Probiotics in obese pregnancy do not reduce maternal fasting glucose: a double-blind, placebo-controlled, randomized trial (Probiotics in Pregnancy Study)[1–3]

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ABSTRACT

Background: Recent studies have reported beneficial effects of probiotics on maternal glycemia in healthy pregnant women. Obesity significantly increases risk of impaired glucose tolerance in pregnancy, but glycemic effects of probiotics in this specific obstetric group require additional investigation.

Objective: The aim of the Probiotics in Pregnancy Study was to investigate the effect of a probiotic capsule on maternal fasting glucose in obese pregnant women.

Design: In this placebo-controlled, double-blind, randomized trial, 175 pregnant women with an early pregnancy body mass index (BMI; in kg/m^2) from 30.0 to 39.9 were recruited from antenatal clinics at the National Maternity Hospital, Dublin, Ireland. Exclusion criteria were BMI <30.0 or >39.9, prepregnancy or gestational diabetes, age <18 y, multiple pregnancy, and fetal anomaly. Women were randomly assigned to receive either a daily probiotic or a placebo capsule from 24 to 28 wk of gestation in addition to routine antenatal care. The primary outcome was the change in fasting glucose between groups from preintervention to postintervention. Secondary outcomes were the incidence of gestational diabetes and neonatal anthropometric measures.

Results: In 138 women who completed the study (63 women in the probiotic group; 75 women in the placebo group), mean (±SD) early pregnancy BMI was 33.6 ± 2.6, which differed significantly between probiotic (32.9 ± 2.4) and placebo (34.1 ± 2.7) groups. With adjustment for BMI, the change in maternal fasting glucose did not differ significantly between treated and control groups [−0.09 ± 0.27 compared with −0.07 ± 0.39 mmol/L; $P = 0.391$; $B = -0.05$ (95% CI: −0.17, 0.07)]. There were also no differences in the incidence of impaired glycemia (16% in the probiotic group compared with 15% in the placebo group; $P = 0.561$), birth weight (3.70 kg in the probiotic group compared with 3.68 kg in the placebo group; $P = 0.723$), or other metabolic variables or pregnancy outcomes. A secondary analysis of 110 women, excluding antibiotic users and poor compliers, also revealed no differences in maternal glucose or other outcomes between groups.

Conclusion: Probiotic treatment of 4 wk during pregnancy did not influence maternal fasting glucose, the metabolic profile, or pregnancy outcomes in obese women. This trial was registered at Current Controlled Trials as ISRCTN97241163 (part A). *Am J Clin Nutr* 2014;99:1432–9.

INTRODUCTION

The prevalence of maternal obesity in pregnancy has increased worldwide, with the rates closely following the rising obesity rates in the general population (1, 2). In 2011, the prevalence of obesity [BMI (in kg/m^2) >30] in women aged 18–35 y in Ireland was 13.3% (3), whereas the prevalence in pregnant women who were attending the National Maternity Hospital in Dublin was 13% (4). Maternal obesity increases risk of various complications in pregnancy including the development of gestational diabetes mellitus (GDM)[4], thromboembolism, preeclampsia, hypertensive disorders, cesarean delivery, miscarriage, and stillbirth (5).

GDM is an important complication of pregnancy because of the significant risks it poses for pregnancy outcomes and the long-term health of both the mother and infant. In the short term, GDM can result in preeclampsia, birth of a macrosomic baby, emergency cesarean delivery, shoulder dystocia, admission to the neonatal intensive care unit (NICU), and neonatal hypoglycemia (6). Long-term complications of GDM include obesity, diabetes, and cardiovascular disease for both the mother and baby (6, 7). Many of these adverse outcomes arise from maternal hyperglycemia that drives excess fetal growth and adiposity, and a positive correlation between these factors has also been shown in women without GDM (8). Furthermore, the Hyperglycemia and Pregnancy Outcome study reported increased pregnancy complications associated with maternal glucose concentrations below those traditionally used as diagnostic cutoffs for GDM (9). Thus, the prevention of GDM is highly desirable, but even small reductions in maternal glucose in

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[4] Abbreviations used: CRP, C-reactive protein; GDM, gestational diabetes mellitus; IGT, impaired glucose tolerance; NICU, neonatal intensive care unit; OGTT, oral-glucose-tolerance test.

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non-GDM women, and particularly in women at high risk of GDM, may provide significant benefit to pregnancy outcomes and the future health of offspring.

