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Effects of Probiotics on the Incidence of Gestational Diabetes Mellitus During Pregnancy: A Randomized and Placebo-controlled Trial

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Abstract We aim to observe whether the supplementation of multiple probiotics in the second trimester of pregnancy affect the health in pregnant women, and improve the maternal and infant outcomes. Pregnant women were randomly divided into probiotics group and placebo group by double-blind method after signing the informed consent. At the same time, pregnant women in the negative control group who did not participate in the research were enrolled. The pregnant women in the probiotics group began to take probiotics including Lactobacillus acidophilus, Bifidobacterium lactis and Lactobacillus plantarum total 400 CFU twice a day from the 16th week of pregnancy to the 23rd week of pregnancy. There were 72 pregnant women in probiotics group, 64 in placebo group and 59 in negative control group in research. There was no significant difference in the incidence of GDM among the groups ($\chi 2$=0.197, P=0.906). In the second trimester, the vitamin D level in the probiotics group was significantly higher than that in the other two groups ($p<0.05$).The BMI, fat weight and body fat percentage of pregnant women in the probiotics group at 24 weeks of pregnancy were significantly lower than those in the other two groups ($p<0.05$). In conclusion, the intervention of multiple probiotics in pregnant women from 16 to 23 weeks of gestation did not significantly reduce the incidence of GDM, but the supplementation of multiple probiotics may affect the incidence of postpartum hemorrhage, the growth of pregnant women's weight, especially body fat and the level of blood lipids and vitamin D.

Keywords: *probiotics, Gestational diabetes mellitus, body fat percentage, vitamin D, pregnancy outcome*

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1. Introduction

GDM is one of the common complications during pregnancy. With the change of lifestyle, the increase of the incidence of overweight/obesity during pregnancy and the increase of gestational age, the prevalence of GDM has increased year by year, which has become one of the important factors profound affecting maternal and infant health.

At present, there is no specific and effective preventive measures for the prevention of GDM. Studies have found that the imbalance of intestinal flora is closely related to metabolic diseases such as diabetes, obesity, hypertension and coronary heart disease. Intestinal microorganisms can regulate the host's immune, inflammatory state and glucose and fat metabolism. Due to the good tolerance and safety of probiotics, many studies have found that probiotics supplementation during pregnancy can improve the inflammatory response and glucose metabolism [1,2], reduce the fasting blood glucose, insulin resistance and insulin concentration of pregnant women, and the therapeutic effect in GDM is positive [3,4,5,6]. But the preventive effect of probiotics supplement on GDM is still inconclusive.

Some studies believe that probiotics intervention has preventive effect on GDM. For example, Bartow et al. [7,8] (supplemented with Lactobacillus rhamnosus hn001 (6 × 109 CFUs))and luoto et al. [9] (combined supplementation of Lactobacillus rhamnosus and Lactobacillus Bb12) found that the use of probiotics reduced the incidence of GDM and improved glucose metabolism and insulin sensitivity in pregnant women.

However, some studies have found that probiotics have no preventive effect on GDM. For example, Lindsay et al. [10] supplemented Lactobacillus salivarius UCC118 at a dose of 109 CFUs, Callaway et al. [11] supplemented Lactobacillus rhamnosus (LGG) and Lactobacillus animalis subspecies (Bb-12) at a dose of >1 × 109 CFUs and Pellopper ä [12] supplemented Lactobacillus rhamnosus and Bifidobacterium animalis ssp. lactis at a dose of 1010 CFUs in pregnant women. They found no improvement in blood glucose, insulin levels, or HOMA2-

IR levels in the probiotics group, nor the reduction of the incidence of GDM by the probiotic intervention.

Therefore, the health benefits of probiotics on pregnant women and whether they can prevent GDM need further study. The purpose of this study was to investigate the preventive effect of combined supplementing probiotics (Lactobacillus acidophilus, Bifidobacterium lactis and Lactobacillus plantarum) on GDM, and to explore other health benefits such as lipid metabolism and body composition changes of probiotics in pregnant women besides blood glucose control even for non GDM pregnant women.

