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ORIGINAL CONTRIBUTION

Effect of probiotic yoghurt on plasma glucose in overweight and obese pregnant women: a randomized controlled clinical trial

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Abstract

Introduction There is only some evidence about effectiveness of probiotics for preventing gestational hyperglycaemia. This trial examined the effects of probiotic yoghurts containing *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 on maternal plasma glucose (primary outcome) and on some maternal and infant complications (secondary outcomes) in overweight and obese women with no diabetes in pregnancy.

Methods Using stratified block randomization, women with pre- or early-pregnancy BMI ≥ 25 and fasting plasma glucose < 92 mg/dl at 22 weeks of gestation were assigned into probiotic or conventional yoghurt group, consuming 100 g/day from 24 weeks of gestation until delivery. The women and their infants were followed up until 1 month after birth.

Results In each group, one out of 65 women had intra-uterine foetal death and were not analysed for other outcomes. The mean BMI was 29.2 (SD 3.3) in probiotic and 30.3 (SD 4.1) in conventional yoghurt group. Four weeks after initiation of the treatment, plasma glucose levels were significantly lower in the probiotic than in conventional yoghurt group at fasting (mean difference adjusted for the BMI category) and baseline FPG (− 4.0 mg/dl; 95% confidence interval − 6.9, − 1.1) and 2-h OGTT (− 13.9; − 22.8, − 5.0). At the 1-h OGTT, however, the difference was not statistically significant (− 9.8; − 20.6, 0.9). Further, there was a significantly lower infant bilirubin level in the probiotic group on days 3–5 after birth (− 2.2 mg/dl; − 3.3, − 1.2). There were not statistically significant differences between the groups regarding the risk of gestational diabetes (6 vs 11; odds ratio 0.5; 0.2, 1.5), preterm delivery (3 vs 8; 0.3; 0.1, 1.2), and other maternal and infant outcomes.

Conclusion The probiotics supplementation has some beneficial effects on glucose metabolism of overweight and obese pregnant women. Nevertheless, further studies are required to judge the clinical significance of such effects.

Keywords Probiotic · Yoghurt · Blood glucose · Hyperbilirubinemia · Overweight · Obese · Pregnancy

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Introduction

Hyperglycaemia, including diabetes in pregnancy and gestational diabetes mellitus (GDM), is one of the most common metabolic complications of pregnancy. About 21 million women every year (17% of women with live births) in the world experience some form of hyperglycaemia in pregnancy, majority (85%) of which are due to GDM [1]. In Iran, the reported crude prevalence for the hyperglycaemia is 19% and its age-adjusted prevalence is 17% [1]. Rate of GDM is more than twice as frequent among overweight and obese women as normal weight women [2]. Thus, the rate of GDM is on the rise due to the growing burden of maternal obesity [3]. In Iran, prevalence of overweight and obesity is very high among adult women; 34% and 29%, respectively, among women ≥ 20 years based on a study in 2013 [4].

GDM increases the risk of some adverse events in women such as preeclampsia, poly-hydramnios, and emergency caesarean section; along with macrosomia, preterm birth, shoulder dystocia, hypoglycaemia, hyperbilirubinemia, and respiratory distress syndrome in their infants. It is also associated with some delayed morbidities such as diabetes type 2 and cardiovascular diseases [5].

Adopting recommended lifestyle changes is usually difficult and less amenable for pregnant women, especially for those who are overweight or obese due to poor motivation and self-efficacy [6]. Therefore, research on new therapies for glucose control can have significant benefits for the future of hyperglycaemia management and may complement the current diet, exercise, and pharmacological therapies [7].

There is emerging evidence regarding the use of probiotics for preventing GDM especially among high-risk women [8]. Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [9]. They potentially represent a novel mechanism for promoting metabolic health during pregnancy [10, 11]. Probiotics may effectively decrease the adverse metabolic effects related to pathogenic microbial communities, through positive alteration of gut microbiota [11–13].

Changes in the gut microbiota composition have been reported in pregnancy, especially at the third trimester, toward reduction in bacterial richness, which is associated with inflammation and energy loss [14]. The reduced numbers of bifidobacterium and bacteroides are pronounced in overweight and obese women [15].

Use of probiotics containing lactic acid bacteria of the Lactobacillus and Bifidobacterium genera is safe and well tolerated during pregnancy, especially after the organogenesis period [16].