Interventions to prevent GDM have largely focused on dietary and lifestyle modifications with varying degrees of success (10–12). Recently, the potential role of the gut microbiota in glycemic control has come to light (13), and the use of probiotics in pregnancy to improve maternal metabolism through the modification of gut microbiota has been recently reviewed (14). Probiotics are live microorganisms that, when administered in adequate amounts, may confer a health benefit for the host (15), and they are commonly consumed in the diet as a component of some yogurts or in supplement form. A randomized controlled trial of probiotics combined with a dietary intervention in healthy pregnant women showed improved glucose control (16) and a reduction in GDM rates (17) in comparison with women who received a placebo with or without dietary advice. Meanwhile, Asemi et al (18) reported that probiotic yogurt compared with placebo yogurt helped maintain serum insulin concentrations in healthy pregnant women, which the authors hypothesized may help to prevent insulin resistance. However, because obese pregnant women are a particularly high risk group for GDM and its associated complications, there is a requirement to investigate whether positive interventions in healthy pregnant women can translate to this important obstetric group.

The aim of the Probiotics in Pregnancy Study was to investigate effects of a daily probiotic capsule compared with placebo capsule on maternal glycemia and other metabolic indexes in obese pregnant women.

SUBJECTS AND METHODS

Study design and setting

The study was a single-center, double-blind, placebo-controlled, randomized trial with maternal written consent conducted in accordance with the standards of the National Maternity Hospital Ethics Committee, which granted full ethical approval for the trial in November 2011. This trial was registered at Current Controlled Trials as ISRCTN97241163 (part A).

Patient selection

Obese pregnant women were identified at their first antenatal visit to the hospital. Inclusion criteria were as follows: <20 wk of gestation, BMI from 30.0 to 39.9 on the basis of measured weight and height at the first visit, singleton pregnancy, and age >18 y. Women were unsuitable for inclusion if their BMI was outside of the specified range, they had a history of gestational diabetes or non-gestational diabetes (type 1 or 2), a fetal anomaly was present, they were carrying twins or triplets, or they were unable to give full informed consent.

Blinding, masking, and random assignment

Probiotic and placebo capsules were produced and supplied by Alimentary Health Ltd. Each active probiotic capsule contained 100 mg *Lactobacillus salivarius* UCC118 freeze-dried powder to achieve a target dose of 10^9 CFU. This probiotic strain was chosen because it had been previously shown to transit and persist within the intestinal tract of healthy humans and influence their immune systems (19). Both capsules were identical in appearance and packaged in bottles labeled as either capsule A or capsule B, and these capsules were stored at −20°C until dispensed to participants. The identity of capsules was unknown to participants, researchers, and the primary investigator.

Allocation to either one of the capsules was conducted by an independent researcher by using a computer-generated, simple randomization process in a ratio of 1:1. The allocation sequence was concealed from the research dietitian who enrolled and assessed participants in sequentially numbered, sealed, opaque envelopes. After written informed consent was obtained and baseline assessments were completed, the envelope corresponding to each participant study identification number was opened by the research dietitian to reveal the allocation.

Data collection, intervention, and trial management

At the first antenatal visit, patient demographics and details of their medical, family, and obstetric histories were recorded. Weight and height were measured as part of routine practice, and BMI was calculated. Weight was also measured at each subsequent antenatal visit. The research dietitian provided each participant with written information on healthy eating for pregnancy and gestational weight gain, gave a brief explanation of its content by using the food pyramid as a guide, and answered any dietary questions. An information sheet on probiotic-containing supplements and foods on the market was also provided, and participants were asked to avoid such products throughout the remainder of their pregnancies to minimize risk of confounding from the ingestion of other probiotics. A follow-up appointment for 24 wk of gestation was arranged, and the women were instructed to fast from 2200 the previous night for blood taking. The women were telephoned or e-mailed 1 wk before the scheduled appointment as a reminder and to encourage continued participation.

At 24 wk of gestation, fasting glucose was measured, and a serum sample was obtained for later metabolic analyses. Participants were provided with a bottle of their allocated capsules (A or B), with each bottle containing a 2-wk supply (15 capsules). The women were instructed to take 1 capsule/d after a meal of their choice and keep the bottle refrigerated. When possible, a brief follow-up meeting with the research dietitian was arranged for a fortnight, at which time the participant returned the first capsule bottle for a pill count to monitor compliance and was provided with a second bottle to complete the 4-wk intervention period. When such a meeting was not feasible for the patient, 2 bottles of capsules were provided at the 24-wk visit, and subjects were followed up with a phone call or e-mail to encourage compliance.

Participants returned to the antenatal clinic at 28 wk of gestation having completed the capsule intervention period. An additional pill count was performed, and the total number of capsules missed, if any, was recorded. Poor capsule compliance, as assessed by pill counts, was considered to apply when ≥3 capsules were missed during the 4-wk intervention period. Participants arrived in the fasted state, and a 3-h 100-g oral-glucose-tolerance test (OGTT) was performed to test for GDM. A fasting serum sample was collected for later analyses. According to the hospital protocol, a positive diagnosis of GDM was based on ≥2 abnormal values on the OGTT by using Carpenter and Coustan criteria (20). When only one value was raised, women were considered to have impaired glucose tolerance (IGT). A diagnosis of either GDM or IGT resulted in a referral to the diabetes care team for additional follow-up care.