2. Methods

Study Participants and Design

Our study was conducted from April 2020 to December 2021 at the obstetrics department of Beijing Jishuitan Hospital, Beijing, China. At 9-12 weeks of gestation, women who voluntarily participated in the research, planned delivery at Jishuitan Hospital and had a singleton pregnancy were invited to participate. Women with pre pregnancy diabetes or impaired glucose tolerance, in vitro fertilization embryo transfer, severe liver and kidney diseases, heart diseases, autoimmune diseases (such as systemic lupus erythematous and rheumatoid arthritis) or malignant tumors will be excluded. Women who have used other probiotic drugs within 3 months before enrollment or have participated in other studies during pregnancy will also be excluded.

According to the principle of informed consent, pregnant women who met the inclusion and did not meet the exclusion criteria received diet and exercise guidance in the nutrition department, recorded food diary, and monitored body composition (NQA-P, Beijing Sihai Huachen Company) at 16 and 24 weeks of gestation. They were randomly divided into intervention group and placebo group. The intervention group and the placebo group were given 2g powder with the same appearance, color and properties twice a day from the 16th to the 23rd week of pregnancy. The intervention group was given finished sugar homeostasis (Yiran Biology) supplement. The ingredients are fructooligosaccharides, inulin, Lactobacillus acidophilus La28 ($>6.5\times10^9$CFU), Bifidobacterium lactis BAL531 ($>7\times10^9$CFU) and Lactobacillus plantarum LP 45 ($>6.5\times10^9$CFU). The placebo was maltodextrin. Pregnant women who were unwilling to take the research reagent but were willing to accept diet and exercise guidance in the nutrition department and were followed up for a long time were included in the negative control group. The negative control group was not given any oral reagent. The pregnant women in each group did not take antibiotics during the whole pregnancy. In this study, those who did not take the research reagent according to the required dose or time were classified as negative control group. Those who took the reagent for more than 2 weeks or the total dose was more than 56g were still classified as the original group. The inclusion and exclusion algorithm is detailed in Figure 1.