To the best of our knowledge, there are only a small number of randomized controlled trials (RCT) published so far which have directly investigated the glycaemic effects of probiotics, either among healthy [17–20] or obese pregnant women [21]. The results from these trials are inconclusive and further studies have been recommended [18, 22]. A previous randomized trial conducted in Tabriz-Iran indicated that probiotic yoghurt containing Lactobacillus acidophilus La5 and Bifidobacterium lactis Bb12 significantly reduces the mean fasting blood sugar of type 2 diabetic patients [23]. Therefore, this trial aimed to examine the effects of probiotic yoghurt containing Lactobacillus acidophilus La5 and Bifidobacterium lactis Bb12 (consumed daily from 24 weeks of gestation until delivery) on maternal plasma glucose four weeks after initiation of the treatment (primary outcome) and on some maternal and infant complications (secondary outcomes) in overweight and obese women who had normal baseline fasting plasma glucose (FPG).

Methods

Study design

This study was a superiority randomized controlled clinical trial with two-parallel arms in which the participants, data collectors and data analyst were blinded to the intervention type received by each group.

Eligibility criteria

This study was conducted on pregnant women with a pre- or early-pregnancy body mass index (BMI) ≥ 25 aged 18 years or older who had FPG < 92 mg/dl and gestational age of 20–22 weeks at the recruitment time. The exclusion criteria were multiple pregnancy; prior history of GDM; taking any medication likely to influence the metabolism of glucose such as metformin, corticosteroids or immunosuppressant; medical conditions associated with altered glucose metabolism such as Cushing's syndrome and hepatic cirrhosis; regular consumption of probiotics for any reason; smoking; regular use of alcohol or illegal drug; any antibiotic intake during the current pregnancy; illiteracy or low literacy; and established major foetal anomaly.

Study setting

The participants were recruited from five public health centres located in the northwest of Tabriz, Iran and administered by one centre. The principal investigator (PI, the first author) worked at the administration centre as a supervisor of the maternal services provided at the five centres. Therefore, the health centres' staff had high cooperation with her and she could fairly easily identify

and gain access to eligible women. She also followed the participants regularly for the weekly treatment re-supplies and follow-up assessments.

Recruitment of participants

Participant recruitment was started after the approval of Ethics Committee of Tabriz University of Medical Sciences (Code: TBZMED.REC.1394.1208, Date: 2016-03-07), and the trial registration in Iranian registry clinical trial (IRCT201604013706N31).

Potentially eligible women were initially identified using the women's pregnant record books which were available at every health centre and contained brief information of all pregnant women, including age, last menstrual period, and pre- or early-pregnancy BMI calculated as weight in kilograms divided by height in meters squared. Because weight gain during the first trimester of gestation is low (0.5–2.0 kg) [24], according to the national guidelines, when there is no reliable woman weight and height measured at one year before pregnancy, those measured within the first trimester by the health care providers are used to calculate the BMI.

The identified women were called at their 17–19 weeks of gestation. After explaining the study objectives and methods, those who were willing to participate in the study were asked to attend the administration centre when they were at their 20–22 weeks of gestation. In the centre, the PI explained the study in more detail to every woman, assessed eligibility criteria thoroughly using a checklist, and asked the eligible women to sign the informed consent from.

Then, the women completed the baseline questionnaire. They were also referred to a special laboratory for FPG test, following eight hours of fasting. Women who had FPG of less than 92 mg/dl were recruited into the trial.

Random allocation

Allocation sequence was generated by a computerized random number generator. Randomization was stratified by the BMI category (BMI = 25.0–29.9; BMI ≥ 30 kg/m^2) and restricted to randomly varying blocks of four and six. Allocation concealment was maintained using consecutively numbered opaque sealed envelopes containing a sheet with the yoghurt code on it. The sequence generation and the envelop preparation were performed by a person not involved in the participant recruitment, data collection and analysis procedures. The envelopes were opened just before the random allocation (after assessment of all eligibility criteria and collection of baseline data), after writing participant's name on them.

Interventions

The participants were asked to refrain from taking any kinds of probiotic supplements or any nutrients, including yoghurts, enhanced by the addition of extra probiotics, for 2 weeks (22–24 weeks of gestation, run-in period). They received either probiotic or conventional yoghurt packs, 100 g/day from 24 weeks of gestation until delivery. They were also asked not to consume any other probiotic supplements during the study period.

The yoghurts for both groups were produced and identically packaged, in packs of 100 g for daily use, by Pegah Dairy Factory in Tabriz, as ordered by the research team. They were plain yoghurts with no other ingredients such as fruits; had identical appearance, pH of 4.3–4.5, 50 kcal energy, 5.5% carbohydrate, 3.6% protein, and 1.5% fat. Both yoghurts contained Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus in the dosage of 10^7 CFU/g (colony forming units per gram) used for biotransformation of milk (as starter). The yoghurt for the intervention group additionally contained 5×10^8 CFU/g Lactobacillus acidophilus La5 and Bifidobacterium lactis Bb12. Rationale for using the identical starters for the both groups was to enable assessing effect of only Lactobacillus acidophilus La5 and Bifidobacterium lactis Bb12 as probiotic microorganisms. The packages could be identified by the codes which were unknown for both the participants and the investigators.

