

Maternal Consumption of *Lactobacillus rhamnosus* GG Yogurt During Pregnancy Promotes Bifidobacteria Growth in Intestinal Microflora of Infants

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ABSTRACT

Background: Recent studies have found associations between maternal probiotic consumption and early growth of healthy intestinal flora in their newborns. We gave probiotics to mothers during late pregnancy and compared changes in the intestinal flora of their newborns.

Methods: Thirty pregnant women were given 100 g of yogurt containing *Lactobacillus rhamnosus* GG (LGG) from the start of the 33rd week of gestation until parturition (LGG group), and the results obtained from their newborns were compared with those from infants born to 20 mothers who had not received this probiotic treatment. Reverse-transcriptase polymerase chain reaction was used to evaluate the presence and number of bifidobacteria in newborn meconium and in stool after receiving colostrum, at discharge from hospital, and at ages 1, 4, and 6 to 9 months and 1 year.

Results: The LGG group had higher bifidobacteria detection rates, a greater number and variety of flora, and higher detection rates of *Bifidobacterium breve*. In particular, the bifidobacteria detection rate was significantly higher in the LGG group ($p = 0.008$).

Conclusions: Giving LGG yogurt probiotics to pregnant women during late pregnancy significantly increased and maintained bifidobacteria growth in the intestinal flora of their newborns, especially from the start to the end of weaning.

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KEYWORDS: probiotics, *Lactobacillus rhamnosus* GG yogurt, intestinal microflora, prevention, *Bifidobacterium breve*

One reason put forward, in 1989, for the recent worldwide increase in the number of allergic conditions was the hygiene hypothesis,¹⁾ which emphasizes that intestinal flora are important in the healthy development of host immunity and in the promotion of oral immunity. Higher

rates of atopic dermatitis (AD) were observed in newborns with low levels of *Lactobacillus* and bifidobacteria in their stool as well as in infants with few bifidobacteria in their stool.^{2,3)} For this reason, research was begun on preventing and treating allergic conditions using probiotics. In

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1997, Majamaa et al. were the first to report that probiotics were effective in treating pediatric AD,⁴⁾ and in 2001 Kalliomäki et al. reported that probiotic use among pregnant mothers from late pregnancy until cessation of breastfeeding resulted in infants having lower rates of AD through age 2 years.⁵⁾ Although these results could not be reproduced at the same facility, progress was made at other facilities that conducted similar research.

Subsequent reports confirmed the effectiveness of administering probiotics to pregnant mothers from late pregnancy to the end of breastfeeding (duration, usually >6 months) in preventing AD in their infants.⁶⁻¹⁰⁾ However, the findings were usually less encouraging for probiotics given only during pregnancy.^{11,12)} Thus, it has been argued that, rather than passing through the placenta,¹³⁾ probiotics are most effectively received through breast milk, as infants receive soluble CD14 (sCD14) and immunoglobulin A (IgA) via this route.¹⁴⁻¹⁶⁾ However, Lahtinen et al. found that even when probiotics were administered only during late pregnancy, changes in the intestinal flora of newborns included significantly higher numbers of bifidobacteria as compared with mothers who had not had received such probiotics.¹⁷⁾

Among probiotics, *Lactobacillus rhamnosus* GG (LGG) has been shown to be resistant to degradation from stomach and bile acids and to have greater adherence to cultured intestinal tract cells as compared with other lactobacilli and bifidobacteria.¹⁸⁾ By producing natural antibiotics, LGG controls proliferation of anaerobic bacteria such as clostridium as well as aerobic bacteria such as *Enterobacteriaceae*. This is believed to result in greater proliferation of bifidobacteria.¹⁹⁾ Finally, it has also been reported that LGG prevents breakdown of barrier function in intestinal epithelial cells, thereby exerting a protective effect on the intestinal epithelium.²⁰⁾

For these reasons, and because LGG is a specially designed health food containing bacteria that arrive alive into the intestine (a "Food for Specified Health Uses: FOSHU"), we chose to give it as a probiotic to mothers in their third trimester of pregnancy. Then we compared the intestinal flora in their newborns with those in newborns whose mothers had not consumed probiotics.

