Probiotics for the Prevention of Gestational Diabetes Mellitus in Overweight and Obese Women: Findings From the SPRING Double-Blind Randomized Controlled Trial

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OBJECTIVE

Given the role of gut microbiota in regulating metabolism, probiotics administered during pregnancy might prevent gestational diabetes mellitus (GDM). This question has not previously been studied in high-risk overweight and obese pregnant women. We aimed to determine whether probiotics (*Lactobacillus rhamnosus* and *Bifidobacterium animalis* subspecies *lactis*) administered from the second trimester in overweight and obese women prevent GDM as assessed by an oral glucose tolerance test (OGTT) at 28 weeks' gestation. Secondary outcomes included maternal and neonatal complications, maternal blood pressure and BMI, and infant body composition.

RESEARCH DESIGN AND METHODS

This was a double-blind randomized controlled trial of probiotic versus placebo in overweight and obese pregnant women in Brisbane, Australia.

RESULTS

The study was completed in 411 women. GDM occurred in 12.3% (25 of 204) in the placebo arm and 18.4% (38 of 207) in the probiotics arm ($P = 0.10$). At OGTT, mean fasting glucose was higher in women randomized to probiotics (79.3 mg/dL) compared with placebo (77.5 mg/dL) ($P = 0.049$). One- and two-hour glucose measures were similar. Preeclampsia occurred in 9.2% of women randomized to probiotics compared with 4.9% in the placebo arm ($P = 0.09$). Excessive weight gain occurred in 32.5% of women in the probiotics arm (55 of 169) compared with 46% in the placebo arm (81 of 176) ($P = 0.01$). Rates of small for gestational age (<10th percentile) were 2.4% in the probiotics arm (5 of 205) and 6.5% in the placebo arm (13 of 199) ($P = 0.042$). There were no differences in other secondary outcomes.

CONCLUSIONS

The probiotics used in this study did not prevent GDM in overweight and obese pregnant women.

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The incidence of gestational diabetes mellitus (GDM) is increasing in parallel with the rise in overweight and obesity in the obstetric population (1). GDM is associated with adverse maternal and neonatal outcomes. Women with GDM have an increased risk of preeclampsia and delivery by cesarean section (2,3). Maternal GDM increases the risk of excessive fetal growth and adiposity, shoulder dystocia, admission to the neonatal intensive care unit, and neonatal hypoglycemia (4,5). Furthermore, GDM is associated with increased later risk of obesity, type 2 diabetes, and cardiovascular disease in both mother and baby (6–8).

Women who are overweight and obese are at higher risk of GDM (9). GDM therapy is currently focused on normalizing glycemia. While this prevents or reduces the impact of short-term complications, this may not be true for the longer-term complications (10,11). Ideally, GDM would be prevented. A number of strategies to prevent GDM during pregnancy have been explored, including the use of metformin, lifestyle interventions, probiotics, *myo*-inositol, and vitamin D (12). Preconception GDM prevention strategies such as weight loss and lifestyle interventions seem logical but have been hampered by substantial barriers to engagement and behavioral change (13–15).

In recent years, the role of the gut microbiota in regulating metabolism has become a topic of investigation. The composition of the gut microbiota is determined by a large number of factors including disease states (16) and dietary intake (17). The gut microbiota are altered in obesity, although the extent and direction of the changes are variable (18). Pregnancy is associated with large changes to the gut microbiota with diversity decreasing as pregnancy progresses (19). In addition, probiotics—live bacteria with a known beneficial effect on the host—may be able to improve gut microbiota function and thereby the health status of the host (20). Interventions such as probiotics are attractive, as acceptability and compliance appear to be much better than with lifestyle interventions (21). There have been promising data to suggest that probiotics may positively alter measures of glucose metabolism or prevent GDM (22–24). Luoto et al. (24) reported 64% relative reduction in GDM frequency (13% in diet and probiotics, 36% in diet and placebo, and 34% in control groups) in a cohort of 256 pregnant women with a mean BMI of 23.6 kg/m^2. Given the higher risk of GDM in overweight and obese women and the effectiveness of probiotics in normal-weight women (15), we designed and conducted a randomized controlled trial (RCT) of probiotics in overweight and obese women.

RESEARCH DESIGN AND METHODS

SPRING (Study of PRobiotics IN Gestation) was a prospective double-blind RCT of probiotics versus placebo to examine whether probiotics prevented GDM in overweight and obese women following Consolidated Standards of Reporting Trials (CONSORT) guidelines (25) (as per Fig. 1). Detailed methodology including information regarding inclusion and exclusion criteria, sample size, and power calculations has been published (26). Inclusion criteria included a singleton pregnancy at <20 weeks' gestation, BMI of >25 kg/m^2, >18 years of age, able to read and understand English, and able to provide informed consent. Gestational age was determined clinically by the woman's pregnancy care provider, based on the first date of the last menstrual period or, where that was not known, estimated gestational age based on the earliest pregnancy ultrasound scan. As outlined in the published protocol (26), all women underwent a random venous plasma glucose (RVPG) prior to enrollment. Those with RVPG ≥8.0 mmol/L proceeded to a 75-g oral glucose tolerance test (OGTT) and were excluded if any values met or exceeded criteria for GDM. Exclusion criteria included gestational age >20 weeks at recruitment, multiple pregnancy, known preexisting diabetes, impaired fasting glucose or impaired glucose tolerance, taking medications that may influence glucose metabolism (metformin, glucocorticoids, immunosuppressants, antipsychotics), medical conditions known to alter glucose metabolism, known major fetal abnormality on 11- to 13-week ultrasound