To verify that every gram of probiotic yoghurt has in average 5×10^8 CFU Lactobacillus acidophilus La5 and 5×10^8 CFU Bifidobacterium lactis Bb12, the microorganisms count was done in the Pegah Factory's laboratory on the 1st and 7th days of the probiotic yoghurt production on the samples kept at the factory, which were about 10^9 and 10^8 CFU/g of each microorganism, respectively. This counting was replicated three times during the study period and the results were very similar at all the three times.

The yoghurts were re-supplied on a weekly basis. The PI received the supplies weekly from the factory and distributed them to the participants on the same day that they had been produced. The participants attended the administration centre to receive the yoghurts. They were asked to store the yoghurts in refrigerator and consume them daily by themselves, starting from the same day they received. In case of not attending on time to receive the yoghurts, the participants were called and encouraged to attend. In rare cases where a participant was not able to attend the centre, the PI delivered the yoghurts to them at their home. A daily diary was used for recording the yoghurt consumption which was delivered to the investigator at the weekly visit.

All women received routine prenatal care from her own provider, as well as routine visits from a nutritionist. In the public health centres of the country, routine prenatal care

consists of eight visits; starting from 6 to 10 weeks of gestation, followed by visits at 16–20, 24–30, 31–34, 37–38 weeks and every week till delivery. Dietary counselling by the nutritionist was done according to the current national nutrition guidelines for overweight and obese pregnant women.

Outcomes

The primary outcomes were FPG, as well as 1- and 2-h plasma glucose post 75-g oral glucose tolerance test (OGTT) measured at 28 weeks of gestation, a 4-week after initiation of the treatment. The reason for choosing the 4-week intervention period for assessing the primary outcomes was that according to WHO, "the usual window for diagnosing GDM is between 24 and 28 weeks of gestation [25]". Therefore, we did the baseline assessment at 22^0–22^6 weeks of gestation to exclude pre-existing diabetes in pregnancy and assessed post-intervention plasma glucose at 28^0–28^6 weeks (after a 2-week run-in period with participants refraining from taking any kind of probiotics, and a 4-week treatment) to diagnose GDM. Such a 4-week intervention period has also been considered in some previous studies [7, 21, 26, 27] assessing the effect of probiotics on plasma glucose levels.

All participants were asked to complete a 3-day food diary (two working days and 1 day at the weekend) at 28 weeks of gestation, in the week prior to the post-intervention assessment of plasma glucose levels. The dietary intake was quantified by Nutritionist 4 software (First Databank, Inc., Hearst Corporation) as well as a database including the tables of contents and nutritional value information inserted on Iranian food products.

Maternal secondary outcomes included GDM, weight gain over pregnancy, preeclampsia, preterm delivery, delivery mode, and satisfaction with the yoghurts.

The International Association of Diabetes and Pregnancy Study Groups (IADPSG) definition was used to identify GDM. Based on the definition, GDM is diagnosed if FPG ≥ 92 mg/dl, 1-h post 75-g plasma glucose ≥ 180 mg/dl or 2-h plasma glucose ≥ 153 mg/dl [28].

Preterm delivery, preeclampsia, and any side effects during intervention including any infections requiring antibiotics were assessed by a checklist during the weekly attendances to receive the yoghurts. For preeclampsia diagnosis, blood pressure was measured by the providers at each routine prenatal visit, using ALPK2 manometer. In case of systolic blood pressure ≥ 140 mmHg and /or diastolic ≥ 90 mmHg on two occasions, at least 4 h apart, urine protein was measured to diagnose preeclampsia. In case of 0.3 g or more of protein in a 24-h urine collection, preeclampsia was confirmed and the patient was referred to receive necessary treatment.

Infant secondary outcomes were total serum bilirubin (TSB) measured on days 3–5 after birth on heel capillary blood; anthropometric index including weight (assessed by Seca scale with 0.1 kg accuracy), length and head circumference measured by the PI on day 10 ± 1, macrosomia (birth weight > 4000 g), large for gestational age (birth weight greater than the 90th percentile for gestational age); and occurrence of neonatal jaundice, treatments used for the jaundice, and neonatal death within 30 days after birth assessed by interviewing women at all of the three postpartum time-point assessments and also checking hospital records, if needed.