Women that had a negative result on the OGTT continued their antenatal care in routine clinics.

All women received a fetal biometry ultrasonography scan at 34 wk of gestation. This scan included a measurement of the fetal anterior abdominal wall width, which was used as a marker of fetal adiposity (21). At delivery, glucose and serum cord blood samples were collected when possible. Details of the delivery method, gestational age, infant sex, and birth weight, length, and head circumference were recorded. The ponderal index was calculated by using the following formula:

$$\text{Birth weight (kg)} \div \text{length (m}^3\text{)} \qquad (1)$$

Birth-weight centiles were calculated by using the Gestation Network's Bulk Calculator (version 6.6, January 2013; Gestation Network). The trial steering committee met bimonthly, and an independent assessor reviewed the safety of data.

Assessment of dietary and lifestyle habits

At the 24-wk visit, the research dietitian provided and explained a 3-d food diary to each participant. Subjects were instructed to record in detail all food and drink consumed on 3 consecutive days and to include one weekend day within the intervention period. Subjects were also provided with a lifestyle questionnaire that was previously validated for pregnancy and included questions on activity, dietary and smoking habits, alcohol intake, level of education, and an assessment of wellbeing. Women were asked to answer these questions in relation to their lifestyles during pregnancy. Both completed forms were returned either when the second bottle of capsules was collected or at the 28-wk visit. Data from food diaries was entered into the nutritional analysis software (WISP version 3.0; Tinuviel Software) for energy and nutrient intake analysis. Subjects were assessed for underreporting of energy intake by using the Goldberg method with a cutoff of 0.9, which has previously been used to assess underreporting in pregnant populations (22, 23). Underreporters were excluded from additional dietary analyses.

Blood analyses

All maternal fasting glucose, OGTT, and cord glucose samples were analyzed by laboratory staff at the shortest possible interval after sample collection. Additional sera collected at 24 and 28 wk of gestation were centrifuged at 4°C ≤1 h of collection for 5 min at 1409 × *g*. The separated serum was immediately frozen at −20°C, with a subsequent transfer to a −80°C freezer. Serum samples from cord blood were treated similarly although the interval from the time of collection until centrifugation was, in some instances, several hours later, and many samples were hemolyzed on collection. However, only 4 of the hemolyzed cord samples were unsuitable for biochemical analyses. All maternal and remaining cord samples were later analyzed for C-peptide, triglycerides, and total, HDL, and LDL cholesterol. C-reactive protein (CRP) and insulin were also analyzed in maternal samples. Insulin and C-peptide were quantified by using an automated immunoassay (E170; Roche) with typical CVs <5%. Total cholesterol, HDL cholesterol, triglycerides, and CRP were analyzed on a Roche c702 platform (Roche). LDL cholesterol was calculated by using Friedewald's equation (24). The HOMA-IR index was calculated using the following formula (25):

$$\text{Fasting glucose (mmol/L)} \times \text{fasting insulin } (\mu\text{U/mL}) \div 22.5 \quad (2)$$

Outcome measures

The primary outcome was the change in maternal fasting glucose from preintervention to postintervention between probiotic and placebo groups. This outcome was first assessed by using an intention-to-treat analysis, which included all subjects with blood result data and followed by a subsequent analysis that excluded noncompliers and antibiotic users. Secondary outcomes were differences in the incidence of impaired glycemia (GDM or IGT) and neonatal anthropometric measures. Other maternal outcomes assessed included metabolic variables (insulin, HOMA-IR, C-peptide, CRP, and lipids), gestational weight gain, preeclampsia, and delivery complications. Fetal and neonatal outcomes assessed were as follows: cord blood metabolic variables (glucose, C-peptide, and lipids), fetal growth at 34 wk of gestation, 5-min Apgar score, and admission to the NICU.

Sample size

A power analysis indicated that a total sample of 100 subjects (50 subjects/group) was required to detect a 0.4-mmol/L reduction in fasting glucose at the 0.05 level of significance with ≥80% power. Such a reduction in fasting glucose was considered clinically meaningful on the basis of results of the Hyperglycemia and Pregnancy Outcome study, which reported a significantly higher odds of birth weight >90th percentile and raised cord blood C-peptide concentrations when maternal fasting glucose increased by 0.4 mmol/L (9). Researchers aimed to recruit ≥150 participants to account for dropouts, loss to follow-up, poor compliance to capsules, and antibiotic use around the time of the intervention period, which would have negated any potential effects from probiotic capsules.