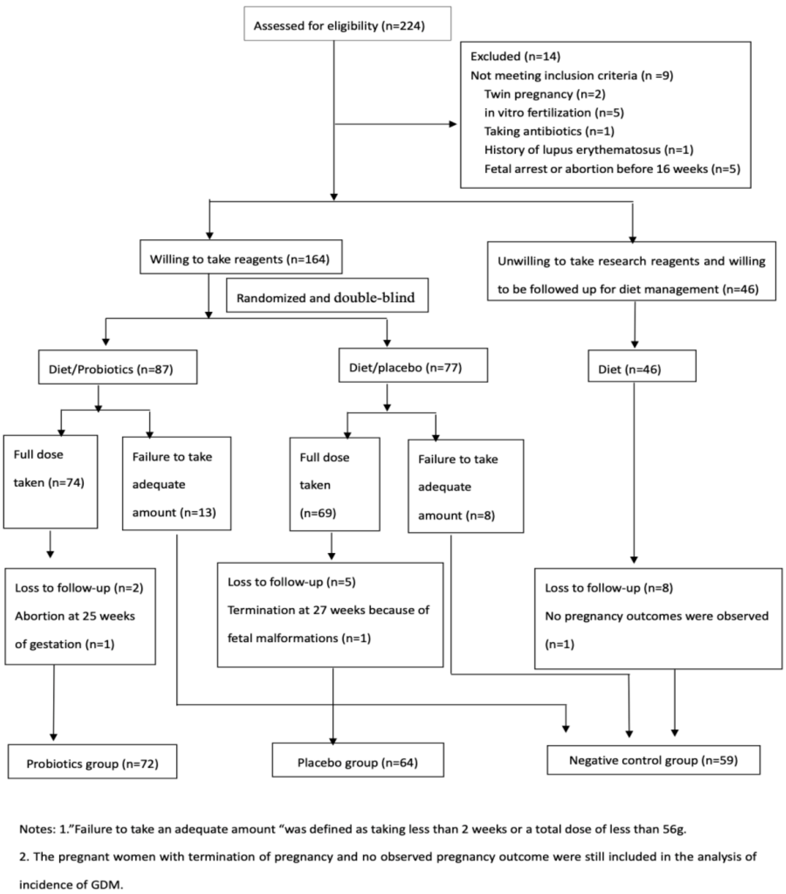

Figure 1. Flow diagram and status of Enrollment participants

The patients were followed up at 12, 16, 20, 24, 30 and 36 weeks of gestation. At the same time, the intervention group, placebo group and negative control group were given face-to-face dietary and lifestyle guidance during pregnancy. Body composition analysis, fecal samples collection and qPCR detection of intestinal flora were performed at 16 and 24 weeks of gestation.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving patients were approved by the the ethics committee of Beijing Jishuitan Hospital (JLKS No. 201909-30-b No. 03). Written informed consent was obtained from all patients.

Baseline Data

The study collected maternal age, pre pregnancy BMI, weight change during pregnancy, primipara or not, previous medical history, blood glucose and lipid status during early pregnancy, family history of diabetes mellitus in immediate family.

Project Recruitment and Follow-up

A total of 224 pregnant women were recruited. Among them, 5 cases were terminated in advance due to abortion, fetal suspension and other reasons, and 9 cases were excluded from the study due to twin pregnancy, artificial in vitro fertilization, antibiotics during pregnancy and systemic lupus erythematosus. In the study, those who did not take the study reagent according to the required dose or time were still analyzed according to the original group according to the time of taking the study reagent for more than 2 weeks or the total dose of more than 56g. In the study, a total of 21 people did not take or did not reach the above-mentioned taking time or dose after signing the informed consent, and 15 people were lost to follow-up. Finally, 72 pregnant women in probiotic group, 64 in placebo group and 59 in negative control group were recruited (Figure 1).

Diagnostic Criteria for GDM

The pregnant women underwent 75g glucose tolerance test within 24-28 weeks. According to the diagnostic criteria of gestational diabetes mellitus in health industry of the People's Republic of China, the normal values of 75g glucose tolerance test at fasting, 1 hour and 2 hours after taking glucose were less than 5.1mmol/l, 10mmol/l and 8.5mmol/l respectively. Any abnormal blood glucose value was diagnosed as GDM.

Statistical Analysis

SPSS21.0 was used for data entry and analysis. Measurement data were expressed as mean and 95% confidence interval, and analysis of variance test was used for comparison between groups. Count data were expressed as rate, and chi-square test was used for comparison between groups. $P<0.05$ represented a statistically significant difference.

3. Results

Basic Information of Patients

It was found that there were no significant differences in age, BMI before pregnancy, family history of diabetes, primipara, premature delivery, cesarean section, pregnancy induced hypertension, premature rupture of membranes, intrauterine infection, fetal distress or growth restriction, and neonatal weight ($p>0.05$) in each group, while the incidence of postpartum hemorrhage in probiotics group was significantly lower than that in the other two groups ($p<0.05$)(Table1)

Incidence of GDM and Blood Glucose Levels During Pregnancy

Chi square test showed that there was no significant difference in the incidence of GDM among pregnant women in each group ($\chi2$ =0.197, P=0.906). Analysis of variance found that there were no significant differences in fasting, 1-hour and 2-hour blood glucose levels, fasting blood glucose, glycosylated hemoglobin and glycated albumin in every trimester of pregnancy among the groups ($p>0.05$) (Table 2).