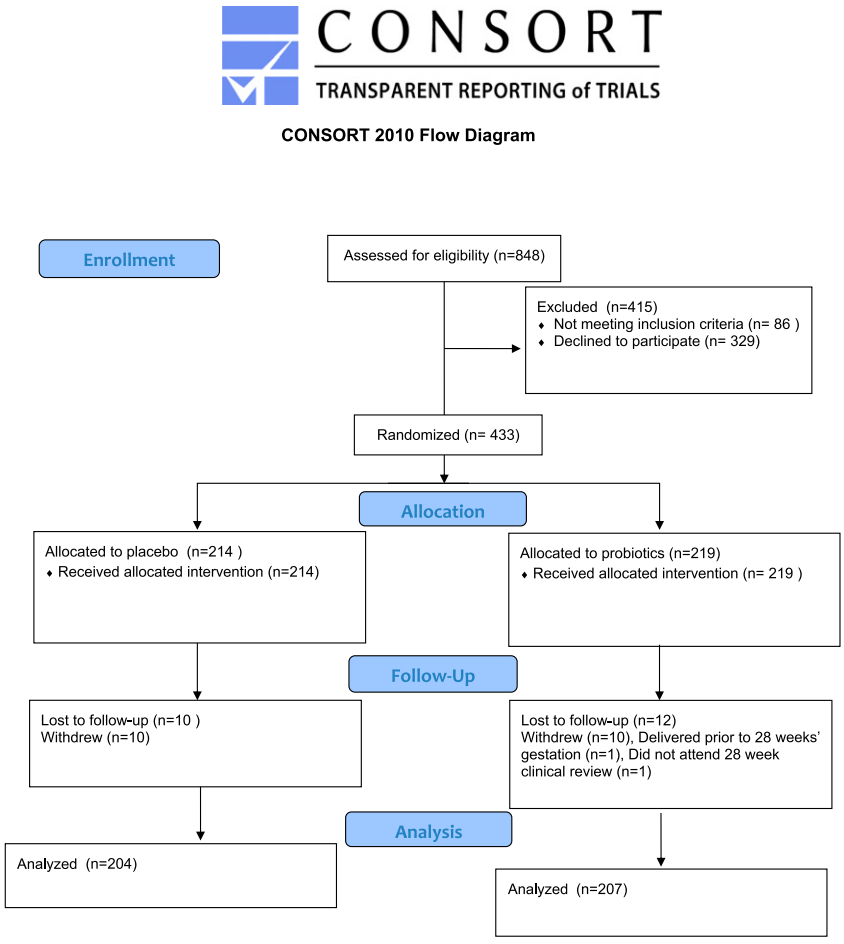

Figure 1—Eligibility, randomization, and follow-up.

scan, and known ingestion of probiotics via capsules or sachets. Overweight and obese women (BMI $\geq$25 kg/m^2, based on height and weight measured at their first study visit) were randomly assigned to probiotics or placebo prior to 20 weeks' gestation. The composition of the probiotics was identical that used by Luoto et al. (24) and consisted of a mixture of *Lactobacillus rhamnosus* (LGG) and *Bifidobacterium animalis* subspecies *lactis* (BB-12) (Chr. Hansen A/S, Hørsholm, Denmark) at a dose of $>1 \times 10^9$ colony-forming units each per day or matched placebo (microcrystalline cellulose and dextrose anhydrate capsules; Chr. Hansen) (27). Capsules were to be taken once daily from enrollment until birth.

Recruitment Facility

This study was conducted at the Royal Brisbane and Women's Hospital (RBWH), Redcliffe Hospital, and the Mater Mothers' Hospital in Brisbane, Australia. The trial commenced recruitment in November 2012.

Randomization Procedure

Participants were randomized using computer-generated random number codes sealed in opaque envelopes. Participants were stratified by center and by BMI category (BMI >25–30, >30–40, and >40 kg/m^2).

Allocation Concealment and Blinding

Matching placebo and probiotic capsules were identically packaged in the RBWH pharmacy by independent pharmacists. All study staff and participants were blinded to the randomized allocation.

Study Medication Compliance

Compliance to study medication adherence was monitored through interviews with all participants. For the subset of participants who supplied a fecal sample at 28 weeks' gestation, presence of BB-12 DNA was tested by end-stage PCR. In brief, DNA was isolated with the repeated bead beating and column (RBB+C) method and a Qiagen AllPrep DNA extraction kit as previously described (28). End-stage PCR was performed using the Bflact2 and Bflact5 primer pair (29). Presence of a band at 680 base pairs was considered confirmation of the presence of BB-12 in the gut microbiota.