Statistical analyses

All statistical analyses were performed with IBM SPSS software for Windows (version 20.0; IBM). Variables were graphically assessed for normality by using histograms, and all data were normally distributed. Independent sample *t* and chi-square tests were initially used to analyze differences for baseline characteristics and dietary intakes between intervention groups for continuous and categorical variables, respectively. Because BMI differed significantly between groups, an additional analysis of primary and secondary outcomes for continuous variables was conducted by using the general linear model with BMI as the covariate and intervention group as the fixed factor. For categorical variables, binary logistic regression was used with placebo capsule as the referent group. After the intention-to-treat analysis, secondary analyses were conducted for all outcomes with the removal of noncompliers and antibiotic users. Statistical significance was set at $P < 0.05$ for comparison of baseline demographics, dietary intakes, and mean change in maternal fasting glucose (primary outcome) between groups. To control for multiple comparisons, a Bonferroni correction was applied to the analyses of all other outcomes. Thus, for the 29 nonprimary outcomes reported, significance was set at $P < 0.0017$.

RESULTS

Recruitment of study participants commenced in March 2012 and ended in March 2013, and the final delivery occurred in August 2013. During the study period, 238 women with BMI >30 at their first antenatal visit were screened for eligibility; 175 women gave consent and were randomly assigned to either the control or intervention group. Thirty-five women dropped out or were excluded from the study before commencing the intervention period at 24 wk of gestation because of a change of mind, miscarriage, multiple pregnancy, or fetal anomaly. Only one woman in each group discontinued the capsules, which represented a dropout rate of 1.4% in women who commenced the intervention period. Thus, the intention-to-treat analysis was conducted for 138 women, and the participant flow from the initial screening to final analysis is shown in **Figure 1**. Baseline demographics of each group are displayed in **Table 1**; the only significant characteristic between groups was early pregnancy BMI ($P = 0.007$).

Dietary intakes could not be assessed for 3 women who did not return food diaries (2 women in the probiotic group and 1 woman in the placebo group). In remaining subjects, 39 women (29%) were identified as underreporters of energy intake [14 women in the probiotic group (22.6%); 25 women in the placebo group (34.7%); $P = 0.123$] and were excluded from additional analyses. Mean intakes of energy, macronutrients expressed as a percentage of total energy, and some key pregnancy micronutrients are displayed in **Table 2**. An analysis between intervention and control groups revealed no significant differences for any of the dietary intakes.

Primary outcome

No significant difference was detected in the change in maternal fasting glucose from preintervention to postintervention between probiotic and placebo groups with early pregnancy BMI controlled for (**Table 3**).

Secondary outcomes

There were no differences between groups in incidences of GDM and IGT or neonatal anthropometric measures (birth weight, birth-weight centile, and ponderal index) (**Table 4**). In total, there were 6 cases of GDM (3 cases from each group) and 15 cases of IGT (7 cases in the probiotic group and 8 cases in the placebo group), which gave an overall rate of glucose intolerance of 15.2%.