Methods

Participants

The participants were recruited from among the 522 pregnant women who made regular visits to our obstet-

rics department during the period from March 2009 through October 2010 and planned to deliver their baby at our hospital. The periods of probiotic administration and control observation were sequentially determined, and participants were recruited during these 2- to 3-month periods. Before participating in the study, all mothers received full and clear descriptions of the study goals and procedures and later signed a consent form indicating that they agreed to participate of their own free will. All procedures in the study were in complete accordance with the Helsinki Declaration and were reviewed and approved by the Toho University Medical Ethics Committee (approval no. 20012).

Exclusion criteria

Women with preeclampsia, pregnancy-induced hypertension, gestational diabetes, allergies to milk products, or infectious diseases (except Group B *Streptococcus* colonization) were excluded from the study.

Design

Thirty pregnant women in the 33rd week of gestation started a probiotic regimen consisting of one 100-g package of yogurt containing LGG per day until parturition. The control group did not receive this probiotic regimen during the same period of their pregnancy. The LGG group therefore received the yogurt for approximately 1 month, starting on the first day of the 33rd week of gestation, and their daily consumption was confirmed by written records. The control group was instructed to eat as little as possible of fermented foods such as *natto* (fermented soybeans) and yogurt. The LGG yogurt (brand name "Onaka e GG") was kindly supplied by the Takanashi Milk Products Co., Ltd. (Yokohama, Japan), whose research confirmed that a 100-g package of yogurt (the daily amount consumed by the LGG subjects) contained more than 1.4×10^{10} colony-forming units (CFUs) of LGG.

The fecal samples obtained were all randomly assigned numbers to ensure anonymity in blinded analysis. After collection, all samples were kept frozen in the sampling containers and were sent, in 1 shipment, to the Takanashi Milk Products Co., Ltd., where reverse-transcriptase polymerase chain reaction (RT-PCR) was used to measure the number of bifidobacteria.

Samples were taken of meconium and of stools after colostrum feeding, at discharge, at age 1, 4, and 6 to 9 months, and 1 year. The meconium sample and samples of colostrum and discharge were collected by nurses at the hospital; the 1-month sample was collected by mothers at

Age (months)	0-a*	0-b*	0-c*	1	4	6-9	12
Stool samples from infant	▲	▲	▲	▲	▲	▲	▲
Questionnaire to mothers				●	●	●	●
No. of entries LGG group	31	30	30	30	19	27	25
Control group	20	20	20	20	10	14	14

*0-a: Meconium, *0-b: after colostrum feeding, *0-c: at discharge

Fig. 1 Study design and progress of participants

Stool samples were taken of meconium, after colostrum feeding, at discharge, and at ages 1, 4, 6 to 9 months, and 1 year. Questionnaires were distributed to mothers at 1, 4, 6 to 9 months, and 1 year. In total, 5 *Lactobacillus rhamnosus* GG (LGG) participants and 6 control participants had withdrawn at the 1-year mark.

home and then brought to the hospital at the time of their newborn's 1-month checkup. All subsequent samples (*i.e.*, 4 months and later) were collected by mothers, using anonymously numbered sample containers that were sent by mail in time for collection of the appropriate sample. Mothers were then requested to send the sample back by mail. Received samples were stored frozen and ultimately sent in 1 shipment for analysis to the Takanaishi Milk Products Co., Ltd. The interval from sampling to freezing was approximately 1-2 days. Fig. 1 shows the planned sampling schedule.