Trial Conduct

We had initially planned to recruit women prior to 16 weeks' gestation. However, owing to changes in hospital policies regarding the timing of first antenatal visit, recruitment was extended up to 20 weeks' gestation in May 2015 after 310 women had been recruited. Sixty-three participants (14.4%) were recruited between 16 and 19 weeks' gestation. A fecal sample was requested at enrollment and at 28 weeks. The requirement for fecal samples was noted to be a barrier to recruitment, and this was removed in May 2015. Initial power calculations based on a 50% reduction in GDM frequency with the use of probiotics (from 18 to 9%), and allowing for 20% attrition, called for the recruitment of 540 women (452 completers). In the end, 436 women were randomized and 411 participants completed the study, reducing the power from 80 to 76% to find the originally predicted 50% reduction in GDM in the probiotic arm. Limited funding prevented prolongation of the study.

Outcomes

The primary outcome of the study was the frequency of GDM at 28 weeks' gestation by a 75-g OGTT using the World Health Organization–endorsed criteria of the International Association of the Diabetes and Pregnancy Study Groups (30).

Prespecified maternal secondary outcomes included gestational weight gain, preeclampsia, hypertensive disorders of pregnancy, cesarean delivery, and gestational age at delivery. Weight gain based on self-report at the beginning of pregnancy and measured weight at 36 weeks' gestation was categorized as excessive or inadequate based on the Institute of Medicine guidelines (31). Planned neonatal outcomes included prematurity, neonatal special care admission, jaundice, hypoglycemia, birth weight, small for gestational age (SGA), large for gestational age, stillbirth, birth injury, congenital anomaly, fat-free mass, and percentage fat (measured in the PEA POD Infant Body Composition System [COSMED, Rome, Italy], using air displacement plethysmography). Australian national birth weight percentiles by sex and gestational age were used. Standard definitions were used for other outcomes (32,33).

Analysis

Baseline characteristics were summarized for each intervention group as *n* (%) for categorical variables, mean (SD) for normally distributed continuous variables, and median (interquartile range [IQR]) for nonnormally distributed continuous variables. Intervention group comparisons were analyzed using binary logistic regression or general linear models as appropriate with adjustment for center and BMI category. Adjusted odds ratios and difference of means with 95% CIs were reported. Where a model did not converge due to small cell counts, the effect estimate and *P* value were not reported. Statistical significance was specified at $\alpha < 0.05$. Data were analyzed using IBM SPSS Statistics 23. All analyses were conducted by an independent statistician at the QIMR Berghofer Medical Research Institute.

Ethics Considerations

Participants provided written informed consent. The study was approved by the human research ethics committees of the RBWH, Mater Health Services, and The University of Queensland. The clinical trial management committee conducted interim analysis to ensure there were no adverse events and, along with all relevant ethics committees, approved all changes to the clinical trial protocol to assist with recruitment.

RESULTS

There were 204 women in the placebo group and 207 women in the probiotics group. Compliance with study medication, assessed by participant interview and monthly telephone follow-up, was reported as >90%.

The presence of the probiotic organism BB-12 in the feces of a subset of study participants randomized to probiotic therapy was confirmed by targeted PCR. Of the women with stool samples obtained at 28 weeks' gestation (*N* = 215 [105 probiotics and 110 placebo]), detection of fecal BB-12 in the probiotics group was similar between women who developed GDM (12 of 16 [75%]) and those who did not (71 of 89 [80%], *P* = 0.74).

Baseline characteristics for each intervention group are outlined in Table 1. Women were recruited into the study at a mean of 15.9 weeks (SD 1.5) and a median of 15.9 weeks (IQR 14.9–16.7). Prerandomization median RVPG was 80 mg/dL (IQR 79–94) in the placebo group and 86 mg/dL (IQR 81–95) in the probiotics group. Fifty-five women in the placebo group and 56 in the probiotics group underwent an early

OGTT, and mean fasting glucose values in both groups were identical at 77 mg/dL (SD 7).

The primary outcome and underpinning glucose measures relating to the primary outcome for this study are presented in Table 2. Rates of GDM were 25 of 204 (12.3%) in the placebo arm and 38 of 207 (18.4%) in the probiotics arm of the study ($P = 0.10$). Mean fasting glucose was higher in women randomized to probiotics (79.3 mg/dL) compared with placebo (77.5 mg/dL) ($P = 0.049$) (difference of means 1.53 [95% CI 0.0095–3.06]). One- and two-hour glucose levels were similar between the groups. There was no indication that rates of GDM were lower in the probiotics arm, and if anything, the results suggested a trend in the opposite direction.

Maternal and neonatal secondary outcomes are presented in Table 3. In women taking probiotics, 9.2% developed preeclampsia compared with 4.9% in the placebo group (0 = 0.09). Excessive weight gain occurred in 32.5% of women in the probiotics group compared with 46% of women in the placebo group ($P = 0.01$). However, there was no difference in mean weight gain in the two groups. There were no other statistically significant differences in any maternal secondary outcome measures between probiotics and placebo. The only statistically significant difference in neonatal outcomes related to SGA (<10th percentile). SGA occurred in 13 of 199 infants in the placebo arm (6.5%) and 5 of 205 infants in the probiotics arm (2.4%) ($P = 0.042$).

CONCLUSIONS

We conclude that probiotics, administered throughout pregnancy from the first half of the second trimester, do not reduce the frequency of GDM at 28 weeks' gestation.