All of the laboratory measurements (plasma glucose, neonatal TSB, and if needed, urine protein) were done at one laboratory. For 4 neonates hospitalized at NICU during 3–5 days after birth, results of TSB were extracted from their hospital records. Enzymatic method was used to measure the plasma glucose and spectrophotometry was employed to measure the TSB. Westgard rules and external quality control were used regularly at the laboratory to control the test quality. In addition, we checked the reliability of plasma glucose test in the laboratory by sending two concurrent blood samples from 10 women to the laboratory and to the referral laboratory in the province. The test reliability was 0.87 and intraclass correlation coefficient was 0.84 (95% CI 0.36–0.96).

Sample size

The sample size was calculated based on the results of the study by Laitinen et al [19], considering a mean FPG of 4.60 mmol and SD 0.46 in the placebo group. To detect 20% change in glucose levels with probiotic supplementation, power of 0.8 and two-sided significance level of 0.05, the estimated sample size was 60 per group. Allowing for 8% attrition in the number of participants, 65 women were recruited for each group.

Statistical analysis

The data were analysed using SPSS version 21. The normality of all quantitative data by the groups was confirmed using one-sample Kolmogorov–Smirnov test. The general linear model (ANCOVA) was used to compare the groups in terms of the primary outcomes (plasma glucose levels) adjusted for stratification factor (the BMI category) and baseline FPG. Binary logistic regression and ANCOVA were used to identify any differences between the two groups regarding the secondary outcomes adjusted for the BMI category. The confidence interval of the main effects was adjusted using Sidak.