Table 1. Basic information and pregnancy outcomes of pregnant women in each group

	Probiotics group [Mean (95%CI)/rate]	The placebo group [Mean (95%CI)/rate]	Negative control group [Mean (95%CI)/rate]	P
Age	31.32(30.45,32.18)	32.03(31.10,32.97)	30.81(29.87,31.76)	0.184
Pre-pregnancy BMI	23.53(22.37,24.68)	24.8(23.77,25.81)	24.95(23.73, 26.17)	0.144
family history of diabetes	15/72	13/64	6/59	0.211
primiparas	13/72	18/64	11/59	0.293
Preterm birth	5/72	1/64	3/59	0.321
Cesarean section	31/71	21/63	25/57	0.386
Hypertension in pregnancy	11/72	10/64	10/59	0.964
Premature rupture of membranes	9/72	7/64	12/59	0.283
Intrauterine infection	3/72	5/64	2/59	0.484
Postpartum Hemorrhage	3/72	11/64	8/59	0.046
Weight of the newborn	3300(3188, 3412)	3330(3238，3422)	3367(3237, 3497)	0.705
Fetal distress or confinement in utero	3/72	3/64	7/59	0.158

Table 2. incidence of GDM and blood glucose levels during pregnancy in each group

	Probiotics group [Mean (95%CI)/rate]	The placebo group [Mean (95%CI)/rate]	Negative control group [Mean (95%CI)/rate]	P
Incidence of GDM	15/72	15/64	14/59	0.93
Fasting OGTT (mmol/L)	4.40 (4.31,4.5)	4.39 (4.25,4.52)	4.53 (4.43,4.63)	0.109
OGTT 1h (mmol/L)	7.62 (7.19,8.05)	8.03 (7.44,8.62)	7.90 (7.53,8.27)	0.442
OGTT 2h (mmol/L)	6.54 (6.21,6.88)	6.94 (6.45,7.43)	6.79 (6.43,7.14)	0.38
First trimester fasting blood glucose (mmol/L)	4.74 (4.63,4.85)	4.71 (4.6,4.82)	4.76 (4.66,4.86)	0.853
Third trimester fasting blood glucose (mmol/L)	4.47 (4.35,4.6)	4.28* (4.13,4.42)	4.36 (4.26,4.47)	0.113
First trimester HbA1c (%)	5.28 (5.21,5.34)	5.25 (5.18,5.33)	5.32 (5.26,5.38)	0.343
Third trimester HbA1c (%)	5.27 (5.18,5.35)	5.22 (5.13,5.31)	5.29 (5.22,5.35)	0.568
First trimester Glycated albumin (%)	13.02 (12.6,13.45)	13.19 (12.67,13.7)	12.88 (12.49,13.26)	0.626
Second trimester Glycated albumin (%)	12.75 (12.45,13.04)	13.20 (12.8,13.6)	12.52** (12.27,12.77)	0.014
Third trimester Glycated albumin (%)	12.54 (12.21,12.87)	13.06 (12.63,13.48)	12.51** (12.22,12.81)	0.092

*Compared with probiotics group, the difference was statistically significant.
**The difference was statistically significant compared with the placebo group.

Table 3. blood lipid and vitamin D levels of pregnant women in different periods of pregnancy