We also conclude that this study provides evidence that daily ingestion of LGG and BB-12 at a dose of $>1 \times 10^9$ colony-forming units each per day does not alter any of the primary or secondary maternal outcomes examined in overweight and obese women. There was a statistically significant reduction in SGA (<10th percentile) in infants born to women taking probiotics.

While there was no statistical difference in GDM between the arms of this study, we noted a higher fasting glucose in women who were randomized to the probiotics arm. Although statistically significant, this small increase in fasting glucose may not be clinically significant. However, if carried through to the population level, even a small increase in fasting glucose could result in higher GDM prevalence. The data from this and other studies will need to be further explored when the Cochrane Review of this topic is updated (34). Although the total number of participants was slightly lower than initially planned, the results clearly do not support efficacy of probiotics.

It is hard to reconcile the lower rates of excessive weight gain in women on probiotics with the other findings from this study. Further, there was no difference in overall weight gain between groups or weight gain per week. However, the role of probiotics in prevention of excessive weight gain will need further exploration.

The lower rates of SGA in infants in the probiotic arm may have related to the fact that 18.4% of these pregnancies were exposed to GDM compared with 12.3% of infants born to women in the placebo group. The higher fasting glucose level in the probiotics arm may have also influenced the lower rate of SGA in these infants. The higher rate of inadequate weight gain and lower rate of excessive weight gain with probiotics may also have contributed. However, the overall rate in both arms was much less than the expected 10%, so the SGA rate is overall low rather than high. No other measure of body composition was consistent with this lower rate of SGA. The alternative explanation is that probiotics have a role in the prevention of SGA. This will need to be explored in future meta-analyses.

For many years, there has been widespread speculative commentary regarding the potential for probiotics to be an elegant and easy solution to GDM prevention (35). They appeared safe and easy to take without any obvious maternal or neonatal side effects (36).

Table 1—Baseline characteristics according to trial group

	Placebo group ($n = 204$)	Probiotics group ($n = 207$)
Age (years)†	31.7 (4.8)	31.3 (4.7)
Age ≥35 years*	57 (27.9)	54 (26.1)
Caucasian*	171 (83.8)	186 (89.9)
Patient center*		
Mater	97 (47.5)	101 (48.8)
Redcliffe	16 (7.8)	18 (8.7)
RBWH	91 (44.6)	88 (42.5)
Gestational age at enrollment (weeks)†§	16.0 (1.4)	15.9 (1.5)
Gestational age at enrollment*§		
12–13 weeks	7 (3.8)	14 (7.3)
14–16 weeks	154 (83.2)	146 (76.4)
17–19 weeks	24 (13.0)	31 (16.2)
Tertiary educated*	95 (46.6)	98 (47.3)
Current smoker*§	6 (3.0)	8 (3.9)
Nulliparous*	85 (41.7)	74 (35.7)
BMI (kg/m^2)*		
25–29	76 (37.3)	66 (31.9)
30–39	101 (49.5)	114 (55.1)
40+	27 (13.2)	27 (13.0)
BMI (kg/m^2)‡	31.6 (7.2)	31.9 (7.5)
Systolic BP (mmHg)†§	109.3 (9.6)	109.6 (10.1)
Diastolic BP (mmHg)†§	67.0 (7.8)	67.2 (7.9)
Previous GDM*§	10 (6.7)	12 (7.9)
Family history of diabetes*§	60 (30.3)	52 (25.4)
Chronic hypertension*§	5 (2.5)	6 (2.9)
Alcohol use in pregnancy*§	12 (5.9)	8 (3.9)

BP, blood pressure. *n (%). †Mean (SD). ‡Median (IQR). §Gestational age at enrollment $n_{placebo} = 185$, $n_{probiotics} = 191$; current smoker $n_{placebo} = 203$, $n_{probiotics} = 207$; systolic blood pressure, diastolic pressure, and chronic hypertension $n_{placebo} = 202$, $n_{probiotics} = 206$; previous GDM $n_{placebo} = 149$, $n_{probiotics} = 152$; family history of GDM $n_{placebo} = 198$, $n_{probiotics} = 205$; alcohol use in pregnancy $n_{placebo} = 203$, $n_{probiotics} = 206$.

Table 2—Primary outcome: GDM at 28 weeks' gestation

	Summary			
	Placebo (n = 204)	Probiotics (n = 207)	Effect (95% CI)‡	P‡
GDM*	25 (12.3)	38 (18.4)	1.62 (0.91–2.89)	0.10
Fasting glucose, mmol/L [mg/dL]†§	4.3 (0.45) [77.5 (8.1)]	4.4 (0.5) [79.3 (9.0)]	0.085 (0.00053–0.17) [1.53 (0.0095–3.06)]	0.049
1-h glucose, mmol/L [mg/dL]†§	7.5 (1.6) [135.1 (28.8)]	7.6 (1.8) [136.9 (32.4)]	0.052 (−0.27 to 0.37) [0.94 (−4.86 to 6.66)]	0.75
2-h glucose, mmol/L [mg/dL]†§	6.3 (1.4) [113.5 (25.2)]	6.4 (1.5) [115.3 (27.0)]	0.13 (−0.15 to 0.40) [2.34 (−2.70 to 7.20)]	0.37

*Summary: n (%). Effect: odds ratio with placebo as referent. †Summary: mean (SD). ‡Effect: difference of means. Adjusted for BMI (25–29, 30–39, and 40+ kg/m^2) and patient center (RBWH, Redcliffe, Mater). §$n_{placebo}$ = 202; $n_{probiotics}$ = 205.