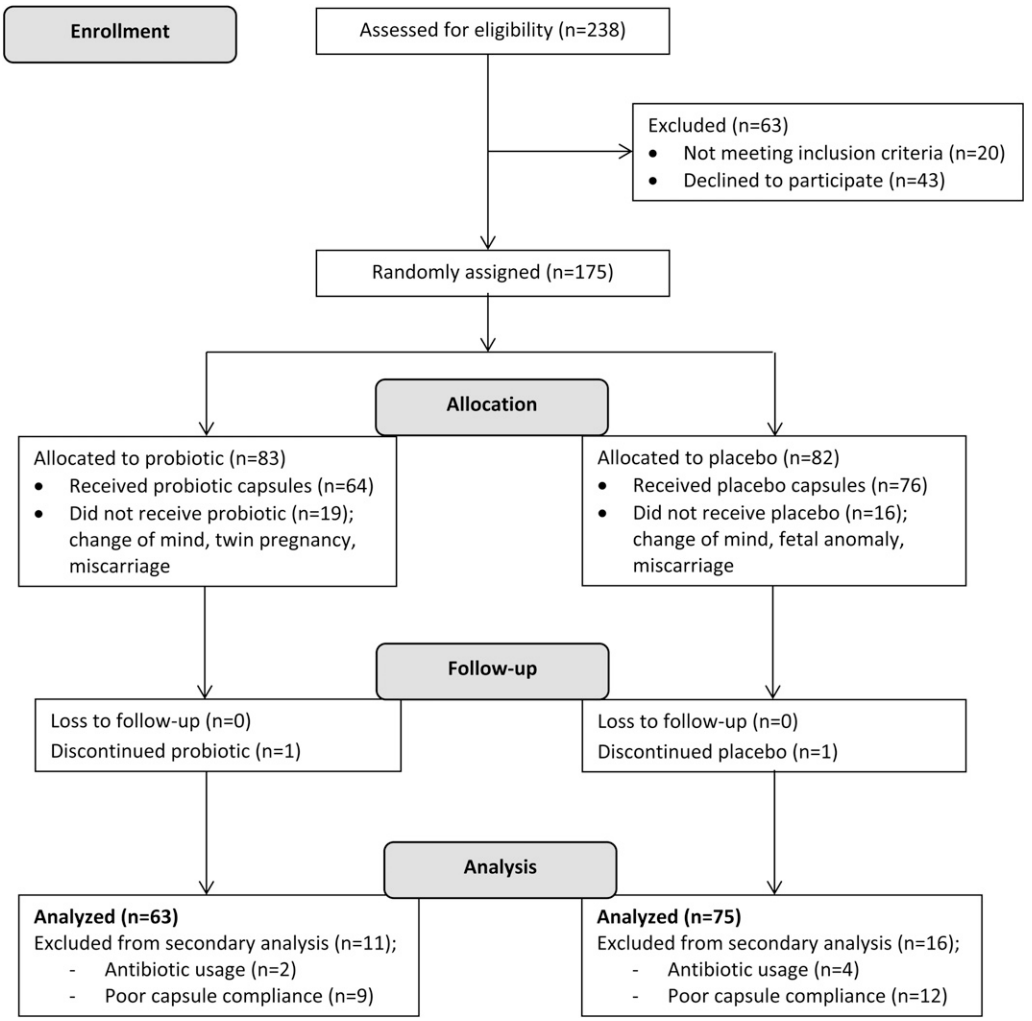

FIGURE 1. Flow diagram of participants

TABLE 1
Baseline demographics by intervention group (n = 138)[1]

	Probiotic group (n = 63)	Placebo group (n = 75)	P
Age (y)	31.4 ± 5.0[2]	31.0 ± 5.2	0.689
Early-pregnancy weight (kg)	89.5 ± 9.1	91.6 ± 10.3	0.221
Height (m)	1.65 ± 0.07	1.64 ± 0.06	0.315
Early-pregnancy BMI (kg/m^2)	32.9 ± 2.4	34.1 ± 2.7	0.007
Gestation at first antenatal visit (wk)	13.8 ± 2.3	13.9 ± 2.4	0.752
Primiparous [n (%)]	31 (49.2)	31 (41.3)	0.354
Irish ethnicity [n (%)]	53 (84.1)	59 (79.7)	0.507
Complete third-level education [n (%)]	25 (43.1)	33 (47.1)	0.648
Current smoker [n (%)]	4 (6.8)	13 (18.3)	0.052

[1] P values were calculated by using an independent-sample t or chi-square test.
[2] Mean ± SD (all such values).

Maternal outcomes

After adjustment for maternal BMI, there were no differences detected between groups in any of the maternal metabolic variables (Table 3) or gestational weight gain, preeclampsia, or delivery outcomes (Table 4). Twelve women in the probiotic group and 14 women in the placebo group had a postpartum hemorrhage (P = 0.929), and there were no preterm deliveries <34 wk of gestation in the study. Three women in the probiotic group and 2 women in the placebo group delivered between 34 and 36 wk of gestation (P = 0.539).

Fetal outcomes

No differences were noted in the third trimester fetal biometry, number of large-for-gestational-age babies (birth weight >90th centile), or admission to the NICU between groups (Table 3). Furthermore, there were no differences between probiotic and placebo groups in gestational age at delivery (mean ± SD: 280.2 ± 10.2 compared with 282.0 ± 10.3 d, respectively; P = 0.477) or Apgar score <7 at 5 min (1.9% compared with 0%, respectively; P = 0.194). The total NICU admission rate was 13% (9 infants from each group), and there were 31 macrosomic infants in total (>4 kg) (15 infants in the probiotic group and 16 infants in the placebo group; P = 0.748). There were no perinatal deaths or congenital malformations in either arm. As concerns cord blood variables, there were no differences between groups in any variable after Bonferroni correction for multiple comparisons (**Table 5**).

Secondary analysis

Six women reported antibiotic usage during the intervention period, and 21 women were identified as poor compliers to the intervention (Figure 1), which left 110 women (52 women in the probiotic group; 58 women in the placebo group) for secondary analyses of all baseline characteristics and primary and secondary outcomes. *See* Supplementary Tables 1–5 under "Supplemental data" in the online issue for results of these analyses which show that no significant differences were detected in any outcome between intervention and control groups.

DISCUSSION

The current study showed no effect of the 4-wk probiotic intervention in obese pregnant women on maternal glycemia by using both an intention-to treat analysis and analysis after exclusion of the confounding variables for noncompliance and antibiotic usage. The intervention also had no effect on neonatal anthropometric measures, other markers of glucose or lipid metabolism, gestational weight gain, and delivery outcomes.