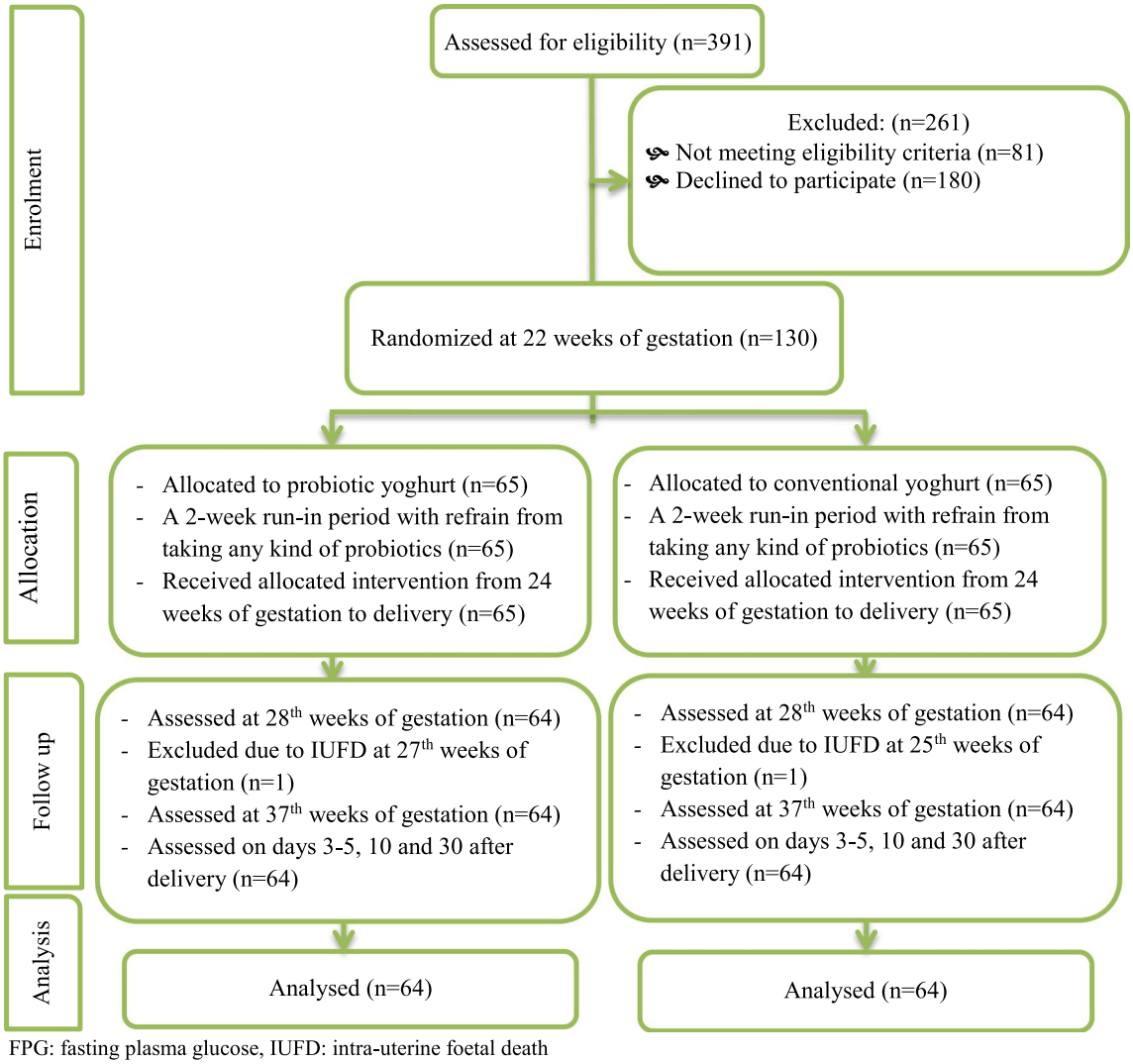
Results

Between Apr 2016 and Sep 2017, 391 women were assessed for eligibility at the health centres, of them 261 were excluded; 81 (21%) due to ineligibility and 180 (46%) due to refusing to participate in the trial, mostly due to inability to regular attendance to receive the weekly supply. No one was excluded during the 2-week run-in period. From the 130 women randomized, 128 completed the trial. Two (one from each group) were excluded after randomization due to intra-uterine foetal death at 25–27 weeks of gestation (Fig. 1).

Most (69%) of participants in both groups were satisfied with consumption of the yoghurts. The mean days of yoghurt consumption prior the primary outcome (plasma glucose levels) assessment was 27.8 (SD 0.8) in the probiotic and 27.6 (SD 1.1) in the conventional yoghurt group. The mean consumption period until delivery was 99.1 (SD 17.4) and 99.7 (SD 16.6), respectively.

The groups were comparable in terms of the baseline socio-demographic and reproductive characteristics. The mean age of the women was 29.5 vs 29.4 years, and their BMI was 29.2 vs 30.3 kg/m^2. About one-third (38% vs 35%) were obese, one-fourth (25% vs 22%) were primigravida and one-fifth (18% in the both groups) reported history of diabetes in a first-degree relative (Table 1).

Among those who completed 3-day food diary (47 in the probiotic and 41 in the conventional yoghurt group), there were no significant differences between the groups in dietary energy and macro-nutrient intake, assessed at the 28–33 weeks of gestation. The mean daily energy intake was 2256 (SD 300) kcal in the probiotic and 2299 (SD 335) in the conventional yoghurt group ($p = 0.524$) (Table 2).



FPG: fasting plasma glucose, IUFD: intra-uterine foetal death

Fig. 1 Flow diagram of the trial

Table 1 Baseline characteristics of the women by study groups

Characteristics	Probiotic yoghurt ($n=65$)	Conventional yoghurt ($n=65$)	P
Age (years)	29.5 (6.2)	29.4 (5.5)	0.940[b]
Primigravida	16 (25%)	14 (22%)	0.677[c]
Education ≥ 12 years	17 (26%)	21 (32%)	0.441[c]
Housewife	63 (97%)	64 (98%)	1.000[d]
Diabetes in first-degree relatives	12 (18%)	12 (18%)	1.000[c]
Pre- or early-pregnancy BMI (kg/m^2)	29.2 (3.3)	30.3 (4.1)	0.090[b]
Overweight (BMI 25-29.9)	40 (62%)	42 (65%)	0.716[c]
Obese (BMI ≥ 30)	25 (38%)	23 (35%)	
Birth weight of previous child (g)[a]	3040 (576)	3160 (346)	0.235[b]
History of caesarean section	26 (40%)	22 (34%)	0.688[c]
History of abortion	21 (32%)	22 (34%)	0.774[c]
History of preterm delivery	2 (3%)	2 (3%)	1.000[d]
History of preeclampsia	0	1 (2%)	1.000[d]
History of Still-birth	2 (3%)	2 (3%)	1.000[d]

Values are number (%) or mean (SD)

[a]Among 45 women in probiotic and 44 women in the conventional yoghurt group who had previous birth

[b]Independent *t* test

cChi square

[d]Fisher exact test

Table 2 The comparison of dietary intake by study groups

Variables	Probiotic yoghurt		Conventional yoghurt		p[a]
	n[b]	Mean (SD)	n[b]	Mean (SD)	
Daily energy consumption (kcal)	47	2256 (300)	41	2299 (335)	0.524
Carbohydrate (% of TE)	47	60.5 (5.9)	41	57.6 (8.2)	0.061
Fat (% of TE)	47	24.8 (5.9)	41	27.5 (8.1)	0.079
Protein (% of TE)	47	15.0 (1.7)	41	14.8 (2.1)	0.495

Calculated using a 3-day food diary (two working days and one day at the weekend), completed at 28–33 weeks of gestation

TE total energy

[a]Independent *t* test

[b]17 women in the probiotic and 23 in the conventional yoghurt group did not completed the 3-day food diary