Despite reassuring safety data, and the logical reasons that probiotics might improve glucose metabolism, our study clearly argues against efficacy of probiotics in GDM prevention.

Consistent with our findings, Lindsay et al. (37) examined the use of *Lactobacillus salivarius* UCC118 for 4 weeks from 24 weeks' gestation in a population of obese women in Ireland and found no difference in any measure of glucose metabolism. Asemi et al. (23) compared probiotic yogurt (*Lactobacillus acidophilus* LA5 and BB-12) with conventional yogurt over a 9-week period during pregnancy. Consistent with our study, there was no improvement in fasting glucose or systolic or diastolic blood pressure in the probiotic yogurt arm.

In the Finnish study, which inspired the current study, probiotic supplementation with LGG and BB-12 reduced the risk of elevated maternal glucose concentrations (38) and the incidence of GDM in women with a mean BMI of 23.6 kg/m^2 (SD 3.8) (24). Our active treatment arm included the same probiotic preparation. There were several key differences between our study and the Finnish study. Firstly, women were selected into the Finnish study based on a personal history of atopic disease or at least one relative with atopic disease; 79% of women in this study had a history of atopic disease. In contrast, participants in our study were selected on

Table 3—Secondary outcomes

	n		Summary			
Secondary outcomes	Placebo	Probiotic	Placebo	Probiotic	Effect (95% CI)‡	P
Mother						
Preeclampsia*	203	206	10 (4.9)	19 (9.2)	2.00 (0.89–4.50)	0.09
Gestational hypertension*	203	206	11 (5.4)	10 (4.9)	0.86 (0.35–2.09)	0.74
Hypertensive disorders of pregnancy*	203	206	26 (12.8)	34 (16.5)	1.35 (0.76–2.37)	0.30
Cesarean*	204	207	80 (39.2)	73 (35.3)	0.85 (0.56–1.27)	0.41
In labor/intrapartum/emergency caesarean*	78	73	36 (46.2)	33 (45.2)	1.00 (0.52–1.93)	1.00
Induced labor*	202	206	62 (30.7)	74 (35.9)	1.23 (0.81–1.87)	0.34
28 weeks systolic BP (mmHg)†	196	197	110.3 (10.6)	110.4 (9.9)	0.003 (−1.90 to 1.91)	1.00
28 weeks diastolic BP (mmHg)†	196	197	65.0 (7.6)	66.4 (7.8)	1.36 (−0.11 to 2.84)	0.070
36 weeks systolic BP (mmHg)†	177	169	114.0 (10.6)	115.7 (11.3)	1.62 (−0.66 to 3.90)	0.16
36 weeks diastolic BP (mmHg)†	177	169	70.5 (8.3)	70.9 (9.8)	0.51 (−1.35 to 2.37)	0.59
36 weeks weight gain from baseline (kg)†	176	169	9.5 (4.3)	8.9 (5.3)	−0.55 (−1.55 to 0.45)	0.28
Excess weight gain*	176	169	81 (46)	55 (32.5)	0.56 (0.36–0.87)	0.01
Inadequate weight gain			28 (15.9)	34 (20.1)		
Weight gain per week from baseline to 36 weeks (kg/week)	176	169	0.40 (0.19)	0.37 (0.23)	−0.03 (−0.08 to 0.01)	0.17
Infant						
Gestational age at delivery (weeks)	180	193	39.32 (1.75)	39.14 (1.88)	−0.18 (−0.55 to 0.19)	0.34
Very preterm (<34 weeks)*	180	193	3 (1.7)	5 (2.6)	1.59 (0.37–6.85)	0.53
Preterm (<37 weeks)*	180	193	12 (6.7)	17 (8.8)	1.36 (0.63–2.96)	0.43
Special care unit admission*	199	207	43 (21.6)	42 (20.3)	0.92 (0.57–1.50)	0.75
Jaundice*	201	205	40 (19.9)	35 (17.1)	0.82 (0.50–1.36)	0.45
Birth injury*	198	203	1 (0.5)	1 (0.5)	—	—
Hypoglycemia*	200	202	27 (13.5)	25 (12.4)	0.90 (0.50–1.63)	0.73
Stillbirth*	204	207	1 (0.5)	0 (0.0)	—	—
Congenital abnormality*	201	204	6 (3.0)	10 (4.9)	1.70 (0.60–4.80)	0.32
Macrosomia (>4,000 g)*	203	206	35 (17.2)	31 (15.0)	0.85 (0.50–1.45)	0.56
Macrosomia (>4,500 g)*	203	206	2 (1.0)	7 (3.4)	—	—
Large for gestational age (>90th percentile)*	180	193	30 (16.7)	35 (18.1)	1.09 (0.64–1.88)	0.75
SGA (<2,500 g)*	203	206	6 (3.0)	7 (3.4)	1.15 (0.38–3.50)	0.81
SGA (<10th percentile)*	199	205	13 (6.5)	5 (2.4)	0.33 (0.12–0.96)	0.042
Birth weight (g)†	203	206	3,541 (514)	3,524 (540)	−15.55 (−118.24 to 87.15)	0.77
Fat-free mass (g)†	105	103	3,011 (357)	3,033 (356)	21.27 (−76.66 to 119.20)	0.67
Percentage fat†	105	105	12.3 (3.6)	12.2 (4.4)	−0.13 (−1.23 to 0.97)	0.82