	Probiotics group [Mean (95%CI)/rate]	The placebo group [Mean (95%CI)/rate]	Negative control group [Mean (95%CI)/rate]	P
First trimester total cholesterol (mmol/L)	4.27(4.07,4.47)	4.2(3.87,4.53)	4.41(4.24,4.57)	0.37
First trimester triglyceride (mmol/L)	1.21(1.06,1.37)	1.1(0.95,1.25)	1.25(1.11,1.39)	0.425
First trimester low density lipoprotein cholesterol (mmol/L)	1.51(1.44,1.59)	1.52(1.42,1.63)	1.48(1.4,1.55)	0.717
First trimester high density lipoprotein cholesterol (mmol/L)	2.44(2.24,2.63)	2.34(2.07,2.61)	2.54(2.37,2.7)	0.405
First trimester apolipoprotein A1 (g/L)	1.66(1.6,1.71)	1.65(1.55,1.75)	1.62(1.54,1.7)	0.729
First trimester apolipoprotein B (g/L)	0.76(0.7,0.82)	0.75(0.66,0.85)	0.81(0.76,0.86)	0.365
First trimester lipoprotein a(mg/L)	275.16(192.04,358.28)	225.79(153.58,298)	207.15(155.52,258.77)	0.311
Third trimester total cholesterol (mmol/L)	6.38(5.61,7.15)	5.57(4.97,6.17)	6.18(5.91,6.46)	0.13
Third trimester triglyceride (mmol/L)	2.47(1.86,3.09)	3.11(2.51,3.71)	3.42* (3.02,3.82)	0.029
First trimester vitamin D (ng/ml)	17.69(15.67,19.71)	15.54(13.6,17.49)	15.9(14.52,17.27)	0.194
Second trimester vitamin D (ng/ml)	25.65(23.41,27.88)	22.02* (19.5,24.54)	22.45* (20.54,24.36)	0.042
Third trimester vitamin D (ng/ml)	24.65(22.2,27.09)	25.04(22.29,27.8)	21.48*/** (19.56,23.4)	0.048

*Compared with probiotics group, the difference was statistically significant.
**The difference was statistically significant compared with the placebo group.

Table 4. body composition of pregnant women at 16 and 24 weeks of gestation in each group

	Probiotics group [Mean (95%CI)/rate]	The placebo group [Mean (95%CI)/rate]	Negative control group [Mean (95%CI)/rate]	P
Basal Metabolic capacity (kcal) at 16 weeks	1357.9(1322.8,1392.9)	1386.5(1357.5,1415.4)	1388.3(1348.2,1428.3)	0.358
BMI (kg/m2) at 16 weeks	24.3(23.1,25.5)	25.2(24.1,26.3)	25.5(24,26.9)	0.386
Muscle Mass (kg) at 16 weeks	39.3(37.9,40.8)	40.4(39.3,41.6)	40(38.2,41.7)	0.523
Fat mass (kg) at 16 weeks	19.5(17.5,21.6)	21.5(19.6,23.4)	21.8(19.3,24.3)	0.237
Free fat mass (kg) at 16 weeks	43.7(42.4,45)	44.7(43.6,45.8)	44.3(42.7,45.9)	0.523
Body fat percentage (%) at 16 weeks	29.4(27.6,31.1)	31.6(29.9,33.3)	31.9(29.6,34.1)	0.104
Basal Metabolic capacity (kcal) at 24 weeks	1393.3(1358.8,1427.7)	1423(1395.3,1450.8)	1455.8*(1408.1,1503.5)	0.073
BMI (kg/m2) at 24 weeks	25.6(24.4,26.7)	26.5(25.5,27.6)	28.3*(26.7,29.8)	0.022
Muscle Mass (kg) at 24 weeks	39.9(38.3,41.4)	41.6(40.5,42.6)	42.3*(40.3,44.4)	0.075
Fat mass (kg) at 24 weeks	21.9(19.8,23.9)	23.9(22.1,25.8)	26.3*(23.5,29.1)	0.027
Free fat mass (kg) at 24 weeks	44.8(43.4,46.1)	45.8(44.8,46.8)	46.5(44.4,48.5)	0.243
Body fat percentage (%) at 24 weeks	31.9(30.2,33.5)	33.6(32.1,35.1)	35.5*(33.3,37.6)	0.026

*Compared with probiotics group, the difference was statistically significant.

Blood lipid and Vitamin D Levels of Pregnant Women in Each Group

There were no significant differences in the levels of blood lipids in the first trimester, total cholesterol in the third trimester and vitamin D in the first trimester among the three groups ($p>0.05$). The triglyceride level in the negative control group in the third trimester was higher than that in the other two groups, and the vitamin D level was significantly lower than that in the other two groups ($p<0.05$). The vitamin D level in the probiotics group in the second trimester was significantly higher than that in the other two groups ($p<0.05$) (Table 3).