Primary outcomes

The mean of baseline FPG was 75.5 mg/dl (SD 7.2) in the probiotic and 74.1 (SD 7.0) in the conventional yoghurt group. After the 4-week treatment, mean change in the FPG was −0.5 (SD 8.1) in the probiotic and 3.9 (SD 8.9) in the conventional yoghurt group and between groups difference of the mean change was statistically significant (−4.4, 95% CI −7.4 to −1.4). In addition, at the 28 weeks of gestation, the plasma glucose levels were significantly lower in the probiotic than in the conventional yoghurt group at the 2-h OGTT (−13.9, −22.8 to −5.0). At the 1-h OGTT, although it was lower in the probiotic than in the conventional yoghurt group but the difference was not statistically significant (−9.8, −20.6 to 0.9). There were no significant interaction effects of BMI category × intervention group on the plasma glucose levels (Table 3, supplement).

Maternal secondary outcomes

There were no statistically significant differences between the groups in frequency of GDM (6 vs 11; OR 0.5, 95% CI 0.2–1.5), and preterm delivery (3 vs 8; 0.3, 95% CI 0.1–1.2). In addition, other maternal outcomes pertaining to gestational weight gain ($p=0.976$), preeclampsia ($p=0.997$), and delivery type ($p=0.695$) did not differ between the groups (Table 4).

Table 3 Comparison of the groups on plasma glucose levels

Primary outcomes	Probiotic yoghurt			Conventional yoghurt			Adjusted difference* (95% CI)	*p**
	Baseline	*n*	Follow-up	Baseline	*n*	Follow-up		
FPG (mg/dl)	75.5 (7.2)	64[a]	74.8 (7.4)	74.1 (7.0)	64[a]	77.9 (11.2)	−4.0 (−6.9 to −1.1)	**0.008**
1-h OGTT (mg/dl)	–	62	128.0 (28.4)	–	63	136.0 (31.7)	−9.8 (−20.6 to 0.9)	0.071
2-h OGTT (mg/dl)	–	59	103.9 (21.0)	–	59	115.5 (26.3)	−13.9 (−22.8 to −5.0)	**0.002**

Bold values are indicating $p < 0.05$ was considered as statistically significant

FPG fasting plasma glucose, *OGTT* Oral Glucose Tolerance Test was done with 75-g oral glucose

Values are mean (SD) unless otherwise stated

The baseline assessment was done at 22 weeks and follow-up assessment at 28 weeks of gestation (after a 2-week run-in period with refrain from taking any kind of probiotics and a 4-week treatment)

Interaction effect of intervention group × BMI category was not statistically significant ($p = 0.64$–0.74)

*Using ANCOVA adjusted for baseline FPG and BMI category (overweight/obese)

[a]In one obese women from each group, the test was not done due to intra-uterine foetal death, missing values at the other time-points were due to lack of tolerance of OGTT

Table 4 The comparison of maternal outcomes between two groups

Outcomes	Probiotic yoghurt ($n = 64$)	Conventional yoghurt ($n = 64$)	OR (95% CI)	*p**
GDM	6 (9%)	11 (17%)	0.5 (0.2–1.5)	0.184
Preeclampsia	1 (2%)	0	–	0.997
Preterm birth	3 (5%)	8 (13%)	0.3 (0.1–1.2)	0.077
Caesarean delivery	33 (52%)	35 (55%)	0.9 (0.4–1.7)	0.695
Weight gain over pregnancy (kg)	9.37 (2.8)	9.34 (3.4)	−0.1 (−1.1 to 1.1)	0.976[a]

Values are number (%) or mean (SD) unless otherwise stated

GDM Gestational Diabetes Mellitus, identified based on 75-g oral glucose tolerance teat results done at the 28 weeks of gestation (4 weeks after treatment) as defined by IADPSG definition

*Logistic Regression

[a]Univariate general linear model (ANCOVA), all analyses were adjusted for BMI category (overweight/obese)

Infant secondary outcomes

The mean neonatal TSB on days 3–5 after birth was significantly lower in the probiotic than the conventional yoghurt group (adjusted difference −2.2 mg/dl, −3.3 to −1.2; 4 out of 64 in the probiotic and 6 out of 64 in the conventional yoghurt group who had no jaundice had not been tested for the neonatal TSB). In addition, the rates of use of all types of treatment as well as treatment with phototherapy (alone or with other treatments) were significantly lower in the probiotic than in the conventional yoghurt group. There were no significant differences between the groups on neonatal anthropometrics (height, weight, head circumference) (Table 5). No neonatal death occurred in the groups, but there was one case of congenital malformation (joint contraction, cleft lip and palate, and hypotonia) resulting in the infant death on day 48 in the conventional yoghurt group. There were four cases of admission to neonatal intensive care unit (two from each group, one with diagnosis of hydronephrosis in the conventional yoghurt group).