*Summary columns: n (%). Effect: odds ratio. †Summary columns: mean (SD). Effect: difference of means. ‡Adjusted for BMI (25–29, 30–39, and 40+ kg/m^2) and patient center (RBWH, Redcliffe, Mater).

the basis of BMI. Women in the Finnish study were relatively lean in comparison with the overweight and obese women in our study. Interestingly, the rate of GDM in our population was much lower than that in the Finnish study (34% in the control arm). GDM rates in the Finnish study were likely to be higher, due to the use of different diagnostic criteria, and this may well have been driven by the lower fasting glucose cutoff (39). In the Finnish study, probiotics were administered in addition to a dietary intervention, whereas in our study, only limited dietary advice was provided: to ensure probiotics were taken with milk or water and not with hot or acidic drinks. It is likely that the women in the Finnish study differed substantially from the women in our population in terms of genetics, obesity, immune system characteristics, dietary patterns, and levels of exercise. These factors could individually and collectively affect both the baseline microbiome as well as changes during pregnancy. It has been shown that there is large variability in which bacterial species become more or less abundant in pregnancy (19). Therefore, multiple factors may have influenced the conflicting outcomes and will need to be explored through future research.

Consistent with the Finnish study, a recent New Zealand study of women or partners at risk for atopic disease who were administered *Lactobacillus rhamnosus* HN001 in pregnancy found that the rate of GDM was 13.8% in the placebo arm and 8.2% in the probiotic arm, with a relative risk of 0.59 (95% CI 0.32–1.08) (22). This study used the same criteria for GDM as was used in our study. The trend toward a lower rate of GDM in this New Zealand study may suggest that probiotic species other than the one used in our study may have different effects. The results of these two studies could suggest that probiotics may be more effective in those with a background of atopic disease compared with the overweight and obese women recruited to our study.

In a recently published meta-analysis (40), unpublished data were obtained from authors of two studies (41,42) and combined with the work of Lindsay et al. (37) to address the issue of probiotics in the prevention of GDM. The combined outcomes of GDM in 365 women across these three different studies were consistent with our large study and demonstrated no effect of probiotics for the prevention of GDM. Surprisingly, two of the studies that assessed GDM outcomes in this meta-analysis commenced probiotics at 32 weeks' (41) and 36 weeks' (42) gestation, respectively. It is unclear how an intervention commenced after 32 weeks could be expected to prevent an outcome that is usually diagnosed by an OGTT at 26–28 weeks' gestation.

In contrast, another recent meta-analysis found an improvement in metabolic outcomes in pregnant women (43). In this meta-analysis, only the Lindsay study, already discussed above, related to GDM prevention in overweight and obese women. This meta-analysis included five studies of probiotics in healthy pregnant women and four studies on the effect of probiotics in women who already had GDM. Probiotics were associated with lower fasting serum insulin, no change in fasting plasma glucose, and conflicting results with the two approaches to measuring insulin resistance.

Our study was designed to be pragmatic with regard to probiotic species, dose, and gestational age at commencement of the intervention. These decisions were guided by feasibility and practicality. Therefore, while our study did not show benefit, other probiotic species might be efficacious in the prevention of GDM. The question of dose of probiotics for pregnant overweight and obese women was not examined in this study, and it is possible that outcomes may have differed (in either direction) with an increased dose of probiotics. In our analysis of the presence of the bacteria in the feces of the women, adherence was 75% and 80% in the women on probiotics who developed GDM and remained normoglycemic, respectively. This suggests that adherence was similar and therefore that the probiotic intervention likely does not affect the development of GDM. The difference in adherence between the fecal analysis (79%) and the self-reporting of intake by the women (90%) could reflect inaccurate self-reporting. However, we have not examined how long the bacteria are detectable in the feces after cessation of the intervention nor do we know the exact sensitivity threshold for the appearance of a band on the agarose gel. Therefore, it is possible that the difference in adherence based on fecal analysis versus self-report is not as large as it appears.

It is also possible that probiotics started prior to pregnancy, or in very early pregnancy, have an effect different from that observed here. Women in the study by Wickens et al. (22) were randomized to probiotics at 14–16 weeks' gestation. Women in the study by Luoto et al. (24) had their first study visit at a median of 14 weeks' gestation (range 7–18) (44). Our median gestation at commencement of intervention was 15.9 weeks' gestation. It is possible that a longer period of intervention may influence outcomes. While earlier commencement of probiotics during pregnancy would be important to explore, this poses practical difficulties in routine clinical practice. Even if very early institution of probiotics was found to be useful, there would be significant logistic barriers to the translation of positive findings. This is a question that will need to be explored in other studies.