Changes in Body Weight and Body Composition During Pregnancy

There was no significant difference in body composition data of pregnant women in each group at 16 weeks of pregnancy ($p>0.05$). The BMI, fat weight and body fat percentage of pregnant women in the probiotics group at 24 weeks of pregnancy were significantly lower than those in the other two groups ($p<0.05$).The body composition analysis data of subjects at 24 and 16 weeks of gestation were compared, and there was no significant difference among the groups ($p>0.05$) (Table 4).

Fecal Intestinal Flora

It was found that the probiotics group had a higher total flora at 24 weeks of gestation ($P<0.05$), and there was no significant difference in other groups and other species of bacteria in Fecal microbiota analysis of qPCR.

4. Discuss

Probiotics and GDM

GDM is one of the most common perinatal diseases, with a prevalence rate of 5~20%. Genetic, environmental and pregnancy related factors such as excessive fat storage and increased inflammatory reactions all play important roles in the pathogenesis of GDM. More scientific data show that intestinal flora imbalance plays an important role in the occurrence and development of abnormal glucose tolerance during pregnancy [13]. Due to the good tolerance and safety of probiotics, many studies have found that probiotics supplementation during pregnancy can improve the inflammatory response and glucose metabolism [1,2], reduce the fasting blood glucose, insulin resistance and insulin concentration of pregnant women, and the therapeutic effect in GDM is positive [3,4,5,6]. However, the preventive effect of probiotics on the incidence of GDM is still inconclusive. Some studies believed that probiotics intervention had preventive effect on the risk of GDM [7,8,9]. While some studies have found that probiotics had no effect on it [10,11,12].

In this study, it was not observed the decrease of the incidence of GDM in the probiotics group after supplementation of Lactobacillus acidophilus La28 (1.3×1010CFUs), Bifidobacterium lactis Bal531 (1.4×1010CFUs) and Lactobacillus plantarum LP45 (1.3×1010 CFUs) in the second trimester of pregnancy for 8 weeks, twice a day.

Although some studies have confirmed the beneficial effects of Lactobacillus acidophilus and Bifidobacterium [5] on glucose metabolism in patients with GDM, a recently published Israeli study [14] found that Bifidobacterium, Bifidobacterium lactis, Lactobacillus acidophilus, Lactobacillus paracasei, Lactobacillus rhamnosus and Streptococcus thermophilus (>1.2 ×1010CFU Probiotic capsules) had no effect on patients with GDM. A clinical trial conducted in Iran [15] explored the preventive effect of Lactobacillus acidophilus La1 (7.5 × 109 CFU), Bifidobacterium longum sp54 CS (109CFU) and Bifidobacterium SP9 CS (6 ×109CFU) on GDM. The intervention lasted from 14 to 24 weeks of gestation. The conclusion is that this probiotic intervention method does not reduce the risk of GDM, nor improve other maternal and infant outcomes. However, there are relatively few studies on the intervention or prevention of GDM with Lactobacillus plantarum. At present, only one literature about Lactobacillus plantarum and GDM has been retrieved, and the test contains a variety of probiotics. Nabhani et al. [16] used synbiotics capsules composed of Lactobacillus acidophilus, Lactobacillus plantus, Lactobacillus fermentans, Lactobacillus gasseri (1.5-7.0 ×109-10 CFU/g) and fructooligosaccharides (38.5 mg) to intervene patients with GDM for 6 weeks. The results showed that the probiotic capsules did not improve the blood glucose of GDM patients. However, some basic studies suggest that Lactobacillus plantarum can regulate blood glucose. For example, Zhang et al. [17] found that Lactobacillus plantarum and its metabolite acetate could play a role in type 1 diabetes by jointly regulating NLRP3 through animal experiments.

The large differences in the results of different studies may be related to the specific strains, doses and taking time of probiotics used, as well as the high heterogeneity of diet, age, genetic background and intestinal flora distribution in different populations [13,18].