The background characteristics of mothers and newborns were analyzed retrospectively, based on their hospital records. Information on mothers included age, history of allergies, complications during pregnancy, use of antibiotics during pregnancy, turbidity of amniotic fluid, and parturition status. Information on newborns included sex, gestational weeks, delivery mode, height and weight at birth, and any complications before or around the time of delivery. Criteria used in the analysis of fecal samples included presence and number of bifidobacteria, number of species found, and presence of *Bifidobacterium breve* (*B. breve*). RT-PCR used 16S ribosomal ribonucleic acid (rRNA) genetic marking, for which a *Bifidobacterium*-specific primer was created. With the aid of this primer, the RT-PCR produced amplification curves and standard curves. The amount of *Bifidobacterium* DNA among the total DNA extracted was then determined by reverse transcription.²¹⁾

Statistical analysis

The *t*-test was used to analyze data from mothers and newborns at and around the time of delivery. Bifidobacteria and *B. breve* detection rates were evaluated with Cochran's *Q* test and the χ^2 test. Finally, the number and species of bifidobacteria detected were compared between groups using 2-way factorial analysis of variance. A 2-tailed *p* value of less than 0.05 was considered to indicate statistical significance. The software package SPSS for Windows, version 18 (SPSS Inc., Chicago, IL, USA) was used for the data analysis.

Results

A total of 81 pregnant women received a complete explanation of the study (31 in the LGG group and 50 controls); 31 in the LGG group and 20 controls consented to participate. One of the LGG participants withdrew after beginning consumption of the probiotic yogurt, which left 30 LGG and 20 control participants at the start of fecal sampling. Five LGG participants and 6 control participants later withdrew at the 1-year mark (1 and 2, respectively, had moved away from the area of the hospital; Fig. 1). As shown in Fig. 1, after 4 months, even if a sample could not be obtained at a given time, the subject was not excluded from the analysis if a later sample could be obtained from that subject. Instead, calculations were done at each stage without missing values from the subjects who had not submitted samples at that point. For this reason, there are some differences in the number of subjects at each of the

Table 1 Characteristics of mothers (M) and newborns (N)
Regarding mothers, no significant differences were seen between the *Lactobacillus rhamnosus* GG (LGG) and control groups.
No significant differences were seen between the LGG group and control newborns.

	LGG (n = 30)	Control (n = 20)	
M	Age (years)	32.7 ± 4.4	32.9 ± 4.6
	Allergy	13	8
	Maternal complications	8	5
	Antibiotics	7	4
	Meconium staining	5	3
	Abnormal labor	2	2
N	Male : Female	16 : 14	13 : 7
	Gestational weeks	39W4d	39W3d
	Height (cm)	49.2 ± 1.6	48.7 ± 1.4
	Weight (g)	3175.5 ± 361.2	3073.4 ± 351.5
	Cesarean section	4	3
	Perinatal abnormality	5	4

W: weeks, d: days

assessed time points.

Background information on mothers and newborns (Table 1)

Regarding the mothers, no significant differences were seen between the LGG and control groups with respect to age, history of allergies, complications during pregnancy, antibiotic use during pregnancy, turbidity of amniotic fluid, or parturition status. Complications during pregnancy included anemia in both groups (5 cases in the LGG group and 3 in the control group), which was treated with an iron supplement (Ferromia®, Eisai Co. Ltd., Tokyo, Japan). Antibacterials were given to both groups (7 LGG participants and 4 controls) in cases of Group B *Streptococcus* positivity, premature rupture of membranes, and caesarean delivery.

No significant differences were seen between the LGG group and control newborns with respect to “male: female” ratio, gestational weeks, height, weight, or rate of complications. Five newborns developed complications in the LGG group: 3 cases of jaundice, 1 case of fever (which resolved spontaneously without treatment), and 1 case of cleft palate. In the control group, 4 newborns developed complications (4 cases of jaundice).

Bifidobacterium detection rate

At 1 month after birth, the LGG group had a higher rate of *Bifidobacterium* as compared with the controls: the rate continued to increase in the LGG group until month 4 and in controls until the 1-year sample (Fig. 2). At 4 months, Bi-

fidobacteria had been detected in 100% of LGG group samples and in 66.7% of control samples, which was the greatest difference between groups. The difference between the LGG group and controls was statistically significant at month 4 and at months 6 to 9 ($p = 0.008$ and 0.047 , respectively).