Side effects

There were two cases of stomach ache requiring medical treatment (one from each group), one case of urinary tract infection in the conventional yougurt group requiring treatment with antibiotic (500 mg cephalexin capsules for 2 weeks), one case of dystocia resulting in neonatal asphyxia in the probiotic group, two cases (one from each group) of maternal admission at hospital due to amniotic fluid reduction which resulted in premature caesarean section in the case of probiotic group, one neonatal surgery with diagnosis of Crohn's disease in the probiotic group, and one case of maternal admission to intensive care unit just after delivery for 3 days with diagnosis of probable pulmonary embolism in the conventional yoghurt group. Only one person received antibiotic during pregnancy.

Table 5 The comparison of infant outcomes between the study groups

Outcomes	Probiotic yoghurt ($n=64$)	Conventional yoghurt ($n=64$)	Mean difference (95% CI)	p*
Birth weight (g)	3270 (495)	3260 (435)	14 (−149 to 177)	0.866
Birth weight > 4000 g	3 (5%)	3 (5%)	0.9 (0.2–51.8)[a]	0.999[b]
Large for gestational age	13 (20%)	13 (20%)	1.0 (0.4–2.4)[a]	0.956[b]
Height at birth (cm)	50.3 (2.5)	50.2 (2.2)	0.1 (−1.0 to 0.7)	0.763
Head circumference at birth (cm)	34.8 (1.4)	34.9 (1.9)	−0.1 (−0.7 to 0.5)	0.685
Total bilirubin on days 3–5 after birth[c] (mg/dl)	9.1 (3.0)	11.3 (2.7)	−2.2 (−3.3 to −1.2)	**< 0.001**
Treatment for hyperbilirubinemia	22 (36%)	41 (59%)	0.33 (0.16–0.7)[a]	**0.001**[b]
Phototherapy for hyperbilirubinemia[d]	10 (16%)	27 (42%)	0.25 (0.11–0.58)[a]	**0.001**[b]
Intrauterine foetal death[e]	1 (2%)	1 (2%)	1.0 (0.1–17.9)[a]	0.974[b]
Admission to NICU	2 (3%)	2 (3%)	0.9 (0.1–7.2)[a]	0.980[b]

Bold values are indicating $p < 0.05$ was considered as statistically significant

Data present n (%) or mean (SD) unless otherwise mentioned

NICU Neonatal Intensive Care Unit

*ANCOVA

[a]OR (95% CI)

[b]Logistic Regression (all analysis were adjusted for BMI category)

[c]60 in probiotic and 58 in conventional yoghurt group for bilirubin level

[d]Phototherapy in home and/or hospital

[e]$n=65$ in each group

Discussion

In this study, the participants were women with pre- or early-pregnancy BMI ≥ 25 who were normoglycemic at the baseline assessment on week 22^0–22^6 of gestation. At 28 weeks of gestation, after a 4-week treatment, the FPG and the 2-h OGTT were significantly lower in the probiotic group than in the conventional yoghurt group. Regarding the 1-h OGTT, it was lower in the probiotic group but the difference was not statistically significant. The mean neonatal TSB on days 3–5 after birth and frequency of treatment due to hyperbilirubinemia were significantly lower in the probiotic than in conventional yoghurt group. There were no statistically significant differences between the groups in risk of GDM, preterm delivery and the other maternal and infant outcomes.

This study results pertaining to reducing effects of the probiotics on the plasma glucose levels are consistent with results of some other trials including a trial conducted in Finland which showed significantly lower plasma glucose levels during pregnancy in the diet and probiotics (Lactobacillus rhamnosus GG and Bifidobacterium lactis Bb12) group than in the diet and placebo group [19]. Another study in New Zealand indicated significantly lower FPG, but no significant difference on 1-h and 2-h post 75-g plasma glucose levels, at 24–30 week of gestation in pregnant women taking capsules containing Lactobacillus rhamnosus HN001 daily (6×10^9 CFU) from 14 to 16 weeks of gestation compared to the placebo [18]. However, our study results are inconsistent with those of some other trials on normal weight [17, 20] and obese [21] pregnant women which revealed no significant effect brought by probiotics on FPG.

As suggested in the literature [29], these discrepancies in the effects may be related to strain-specificity of probiotic effects, as well as the viable counts of probiotic cells in the yoghurt at time of consumption. In the Irish study [21] which found no effect of probiotics on FPG, the strain used (Lactobacillus salivarius UCC118) might have not been optimal for use in pregnant obese women. In addition, insufficient power of the trial (only 15 cases of impaired glucose tolerance) may have contributed to the insignificant results. The significantly lower plasma glucose levels in the probiotic group observed in our study may be related to species of the probiotic bacteria used (i.e. Lactobacillus acidophilus La5 and Bifidobacterium lactis Bb12).