Comparison with existing literature

To our knowledge, there have been only 2 other published trials that have investigated the effects of a probiotic intervention in pregnancy on maternal glycemia and metabolism. First, Laitinen et al (16) compared 3 intervention groups in a Finnish population of

TABLE 2
Nutrient intakes of intervention and control groups in normal reporters of energy intake (n = 95)[1]

Nutrient	Probiotic group (n = 48)	Placebo group (n = 47)	P
Energy (kcal)	1951 ± 401 (1834.1, 2067.0)	1898 ± 304 (1809.0, 1987.7)	0.478
Protein (% of TE)	17.78 ± 3.24 (16.8, 18.7)	18.41 ± 3.49 (17.4, 19.4)	0.365
Carbohydrate (% of TE)	46.51 ± 6.70 (44.6, 48.5)	46.42 ± 5.98 (44.7, 48.2)	0.946
Total fat (% of TE)	36.53 ± 6.75 (34.6, 38.5)	36.30 ± 4.81 (34.9, 37.7)	0.848
SFA (% of TE)	14.87 ± 3.46 (13.86, 15.87)	14.90 ± 3.31 (13.93, 15.97)	0.963
MUFA (% of TE)	13.14 ± 2.95 (12.28, 14.00)	13.12 ± 2.01 (12.52, 13.71)	0.960
PUFA (% of TE)	6.23 ± 1.73 (5.73, 6.73)	6.07 ± 1.95 (5.50, 6.64)	0.673
Calcium (mg)	986.5 ± 343.8 (886.7, 1086.3)	942.9 ± 247.5 (870.2, 1015.5)	0.481
Iron (mg)	11.48 ± 3.54 (10.45, 12.51)	10.82 ± 2.90 (9.97, 11.67)	0.324
Folate (μg)	303.67 ± 108.97 (272.02, 335.31)	277.69 ± 100.58 (248.16, 307.22)	0.231
Vitamin D (μg)	3.13 ± 2.57 (2.38, 3.87)	2.73 ± 2.08 (2.12, 3.35)	0.415
Vitamin B-12 (μg)	4.68 ± 1.94 (4.12, 5.24)	4.61 ± 1.85 (4.07, 5.16)	0.859

[1] All values are means ± SDs; 95% CIs in parentheses. P values were calculated by using an independent-sample t test. TE, total energy.

TABLE 3
Metabolic profile before and after the intervention (n = 138)[1]

	Probiotic group (n = 63)		Placebo group (n = 75)			
	Before	After	Before	After	Coefficient for group (95% CI)	P
Fasting glucose (mmol/L)	4.69 ± 0.43[2]	4.60 ± 0.40	4.76 ± 0.47	4.69 ± 0.46	−0.05 (−0.17, 0.07)	0.391
Insulin (mU/L)	13.85 ± 4.62	15.63 ± 6.35	16.67 ± 7.85	16.88 ± 5.75	2.06 (−0.46, 4.58)	0.108
HOMA-IR	2.94 ± 1.17	3.26 ± 1.58	3.54 ± 1.91	3.53 ± 1.32	0.47 (−0.15, 1.08)	0.135
C-peptide (ng/mL)	2.83 ± 0.89	3.32 ± 1.14	3.03 ± 0.76	3.37 ± 0.76	0.18 (−0.09, 0.45)	0.184
Total cholesterol (mmol/L)	6.25 ± 1.02	6.33 ± 1.12	6.43 ± 1.04	6.60 ± 1.16	−0.06 (−0.28, 0.15)	0.571
HDL cholesterol (mmol/L)	2.04 ± 0.47	1.90 ± 0.47	2.01 ± 0.55	1.87 ± 0.41	0.00 (−0.11, 0.12)	0.967
LDL cholesterol (mmol/L)	3.38 ± 0.91	3.55 ± 0.95	3.54 ± 0.97	3.78 ± 1.16	0.02 (−0.24, 0.20)	0.839
Triglycerides (mmol/L)	1.73 ± 0.56	1.94 ± 0.67	2.00 ± 0.69	2.11 ± 0.59	0.10 (−0.06, 0.25)	0.218
C-reactive protein (mg/L)	7.92 ± 8.38	7.27 ± 6.49	8.42 ± 5.85	7.52 ± 4.75	0.21 (−2.85, 2.43)	0.873

[1] P values reflect differences in the change in each variable between groups from preintervention to postintervention calculated using a general linear model with adjustment for maternal BMI. Significance was set at $P < 0.0017$ after Bonferroni correction for multiple comparisons.
[2] Mean ± SD (all such values).

256 healthy pregnant women (diet/probiotic, diet/placebo, and control/placebo groups). The daily probiotic supplement capsule contained a combination of *Lactobacillus* and *Bifidobacterium*, administered from the first trimester until the postpartum period. The trial showed that the diet/probiotic group had significantly reduced risk of elevated plasma glucose in the third trimester (P = 0.013), a lower incidence of GDM (13% compared with 36% in the diet/placebo group and 34% in the control/placebo group; P = 0.003), reduced insulin resistance, and improved insulin sensitivity. Similar to in our study, the trial reported no differences between groups in the duration of gestation, cesarean delivery, or birth weight.