Number of *Bifidobacterium* (\log_{10} cells/g of feces)

The number of *Bifidobacterium* generally increased with time (Fig. 3). At 1 month, the average number of *Bifidobacterium* was higher in the LGG group than in the controls, and the greatest difference was seen at months 6 to 9 (9.47 in LGG vs 7.14 in controls). Similar to the detection rate discussed above, the number of cells steadily increased until month 4 in the LGG group and until 1 year in the controls. However, no statistically significant differences were found.

Number of *Bifidobacterium* species

The LGG group had more *Bifidobacterium* species than did the controls at every sampling point, and the number of species gradually increased in both groups during the study period (Fig. 4). The difference between groups became more evident at 4 months (average number of species: 2.53 for LGG vs 1.7 for controls). At months 6 to 9, average number of species was 2.89 in the LGG group and 1.79 in the controls; however, at 1 year, the gap had closed slightly (3.29 for LGG vs 2.63 for controls). There was no statistically significant difference between groups.

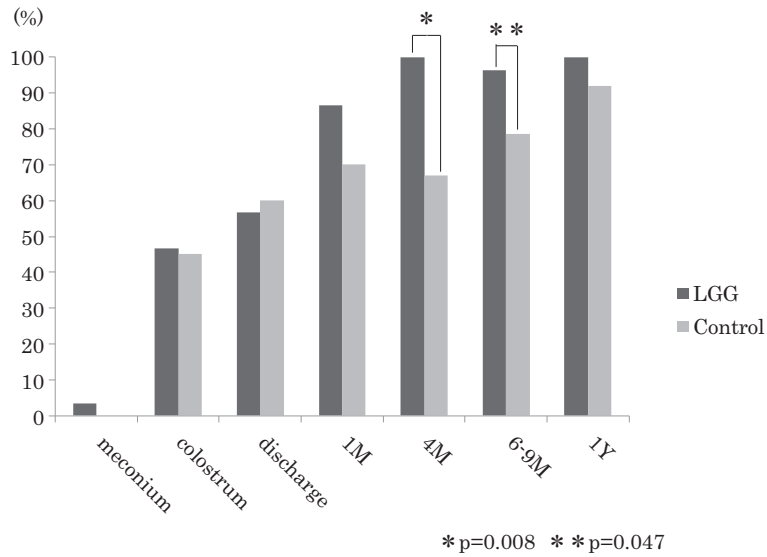


Fig. 2 *Bifidobacterium* detection rate

The *Lactobacillus rhamnosus* GG (LGG) group had a higher rate of *Bifidobacterium* as compared with controls. The difference between the LGG group and controls was statistically significant at month 4 and at months 6 to 9.

M: months, Y: years

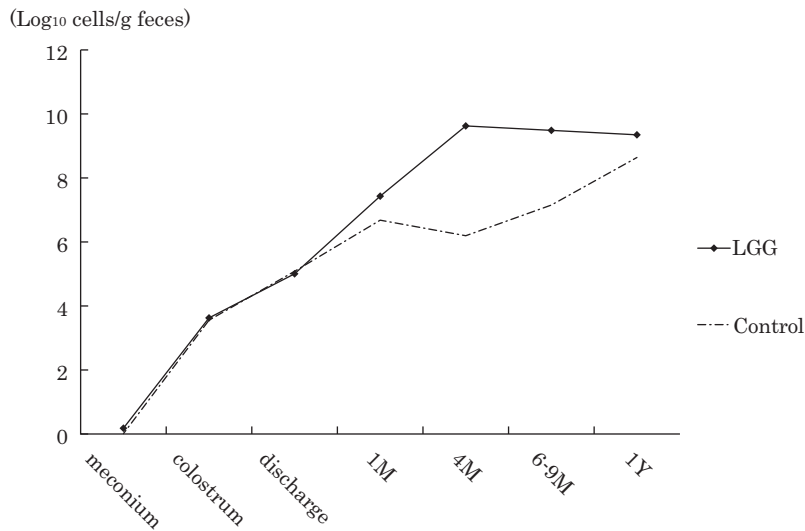


Fig. 3 Number of *Bifidobacterium*

The average number of *Bifidobacterium* was higher in the *Lactobacillus rhamnosus* GG (LGG) group than in the controls, but no statistically significant differences were found.