The mean TSB on days 3–5 after birth and frequency of neonatal jaundice treatment were significantly lower in the probiotics group than in the conventional yoghurt group. We found no similar study assessing the effect of probiotic consumption during pregnancy on neonatal bilirubin. However, a recent systematic review analysing six prophylactic ($n=1761$) and three therapeutic ($n=279$) trials with low to high risk of bias indicated that TSB was significantly reduced after probiotic treatment of neonates [30]. In addition, another systematic review on 13 RCTs involving 1067 neonate with jaundice revealed positive effect of probiotics on reduction of TSB level [31]. The protective effect against

hyperbilirubinemia has been attributed to the effect of probiotics on intestinal motility and intestinal microbiota [31]. The reduced TSB in our study may be related to the microbial transfer at the feto-maternal interface [32, 33] and/or the larger number of beneficial bacteria in the breast milk of mothers in the probiotic group, although the milk microbial content was not examined in this study. A study by Tuzun et al. showed higher Bifidobacterium bifidum contents in the breast milk and greater B adolescentis, B bifidum, and B longum contents in the faecal samples of the neonates with no jaundice compared with those with jaundice [34]. Infant TSB and neonatal jaundice treatment in this study were among the secondary outcomes and the probability of error I in the results was high due to multiplicity. Therefore, it is suggested to assess such effects in future trials.

Similar to the results of the recent review [22], we also found no evidence that taking the probiotics either significantly increases or reduces most maternal outcomes such as preeclampsia, weight gain over pregnancy, and caesarean delivery; along with most infant outcomes such as macrosomia, large for gestational age and neonatal anthropometrics at birth. Nevertheless, the sample size in this study was too small to provide precise evidence on these secondary outcomes. Such results could be combined with results of similar studies in future meta-analyses to provide precise evidence in such areas.

We tried to optimize the validity of our study findings using high quality methodology including valid assessment methods and applying all measures to minimize the risk of bias including random sequence generation, allocation concealment, blinding of participants, investigators and data analyst, follow-up of all participants, and assessment of most outcomes in all participants. The weekly re-supply of yoghurts to maximize probiotic viability, and assessment of some definitive endpoints in addition to some surrogate endpoints can also be mentioned as the strengths of this study.

All outcomes reported in this paper have been preplanned, as well as all outcomes measured in this study have been reported in this paper. However, due to funding limitation, we did not assess the effect of the intervention on some other outcomes such as concentrations of lipids and inflammatory biomarkers in maternal blood sample, and microbiota and inflammatory markers in maternal and infant fecal samples. In addition, due to time constraints, we did not assess the long-term effects of intervention on the maternal and infant outcomes beyond 1 month after birth. Relatively low sample size can be mentioned as another limitation of this study, making the results inconclusive about most secondary outcomes.

Dietary intake was measured only once, at 28–33 weeks of gestation (at the week of of the primary outcome measurement until maximum 4 weeks following the week), with relatively low reponsiveness (73% in the probiotic and 64% in the conventioal yoghurt group). With the current data it is not possible to clearly state about similarity of this background data, and if and how it changed over the intervention period. It is also of note that all participants received dietary counselling and that individual responsiveness to this may vary.

The relatively high number of inclusion and exclusion criteria, relatively high number of eligible women unwilling to participate in the trial, and conducting the study only in north-west of Tabriz may compromise the generalizability of the study results.

Conclusions

Based on the results, it seems that the probiotics supplementation has some beneficial effects on glucose metabolism of overweight and obese pregnant women, and with a lesser certainty on the prevention of hyperbilirubinemia in their infants. Further studies in different settings with a larger number of participants, consideration of infant bilirubin as the primary outcome, and assessment of more biomarkers and definitive long-term outcomes in mothers and infants are recommended to judge the possible mechanism of the effects and clinical significance of probiotics intake during pregnancy for overweight and obese pregnant women and their infants.

Author contributions All of the authors contributed to the conception and design of the study. HA conducted the experiment and wrote the first draft of the paper. HA, SMAC analysed the data. SMAC, AHR and MM revised the manuscript. All the authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

Ethical standards This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all the procedures involving human subjects. It was approved by the Ethics Committee of Tabriz University of Medical Sciences (Ethics committee reference number: TBZMED.REC.1394.1208, Date 2016-03-07) and registered in http://apps.who.int/trialsearch/Trial3.aspx?trialid=IRCT201604013706N31 prior to participant recruitment. We followed all Helsinki declaration and national ethical standards.

Informed consent Written informed consent was obtained from all the participants.

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