The strengths of this study are that it is the largest double-blind RCT conducted to date examining the role of probiotics for the prevention of GDM in a high-risk group. It used a probiotic that had previously been shown to prevent GDM (24). The results are quite clearly negative.

There are some limitations to our study. Our protocol did not require a formal OGTT for every participant prior to enrollment, so although available measures of baseline glycemia were similar between the groups, we are not able to exclude more subtle differences. It is possible, given the low rates of smoking and high rates of tertiary education, that the overweight and obese women in this study were healthier than average. Therefore, the trial population may not be representative of all overweight and obese women. Our original recruitment targets were not achieved, but it is very unlikely that recruitment of additional participants would have altered the conclusions of this study. Owing to slow recruitment and altered hospital routines, we changed the gestational age at enrollment to include participants up to 20 weeks' gestation, which was approved and supported by all of the relevant regulatory authorities and the trial management committee. This lowered the total exposure time to probiotics for some women (14.4% of the total study population) by 1–4 weeks. However, given the outcomes of this study,

it is hard to envisage that a longer period of probiotic exposure in the intervention arm would have altered our findings. Nonetheless, the impact of probiotics from very early in pregnancy remains an unanswered question.

Based on the findings of this double-blind randomized placebo controlled trial, we conclude that probiotics administered from the second trimester of pregnancy do not prevent GDM, or improve secondary outcomes, in overweight and obese pregnant women.

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References

1. Buchanan TA, Xiang AH, Page KA. Gestational diabetes mellitus: risks and management during and after pregnancy. Nat Rev Endocrinol 2012;8:639–649
2. Dempsey JC, Ashiny Z, Qiu CF, Miller RS, Sorensen TK, Williams MA. Maternal pre-pregnancy overweight status and obesity as risk factors for cesarean delivery. J Matern Fetal Neonatal Med 2005;17:179–185
3. Carr DB, Newton KM, Utzschneider KM, et al. Gestational diabetes or lesser degrees of glucose intolerance and risk of preeclampsia. Hypertens Pregnancy 2011;30:153–163
4. Wendland EM, Torloni MR, Falavigna M, et al. Gestational diabetes and pregnancy outcomes–a systematic review of the World Health Organization (WHO) and the International Association of Diabetes in Pregnancy Study Groups (IADPSG) diagnostic criteria. BMC Pregnancy Childbirth 2012;12:23
5. Esakoff TF, Cheng YW, Sparks TN, Caughey AB. The association between birthweight 4000 g or greater and perinatal outcomes in patients with and without gestational diabetes mellitus. Am J Obstet Gynecol 2009;200:672.e1–4
6. Kim C. Gestational diabetes mellitus and risk of future maternal cardiovascular disease. Expert Rev Cardiovasc Ther 2010;8:1639–1641
7. Crume TL, Ogden L, West NA, et al. Association of exposure to diabetes in utero with adiposity and fat distribution in a multiethnic population of youth: the Exploring Perinatal Outcomes among Children (EPOCH) Study. Diabetologia 2011;54:87–92
8. Malcolm J. Through the looking glass: gestational diabetes as a predictor of maternal and offspring long-term health. Diabetes Metab Res Rev 2012;28:307–311
9. McIntyre HD, Gibbons KS, Flenady VJ, Callaway LK. Overweight and obesity in Australian mothers: epidemic or endemic? Med J Aust 2012;196:184–188
10. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS; Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS) Trial Group. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med 2005;352:2477–2486
11. Landon MB, Spong CY, Thom E, et al.; Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. A multicenter, randomized trial of treatment for mild gestational diabetes. N Engl J Med 2009;361:1339–1348
12. Simmons D. Prevention of gestational diabetes mellitus: where are we now? Diabetes Obes Metab 2015;17:824–834
13. Callaway LK, O'Callaghan MJ, McIntyre HD. Barriers to addressing overweight and obesity before conception. Med J Aust 2009;191:425–428
14. Foxcroft KF, Rowlands IJ, Byrne NM, McIntyre HD, Callaway LK; BAMBINO Group. Exercise in obese pregnant women: the role of social factors, lifestyle and pregnancy symptoms. BMC Pregnancy Childbirth 2011;11:4
15. Callaway LK, Colditz PB, Byrne NM, et al.; BAMBINO Group. Prevention of gestational diabetes: feasibility issues for an exercise intervention in obese pregnant women. Diabetes Care 2010;33:1457–1459
16. Bull MJ, Plummer NT. Part 1: the human gut microbiome in health and disease. Integr Med (Encinitas) 2014;13:17–22
17. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014;505:559–563
18. Bouter KE, van Raalte DH, Groen AK, Nieuwdorp M. Role of the gut microbiome in the pathogenesis of obesity and obesity-related metabolic dysfunction. Gastroenterology 2017;152:1671–1678
19. Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell 2012;150:470–480
20. Floch MH. The effect of probiotics on host metabolism: the microbiota and fermentation. J Clin Gastroenterol 2010;44(Suppl. 1):S19–S21
21. Lindsay KL, Brennan L, McAuliffe FM. Acceptability of and compliance with a probiotic capsule intervention in pregnancy. Int J Gynaecol Obstet 2014;125:279–280
22. Wickens KL, Barthow CA, Murphy R, et al. Early pregnancy probiotic supplementation with Lactobacillus rhamnosus HN001 may reduce the prevalence of gestational diabetes mellitus: a randomised controlled trial. Br J Nutr 2017;117:804–813
23. Asemi Z, Samimi M, Tabassi Z, et al. Effect of daily consumption of probiotic yoghurt on insulin resistance in pregnant women: a randomized controlled trial. Eur J Clin Nutr 2013;67:71–74
24. Luoto R, Laitinen K, Nermes M, Isolauri E. Impact of maternal probiotic-supplemented dietary counselling on pregnancy outcome and prenatal and postnatal growth: a double-blind, placebo-controlled study. Br J Nutr 2010;103:1792–1799
25. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. BMJ 2010;340:c869
26. Nitert MD, Barrett HL, Foxcroft K, et al. SPRING: an RCT study of probiotics in the prevention of gestational diabetes mellitus in overweight and obese women. BMC Pregnancy Childbirth 2013;13:50
27. Barrett HL, Callaway LK, Nitert MD. Probiotics: a potential role in the prevention of gestational diabetes? Acta Diabetol 2012;49(Suppl. 1):S1–S13
28. Yu Z, Morrison M. Improved extraction of PCR-quality community DNA from digesta and fecal samples. Biotechniques 2004;36:808–812
29. Ventura M, Reniero R, Zink R. Specific identification and targeted characterization of Bifidobacterium lactis from different environmental isolates by a combined multiplex-PCR approach. Appl Environ Microbiol 2001;67:2760–2765
30. Metzger BE, Gabbe SG, Persson B, et al.; International Association of Diabetes and Pregnancy Study Groups Consensus Panel. International Association of Diabetes and Pregnancy Study Groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010;33:676–682
31. American College of Obstetricians and Gynecologists. ACOG Committee opinion no. 548: weight gain during pregnancy. Obstet Gynecol 2013;121:210–212
32. Feig DS, Corcoy R, Jensen DM, et al.; International Association of Diabetes in Pregnancy Study Group (IADPSG) Working Group on Outcome Definitions. Diabetes in pregnancy outcomes: a systematic review and proposed codification of definitions. Diabetes Metab Res Rev 2015;31:680–690