M: months, Y: years

Detection of *B. breve*

The *B. breve* detection rate continued to increase in both groups until month 4, but declined temporarily at months 6 to 9 (Fig. 5). The detection rate was higher in the LGG group until months 6 to 9, but the difference had disappeared at 1 year. No statistically significant differences

were found at any time point, although the difference at 6 to 9 months was of borderline significance ($p = 0.087$).

Discussion

The present study confirmed that LGG yogurt given to pregnant mothers during the last month of pregnancy en-

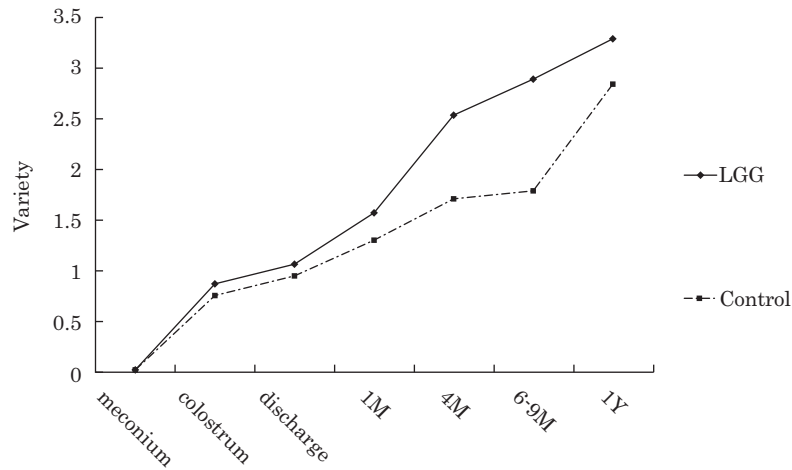


Fig. 4 Number of *Bifidobacterium* species (mean)

The *Lactobacillus rhamnosus* GG (LGG) group had more *Bifidobacterium* species than did the controls at every sampling point, but no statistically significant differences were found.

M: months, Y: years

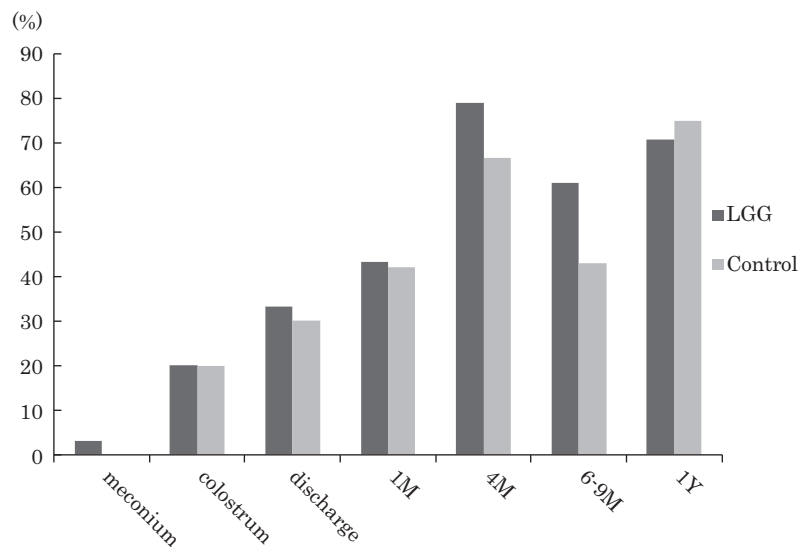


Fig. 5 *Bifidobacterium breve* (*B. breve*) detection rate

The detection rate was higher in the *Lactobacillus rhamnosus* GG (LGG) group until months 6 to 9. The difference between the LGG group and controls was not statistically significant.

M: months, Y: years

couraged growth of healthy intestinal flora in their newborns, especially during the period between the start and end of weaning. This was shown by our finding that during the period from month 4 to 9, the bifidobacteria detection rate was higher in the LGG group than in controls, and by the fact that the LGG group also had higher numbers and a greater variety of *Bifidobacterium* flora, although

these differences were not statistically significant.