In our study, the three groups of participants were given dietary guidance intervention and the dosage of probiotic intervention is sufficient (80 billion per day). However, the above three strains were not observed to reduce the incidence of GDM during 16-23 weeks of gestation. Possible reasons for the heterogeneity include insufficient sample size, individual differences, strain survival and colonization in the gut, as well as the timing and duration of intervention. In conclusion, there are many factors affecting the incidence of GDM, and the preventive effect of probiotics on GDM is not well studied at present, and more systematic studies are needed.

Probiotics and Glucose and Lipid Metabolism

Although the improvement effects of probiotics on prevention of GDM and OGTT test, fasting blood glucose, glycosylated hemoglobin and glycosylated albumin in each pregnancy were not observed, this study found the relationship between probiotics and lipid metabolism, nutrients, pregnancy outcome and body composition indicators, suggesting the benefits of probiotics for pregnant women.

This study found significant differences in triglyceride levels in the third trimester among the three groups of pregnant women, and the triglyceride levels in the probiotics group were significantly lower than those in the negative control group. Some studies have found that Lactobacillus and Bifidobacterium can reduce human cholesterol [18]. Amirani et al. found that the combination of Lactobacillus acidophilus, Bifidobacterium, Bifidobacterium lactis, Bifidobacterium longum and selenium can reduce fasting blood glucose, improve insulin resistance and reduce blood lipids in pregnant women [19]. Karamali et al. found that Lactobacillus acidophilus, Lactobacillus casei and Bifidobacterium can significantly reduce triglyceride and very low density cholesterol levels in GDM patients [20]. Probiotic capsules of Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium and Lactobacillus fermentans by Babadi et al. can significantly reduce triglyceride, very low density lipoprotein cholesterol and total cholesterol/high density lipoprotein cholesterol ratio, and significantly increase the level of high density lipoprotein cholesterol in

GDM patients [21]. However, a meta-analysis [22] found that probiotics can improve the total cholesterol level of GDM patients, but did not find the improvement effect of probiotics on triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol. In a word, combined with previous studies and the results of this study, we believed that probiotics could improve the blood lipids of pregnant women.

Probiotics and Pregnancy Complication

A Previously study have found that probiotics can increase the risk of preeclampsia [23], and it is inferred that the use of probiotics during pregnancy has harmful risks. This study investigated the incidence of hypertension in subjects, and the results showed that probiotics intervention can not increase the risk of pregnancy induced hypertension. In addition, this study also investigated the effect of probiotic intervention on other pregnancy outcomes. The results showed that probiotic intervention had no effect on the pregnancy outcomes of premature delivery, premature rupture of membranes, intrauterine infection and fetal distress. There was no effect on neonatal weight. However, the probability of postpartum hemorrhage in pregnant women with probiotic intervention was significantly lower than that in the negative control group and the placebo group. Shiao Yong Chan et al. [24] found that for pregnant women in the UK, Singapore and New Zealand, continuous supplementation of inositol, probiotics and micronutrients from before pregnancy and during pregnancy can shorten the duration of the second stage of labor, the risk of surgical delivery due to the delay of the second stage of labor, and blood loss during delivery. Combined with the results of this study, it is suggested that probiotic intervention can improve pregnancy outcomes.

Probiotics and Body Composition

Most studies generally believed that excessive fat increase during pregnancy leaded to rapid weight gain during pregnancy, which was a risk factor for GDM. [25] Compared with non GDM pregnant women, GDM pregnant women had higher body fat percentage and waist hip ratio, faster weight gain, and lower adiponectin (APN) level [26]. So appropriate weight gain during pregnancy can reduce the incidence of adverse pregnancy outcomes such as premature birth and large for gestational age infants.