33. Lowe SA, Bowyer L, Lust K, et al.; Society of Obstetric Medicine of Australia and New Zealand. The SOMANZ Guidelines for the Management of Hypertensive Disorders of Pregnancy 2014. Aust N Z J Obstet Gynaecol 2015;55:11–16
34. Barrett HL, Dekker Nitert M, Conwell LS, Callaway LK. Probiotics for preventing gestational diabetes. Cochrane Database Syst Rev 2014;CD009951
35. Isolauri E, Rautava S, Collado MC, Salminen S. Role of probiotics in reducing the risk of gestational diabetes. Diabetes Obes Metab 2015; 17:713–719
36. Allen SJ, Jordan S, Storey M, et al. Dietary supplementation with lactobacilli and bifidobacteria is well tolerated and not associated with adverse events during late pregnancy and early infancy. J Nutr 2010;140:483–488
37. Lindsay KL, Kennelly M, Culliton M, et al. Probiotics in obese pregnancy do not reduce maternal fasting glucose: a double-blind, placebo-controlled, randomized trial (Probiotics in Pregnancy Study). Am J Clin Nutr 2014;99:1432–1439
38. Laitinen K, Poussa T, Isolauri E; Nutrition, Allergy, Mucosal Immunology and Intestinal Microbiota Group. Probiotics and dietary counselling contribute to glucose regulation during and after pregnancy: a randomised controlled trial. Br J Nutr 2009;101:1679–1687
39. Metzger BE, Coustan DR. Summary and recommendations of the Fourth International Workshop-Conference on Gestational Diabetes Mellitus. The Organizing Committee. Diabetes Care 1998;21(Suppl. 2):B161–B167
40. Jarde A, Lewis-Mikhael AM, Moayyedi P, et al. Pregnancy outcomes in women taking probiotics or prebiotics: a systematic review and meta-analysis. BMC Pregnancy Childbirth 2018;18:14
41. Rautava S, Kainonen E, Salminen S, Isolauri E. Maternal probiotic supplementation during pregnancy and breast-feeding reduces the risk of eczema in the infant. J Allergy Clin Immunol 2012;130:1355–1360
42. Mastromarino P, Capobianco D, Miccheli A, et al. Administration of a multistrain probiotic product (VSL#3) to women in the perinatal period differentially affects breast milk beneficial microbiota in relation to mode of delivery. Pharmacol Res 2015;95-96:63–70
43. Zheng J, Feng Q, Zheng S, Xiao X. The effects of probiotics supplementation on metabolic health in pregnant women: an evidence based meta-analysis. PLoS One 2018;13: e0197771
44. Aaltonen J, Ojala T, Laitinen K, Piirainen TJ, Poussa TA, Isolauri E. Evidence of infant blood pressure programming by maternal nutrition during pregnancy: a prospective randomized controlled intervention study. J Pediatr 2008; 152:79–84, 84.e1–2