Past reports showed that probiotic administration to mothers from late pregnancy until the end of weaning tended to reduce the development of AD symptoms.^(6-9, 22) However, nonspecific immunoglobulin E (IgE) production was not suppressed. Thus, it seems that the benefit with respect to AD may not apply to other allergic condi-

tions.^{7,13)} Furthermore, a meta-analysis in 2012 concluded that AD prevention resulting from maternal administration of probiotics was solely due to lactobacillus flora in the intestines of neonates.²³⁾ However, it should be noted that the above studies did not analyze cases in which probiotics were administered only during pregnancy. Some studies of probiotic administration found higher rates of allergic rhinitis and asthma and higher rates of food allergies,²⁴⁻²⁶⁾ while others highlighted the need to consider the optimal period and type of maternal probiotic administration.^{22,27)} A recent report found that probiotics can be useful in persons with milk allergies.²⁸⁾

In contrast, a meta-analysis of the effects of probiotics on AD prevention by Pelucchi et al. revealed preventive effects irrespective of the way probiotics were administered, *i.e.*, AD prevention was not related to the timing of probiotic administration (*e.g.*, during pregnancy only, during pregnancy and breastfeeding, or during breastfeeding only) or to the recipient of probiotics (mother only, mother and newborn, or newborn only).²⁹⁾ Other reports indicate that the critical period for the allergy prevention effects of probiotics is during pregnancy and breastfeeding.¹⁰⁾ We did not investigate the development of pediatric allergies, but the differences between the LGG and control groups in the production of intestinal bifidobacteria during early infancy suggest that the LGG group will have fewer difficulties related to AD.

Other studies also found that maternal LGG administration promoted growth of healthy intestinal flora in newborns, especially bifidobacteria formation. Gueimonde et al. gave LGG to mothers during the period from late pregnancy to 3 months after parturition and reported significantly higher detection rates of *B. breve* in neonate fecal samples from an LGG group 5 days after birth.³⁰⁾ In addition, Grönlund et al. reported higher rates of *Bifidobacterium bifidum* (*B. bifidum*) and *B. breve* in fecal samples collected at 1 and 6 months after birth from infants whose mothers had received a probiotic regimen from late pregnancy through the breastfeeding period.³¹⁾ Another report found that maternal LGG administration resulted in higher numbers of *Bifidobacterium longum* (*B. longum*) and *B. breve* and lower numbers of *Bifidobacterium adolescentis* (*B. adolescentis*) in newborns,¹⁷⁾ although the mechanisms involved remain unclear. With respect to this mechanism, it was suggested that babies ingest maternal intestinal flora while passing through the birth canal during childbirth.³²⁾ However, recent evidence indicates that dendritic cells

and microphages break down maternal intestinal flora and that these flora then spread throughout the mother's body via the lymphatic and circulatory systems and are transmitted to the fetus through the placenta and amniotic fluid and to the newborn via mother's milk.³³⁾ The results of the present study are in agreement with this theory. In addition, we are currently planning further investigations of cytokines and other constituents in umbilical cord blood and mother's milk.

We focused on *B. breve* because a previous report found that Japanese infants with confirmed *B. breve* had a greater amount, and more species, of bifidobacteria than did infants without *B. breve*.³⁴⁾ The number of intestinal bifidobacteria cells was low in infants with AD, and symptom severity appeared to be inversely related to the amount of bifidobacteria.^{35,36)} It therefore seems reasonable to assume that AD would also be prevented in infants with higher numbers of *B. breve*, as the presence of *B. breve* is associated with greater quantities and more varieties of AD-preventing bifidobacteria in general.

B. breve administration results in dendritic cell induction in mice, which increases interleukin-10 (IL-10) production and downregulates expression of inflammation-related genes.³⁷⁾ Another report found that *B. breve* administration promoted an intestinal flora environment with a preponderance of bifidobacteria.³⁸⁾ Therefore, an increase in *B. breve* detection rate due to probiotic administration could result in a general increase in bifidobacteria and a subsequent healthy balance of intestinal flora.