Meanwhile, Asemi et al (18) used probiotic yogurt that contained a mix of *Lactobacillus* and *Bifidobacterium* as the intervention over a 9-wk period compared with conventional yogurt with no dietary intervention in 70 pregnant women. Similar to in our study, the trial did not report any effect of the probiotic intervention on fasting plasma glucose and lipid concentrations, CRP, or gestational weight gain. However, a smaller increase in serum insulin was noted in the probiotic yogurt compared with control groups. GDM rates, birth weight, and other pregnancy outcomes were not reported.

Differences in findings between our study and that of Laitinen et al (16) might have been attributed to several factors. Most importantly, the current study consisted of an obese cohort, who was already at high risk of poor glycemic control arising from the metabolic milieu associated with obesity. Indeed, baseline concentrations of insulin and HOMA-IR were raised compared with in other studies that measured these variables in nonobese, glucose-tolerant women (26, 27), which suggested a degree of insulin resistance was present before the intervention. In contrast, Laitinen et al (16) enrolled lean, healthy pregnant women with mean (±SD) BMI of 23.6 ± 3.8. Despite this difference, the incidence of GDM in the Finnish trial was surprisingly high at 27.5% (17), which compares to an incidence of 15% in the obese cohort in the current study. These different rates could in part be explained by genetic differences between the 2 population

TABLE 4
Maternal, fetal, and neonatal outcomes associated with a probiotic intervention in obese pregnant women (n = 138)[1]

	Probiotic capsule (n = 62)	Placebo capsule (n = 74)	Effect size (95% CI)	P
Maternal total GWG (kg)	11.1 ± 6.2	9.4 ± 5.6	0.76 (−1.37, 2.89)	0.479
Fetal 34-wk AAW width (mm)	5.3 ± 1.4	5.5 ± 1.4	−0.20 (−0.70, 0.31)	0.447
Fetal 34-wk weight (g)	2537.1 ± 321.0	2542.9 ± 281.6	9.16 (−95.1, 113.4)	0.862
Birth weight (kg)	3.70 ± 0.52	3.68 ± 0.51	0.03 (−0.15, 0.22)	0.723
Birth weight percentile	45.9 ± 27.2	45.0 ± 30.3	0.64 (−9.67, 10.96)	0.902
Ponderal index (kg/m^3)	28.1 ± 3.4	27.6 ± 3.5	0.58 (−0.71, 1.86)	0.375
Meets ACOG exercise guidelines [n (%)]	22 (37.3)	23 (32.9)	0.06 (−0.11, 0.24)	0.474
Excess GWG (>9 kg) [n (%)]	41 (69.5)	35 (50.7)	−0.12 (−0.29, 0.05)	0.157
Diagnosis of IGT/GDM [n (%)]	10 (16.1)	11 (14.9)	0.04 (−0.09, 0.16)	0.561
Preeclampsia [n (%)]	3 (4.8)	2 (2.7)	0.03 (−0.04, 0.10)	0.366
PIH [n (%)]	5 (7.9)	3 (4.0)	0.04 (−0.04, 0.13)	0.289
Cesarean delivery [n (%)]	20 (32.8)	25 (34.7)	−0.03 (−0.20, 0.14)	0.744
Induction of labor [n (%)]	15 (24.2)	22 (30.1)	−0.04 (−0.20, 0.12)	0.628
Admission to NICU [n (%)]	9 (14.8)	9 (12.3)	0.24 (−0.10, 0.15)	0.691
Birth weight >90th percentile [n (%)]	6 (9.8)	7 (9.7)	0.01 (−0.10, 0.12)	0.844

[1] Continuous variables are reported as means ± SDs with the corresponding coefficient for the effect size; P values were calculated by using a general linear model with adjustment for maternal BMI. Categorical variables are reported as n (%) with the corresponding OR for the effect size; P values were calculated by using binary logistic regression with adjustment for maternal BMI. Significance was set at $P < 0.0017$ after Bonferroni correction for multiple comparisons. AAW, anterior abdominal wall; ACOG, American College of Obstetricians and Gynecologists; GDM, gestational diabetes mellitus; GWG, gestational weight gain; IGT, impaired glucose tolerance; NICU, neonatal intensive care unit; PIH, pregnancy-induced hypertension.