Although the effect of probiotics on the incidence of GDM was not observed in this study, it was observed that the increase of BMI, fat weight and body fat percentage of pregnant women after taking probiotics for 8 weeks was less than that of non probiotics group, and the difference was statistically significant ($p<0.05$), indicating that probiotics have a certain effect on reducing the body fat rate of pregnant women, and may play a certain preventive role in the occurrence of GDM.

Probiotics and Vitamin D

It was an unexpected research finding that there was no difference in serum vitamin D levels among pregnant women in each group in the first trimester, but the vitamin D levels of pregnant women were significantly increased after probiotics supplementation, and those who took probiotics and consolation still maintained higher vitamin D levels in the third trimester.

Previous studies have found that pregnant women with GDM have a higher rate of vitamin D deficiency, 25 (OH) D3 levels in pregnant women with GDM have a significant negative correlation with insulin resistance index, TG and LDL-C [27]. Meta analysis [28] showed that GDM women had lower serum vitamin D than non GDM women. There are also clinical trials [29,30,31] that vitamin D supplementation could significantly reduce fasting blood glucose, HbA1c and serum insulincan and prevent the occurrence of GDM. And studies have found that pregnant women with GDM with lower vitamin D levels have a higher incidence of macrosomia [32]. Maternal vitamin-D deficiency is significantly associated with a high risk for emergency cesarean section, pre-eclampsia and anemia [33].

These results suggest that vitamin D has a regulatory effect on blood glucose in patients with GDM, which may be related to the regulation of immune and anti-inflammatory effects of vitamin D. The possible reasons for the positive results of this study are probiotics could increase intestinal permeability, regulate the secretion of pro-inflammatory mediators, so as to control local and systemic inflammation [34], thus promoting the absorption of vitamin D. In short, the storage and absorption of vitamin D may be related to glucose and lipid metabolism during pregnancy and improving the vitamin D level of pregnant women after taking probiotics may help to improve the glucose and lipid metabolism of pregnant women.

In addition, some studies have found that people with vitamin D deficiency have higher incidence of obesity [35]. And neonates of Asian mothers with mid-gestation 25(OH)D inadequacy have a higher abdominal subcutaneous adipose tissue volume [36]. In this study, compared with other groups, pregnant women in probiotics group at 24 weeks of gestation have higher vitamin D level and lower pregnancy weight and body fat rate. There may be some correlation between them during pregnancy.

Summary and Possible Mechanism

In conclusion, the intervention of multiple probiotics on pregnant women from 16 to 23 weeks of gestation did not significantly reduce the incidence of GDM, but the supplementation of multiple probiotics may affect the growth of pregnant women's weight, especially fat accumulation, blood lipids, incidence of postpartum hemorrhage as well as the metabolism of vitamin D.

Many studies have found that probiotics can reduce inflammatory response. Vitamin D is considered to be an immune-related nutrient. People with better serum vitamin D levels have lower levels of inflammatory responses in their body. The reduction of inflammatory response can reduce the level of free fatty acids in serum, thereby reducing the level of triglyceride in serum, and reducing the accumulation of fat in the body [37]. Therefore, the weight gain during pregnancy is reduced, and the body fat percentage is lower.

In our research, pregnant women in the probiotics group had lower triglyceride level, higher vitamin D level, lower body fat rate, lower postpartum hemorrhage rate, and better blood lipid level, nutritional status, and pregnancy outcome. Therefore, we believed that although the pathogenesis of GDM is affected by factors such as genetics and islet function, taking probiotics may achieve the above changes by improving the level of inflammatory response in pregnant women. Of course, the specific mechanism needs to be further studied, and the related inflammatory factors and adiponectin in the patient's body should be detected to further verify our conjecture.

The limitations of our study include lack of continued use of probiotics until the end of pregnancy because of lack of funding. In addition, due to the insufficient sample size, the conclusion of the preventive effect of probiotics on gestational diabetes mellitus is not certain. The specific mechanism of probiotics on inflammatory factors and lipid metabolism during pregnancy needs to be further studied, such as detecting related inflammatory factors and adiponectin in patients to further verify our conjecture.

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