As was the case in previous studies, *B. breve* detection rates were higher in our LGG participants, and they had a greater number and more species of *Bifidobacterium* cells. Although the differences between groups were not significant, tendencies were apparent, especially between months 4 to 9. Simple maternal probiotic administration during the last month of pregnancy resulted in sustained presence of *B. breve* for 6 months after parturition, when the infants began to be fed baby food, which is reported to be a critical time in the growth of intestinal flora.³⁹⁾ Furthermore, the presence of *B. breve* was accompanied by a consistent increase in the number and species of bifidobacteria, suggesting that probiotic administration promoted growth and preservation of healthy intestinal flora.

For safety reasons probiotics (which are live bacteria) are not recommended for direct use with newborns; so-called "prebiotics" (including oligosaccharides) are recommended instead. When administering probiotics to new-

borns and infants, the *Lactobacillus* preparation is usually mixed with infant formula ("probiotic milk").⁴⁰⁾ However, as yet there is no commercially available probiotic milk in Japan. Thus, we did not administer probiotics directly to newborns. Instead, mothers were given a probiotic yogurt that had been developed as a commercial FOSHU. This encouraged formation of normal intestinal flora in newborns and was considered a safe and effective administration method for mothers and their newborns.

Conventional yogurt is prepared using 2 bacterial strains: *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The "probiotics" designation is assigned, however, when other beneficial bacteria such as *Lactobacillus casei* and LGG are added to these naturally occurring strains during fermentation. Conventional yogurt therefore also contains *Lactobacillus*, and reports indicate that it thus encourages production of interferon- γ (IFN- γ), suppresses production of IgE antibodies and directly suppresses IL-10. In addition, yogurt was also found to be useful in preventing allergic conditions.^{41, 42)} The yogurt chosen for use in the present study (Onaka e GG) is a probiotic yogurt containing LGG. In the present study, to demonstrate the effect of probiotics, we believe it is necessary to conduct a follow-up study with a control group receiving conventional yogurt.

Other limitations of the present study include the small sample size and the fact that all research was conducted at only 1 center. Future studies should be randomized, include several centers, and enroll a larger number of participants.

At the time of fecal sampling, we also included a questionnaire asking about development of skin rashes, wheezing, and AD in newborns. This study is designed to follow newborns until age 3 years and to gather allergy-related data and fecal samples for bifidobacteria (especially *B. breve*) testing in order to evaluate the long-term effectiveness of maternal probiotic therapy administered during the last month of pregnancy in reducing AD in their infants.

Conclusion

Probiotic LGG yogurt given to mothers during the last month of pregnancy resulted in growth of healthy intestinal flora in their newborns at early weaning.

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妊婦の *Lactobacillus rhamnosus* GG ヨーグルト 摂取による乳児期早期の腸内 *Bifidobacterium* 属菌形成促進

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要約

目的: 妊娠後期の母体にプロバイオティクスを摂取させ、出生した児の腸内細菌叢の変化を比較検討した。

対象および方法: 妊娠 33 週の妊婦 30 例に *Lactobacillus rhamnosus* GG (LGG) ヨーグルト (菌) を出産まで毎日 1 個摂取させた群を LGG 群、摂取していない妊婦 20 例を対象群とした。出生した児の便中の *Bifidobacterium* 属菌を reverse-transcriptase polymerase chain reaction (RT-PCR) を用いて定量し経過観察した。検査の時期は、胎便、初乳後、退院時、生後 1, 4, 6~9 カ月、1 歳で行った。

結果: LGG 群の方が、*Bifidobacterium* 属菌の検出率、菌数、菌種数、および *Bifidobacterium breve* (*B. breve*) の検出率が高く、とくに *Bifidobacterium* 属菌の検出率は LGG 群で有意に高かった ($p=0.008$)。

考察: 妊娠後期の母体が、LGG ヨーグルトでプロバイオティクスを摂取し、児の離乳初期から離乳後期における *Bifidobacterium* 属菌優位の腸内細菌叢の形成が促進され、その後も維持された。

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