TABLE 5
Cord blood metabolic profile before and after the intervention (n = 114)[1]

	Probiotic capsule (n = 53)	Placebo capsule (n = 61)	Coefficient for group (95% CI)	P
Glucose (mmol/L)	4.43 ± 1.16[2]	4.04 ± 0.94	0.46 (0.05, 0.87)	0.027
C-peptide (ng/mL)	1.42 ± 0.72	1.52 ± 0.68	−0.15 (−0.41, 0.12)	0.273
Total cholesterol (mmol/L)	1.75 ± 0.55	1.67 ± 0.40	0.10 (−0.08, 0.29)	0.283
HDL cholesterol (mmol/L)	0.72 ± 0.39	0.64 ± 0.23	0.09 (−0.03, 0.21)	0.133
LDL cholesterol (mmol/L)	0.79 ± 0.26	0.78 ± 0.24	0.01 (−0.08, 0.11)	0.787
Triglycerides (mmol/L)	0.50 ± 0.25	0.54 ± 0.31	−0.04 (−0.15, 0.08)	0.522

[1] P values were calculated by using a general linear model with adjustment for maternal BMI. Significance was set at $P < 0.0017$ after Bonferroni correction for multiple comparisons.
[2] Mean ± SD (all such values).

groups as well as the different diagnostic criteria used. Furthermore, the Finnish trial used a different probiotic strain, and it is possible that all probiotic strains do not induce the same influence on glycemic control in pregnant women. The Finnish trial also administered the probiotic/placebo over a longer period. A short intervention period of 4 wk was used in the current study because previous experiments with the same probiotic showed its presence in the human gut after 3 wk (19). However, a longer administration period may have been required for any probiotic effect to be exerted in this specific cohort of obese pregnant women.

The combination of intensive dietary counseling with a probiotic or placebo intervention in the trial by Laitinen et al (16) may also have influenced results because several outcomes were not significantly different between diet/probiotic and diet/placebo groups (16). A recent trial of a healthy, low–glycemic index diet in pregnancy from our own center was shown to reduce gestational weight gain and glucose intolerance (28). In the current trial, we wish to assess the impact of the probiotic only on maternal glycemia and, therefore, did not add a dietary intervention, which could have confounded the results. Minimal dietary advice on healthy eating for pregnancy was provided to all women on recruitment only, and food-diary data reported no differences in energy or nutrient intakes between probiotic and placebo groups.

Neither of the previous 2 trials reported the collection of cord samples for analysis, and to our knowledge, the current study is the first to investigate the effect of probiotics in pregnancy on cord glucose and lipid concentrations. Although no beneficial effects of the probiotic consumption on cord or maternal metabolic variables was observed, the absence of any negative metabolic effects or adverse pregnancy and neonatal outcomes supported the safe use of probiotics in pregnancy similar to in other recent studies (17, 29, 30).

Clinical implications, strengths and limitations

Although the current trial yielded several negative findings, it was, to our knowledge, the first published study to investigate probiotics in obese pregnancy and, therefore, is a valuable addition to the literature on this topic. A recent systematic review of studies that reported on effects of probiotics in pregnancy on maternal metabolic outcomes highlighted the need for studies involving obese women because of their elevated risk of adverse pregnancy outcomes (14). The current study has made the first step in filling this knowledge gap; however, the length of the intervention may have been too short to exert a significant effect, and longer interventions should be considered in the design of future trials in obese pregnancy. The recently published protocol of a new randomized controlled trial of probiotics in pregnancy (SPRING) describes a 24-wk probiotic intervention period in overweight and obese pregnant women with the incidence of GDM as the primary outcome (31). After the negative findings in the current study, results from the SPRING trial will be eagerly awaited to see if the positive effects reported by Luoto et al (17) and Laitinen et al (16) may be reproduced in a new population of high-risk pregnant women.

Other important strengths of this study were that it was sufficiently powered, adequately blinded, and had a low dropout rate once commencement of capsules had begun. The intervention was noninvasive, short in duration, yielded no adverse effects on participants or their babies, and good compliance and acceptability in this pregnant population has been demonstrated (32). The inclusion of a dietary analysis during the intervention period also enabled the assessment of any potential dietary confounders. Furthermore, the wide scope of data collection from gestational weight gain to fetal growth and cord blood in each participant allowed numerous outcomes to be examined.

The study had one particular limitation worthy of consideration. Fecal samples were not collected from participants, and thus, it could not be proven that the probiotic bacteria became established in the gut of the intervention group or if there were any changes in the gut flora after the intervention. Therefore, capsule compliance could only be ascertained by self-reported measures. However, previous human trials that used the same *Lactobacillus* UCC118 strain have shown the presence and transit of this probiotic through the human gut (19, 33). Another potential limitation of the study design was that the random assignment of women occurred several weeks before they commenced the capsule intervention, and consequently, the dropout rate during this interval appeared high (35 of 175 women; 20%), despite a low dropout rate after capsule commencement (2 of 140 women; 1.4%).

In conclusion, a probiotic-capsule intervention did not reduce maternal fasting glucose or other metabolic variables in obese pregnant women. Beneficial effects of probiotics previously detected in healthy pregnant women may not be translatable to a more-metabolically unstable, high-risk pregnant population.

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