema, atopic eczema, allergic rhinitis, or asthma were observed between probiotic and placebo groups. Only less IgE-associated allergic disease occurred in cesarean-delivered children receiving probiotics (24.3% compared with 40.5%; OR: 0.47; 95% CI: 0.23, 0.96%) at 5 y of age.

Another clinical double-blind, placebo-controlled trial was carried out to study the preventive effect of the same probiotic, *L. rhamnosus* GG, on the development of atopic dermatitis when administered to pregnant women (n = 94) and their infants in Germany (38) after a dosage regimen similar to that used in previous interventions. In this case, supplementation with *L. rhamnosus* GG during pregnancy and early infancy neither reduced the incidence of atopic dermatitis nor altered the severity of atopic dermatitis in the affected children, but it was associated with an increased rate of recurrent episodes of wheezing bronchitis (26% compared with 9.1%) at the age of 2 y. Another trial was conducted in pregnant women (n = 188) who received *Lactobacillus reuteri* ATCC 55730 (1×10^8 CFU/d) from gestational week 36 until delivery and in their infants from birth until 12 mo of age, who were followed up for 2 y (39). The cumulative incidence of eczema was similar in both the treated and placebo groups; however, the probiotic group had less IgE-associated eczema during the second year of life (8% compared with 20%). Skin-prick test reactivity was also less common in the treated than in the placebo group (14% compared with 31%) only in infants of mothers with allergies. A comparative study of the effects of 2 probiotics was also conducted in pregnant women and their infants (40). Women were randomly assigned to take *L. rhamnosus* HN001, *Bifidobacterium animalis* subsp *lactis* HN019, or placebo daily at gestation week 35 until 6 mo of breastfeeding, and their infants were randomly assigned to receive the same treatment from birth to 2 y (n = 474). Infants receiving *L. rhamnosus* had a significantly reduced risk of eczema [hazard ratio (HR): 0.51; 95% CI: 0.30, 0.85 compared with placebo, but this was not the case for *B. animalis* subsp *lactis* (HR: 0.90; 95% CI: 0.58, 1.41). A mixture of probiotic bacteria (*Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W52, and *Lactococcus lactis* W58) was prenatally administered to mothers of high-risk children 6 wk before delivery and to their offspring for 12 mo after birth, and the follow-up lasted 24 mo (n = 98) (41). Only parental-reported eczema during the first 3 mo of life was significantly lower in the intervention group than in the placebo group (6/50 compared with 15/52; OR: 0.322; 95% CI: 0.108, 0.960); however, between the age of 3–12 mo and 12–24 mo, the incidence of eczema was similar in both groups.

The effects of probiotic supplementation plus dietary counseling on glucose metabolism in pregnant women were also evaluated (16). The study included 3 subgroups of pregnant women (n = 256) in the first trimester of pregnancy. The first group received nutritional counseling to modify dietary intake according to current recommendations (diet/placebo), the second group received nutritional counseling and probiotics (*L. rhamnosus* GG and *B. lactis* Bb12; diet/probiotics), and the third group received placebo without nutritional counseling (control/placebo). Blood glucose concentrations were the lowest in the diet/probiotics group during pregnancy and over the 12-mo postpartum period. Glucose tolerance was also better in the diet/probiotics group than in the control/placebo group during the last trimester of pregnancy and over the 12-mo postpartum period (16); however, the effects on blood pressure in children at 6 mo were unrelated to probiotic intake in another study (42). Finally, the effect of perinatal probiotic intervention on childhood growth patterns and the development of overweight during a 10-y follow-up was also evaluated in 159 women who were randomly assigned and

double-blinded to receive *L. rhamnosus* GG (1×10^{10} CFU) or placebo 4 wk before their expected delivery and for 6 mo postnatally (43). The perinatal probiotic intervention appeared to moderate the initial phase of excessive weight gain (onset during fetal period and continuing until 24–48 mo of age), especially in children who later became overweight, and seemed to reduce the birth weight–adjusted mean body mass index at the age of 4 y; however, the differences were not significant.

CONCLUSIONS

Most clinical trials evaluating the effects of perinatal administration of probiotics to pregnant women and to infants after birth focus on the primary prevention of atopic dermatitis. The findings indicate some positive effects, but there are also conflicting results depending on the strains tested, the conditions of use, and the population groups. Only one clinical trial reported a reduction in respiratory infections and another reported a reduction in total gastrointestinal symptoms in infants. Some observational studies associated changes in gut microbiota composition with body weight and body weight gain during pregnancy, but only one clinical trial reported positive effects of perinatal probiotic administration to pregnant women on blood glucose control. Another 2 interventional studies in pregnant women reported positive effects of probiotics in reducing the risk of genital infection; nevertheless, data on the possible effect on preterm birth and its complications are not available. The need for a larger number of long-term clinical trials to shed light on the possible role played by perinatal and early postnatal administration of certain probiotics in reducing the burden of diseases common in modern life is evident. Moreover, further studies are required to define the mechanisms by which intestinal bacteria may influence a mother's physiology and to define the transmission routes of such effects to the offspring and thus explain and rationally exploit these interactions.

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