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Effect of Probiotic Supplementation on Blood Pressure of Females with Gestational Diabetes Mellitus: A Randomized Double Blind Controlled Clinical Trial

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Abstract

Background: Despite progress in the control and treatment of gestational diabetes mellitus (GDM) in pregnant females, these patients remain at risk of disease complications.
Objectives: The present study aimed at investigating the effect of probiotic supplements on Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) in pregnant females diagnosed with GDM.
Methods: This randomized double-blind, placebo-controlled trial randomly assigned 64 pregnant females with GDM, recruited through convenience sampling, to either a group receiving a probiotic capsule (n = 32) or a group receiving a placebo (n = 32) for 8 weeks in Tabriz, Iran, during the spring and summer months of 2014. Their blood pressure was measured at baseline and at 2-week intervals, up to 8 weeks.
Results: A total of 56 subjects completed the study. There was no significant difference in SBP in the probiotic group at any time compared with that at onset, yet, SBP increased significantly in the placebo group. The declining trend of DBP was evident in the probiotic group at 2 weeks and continued to the end of the study; however, DBP had increased slightly by week 6 in the placebo group. There were significant differences between the probiotic and placebo groups at 6 and 8 weeks, respectively, for SBP (104.828 (2.051) mmHg vs. 112.963 (2.126) mmHg; P = 0.008) and (106.552 (1.845) mmHg vs. 115.185 (1.912) mmHg; P = 0.002) and for DBP (62.414 (1.353) mmHg vs. 70.741 (1.402) mmHg; P < 0.001) and (60.690 (1.540) mmHg vs. 71.296 (1.596) mmHg; P < 0.0010).
Conclusions: The results demonstrated that consumption of probiotic supplements for 8 weeks prevented an increase in SBP and decreased DBP in pregnant females diagnosed with GDM.

Keywords: Probiotic, Gestational Diabetes Mellitus, Blood Pressure

1. Background

Gestational diabetes mellitus (GDM) is carbohydrate intolerance of any degree of severity that begins or is diagnosed during pregnancy (1-4). It has a prevalence of 1% to 14% depending on the screening method, diagnostic criteria, and target population (5). The prevalence of GDM has increased from 16% to 127% in many ethnic groups in the past 20 years (6). In general, Asian females in the United States are at greater risk of developing GDM than other ethnic groups (7). It is estimated that 16.8% of global live births in 2013 were by females with some degree of increased blood sugar during pregnancy (8).

Given the strong link between maternal diabetes and obesity, and the risk of obesity and glucose intolerance in the child, metabolic conditions of the intrauterine environment are considered to be critical risk factors for diabetes and cardiovascular disease (9-12). Studies have shown that fasting hyperinsulinemia in mid-pregnancy and preeclampsia and pregnancy-induced hypertension unrelated to Body Mass Index (BMI) are relevant for females (13, 14). Even in non-diabetic pregnancies, post-prandial glycaemia in mid-pregnancy is positively correlated with increased risk of preeclampsia hypertension. A recent study on pregnant females with GDM in Iran showed that high blood pressure and preeclampsia cesarean section is

the most common complication of GDM (15).

Existing treatments focusing on normalizing blood glucose levels have shown varied success in reducing the short-term complications of GDM and may have no effect on long-term complications (1, 16). The limited results available on traditional risk factors, diet and physical activity, and poor acceptance of lifestyle interventions heighten the need for new solutions. Evidence indicates that significant changes occur in the microbial environment in the gut of the mother from the first to the third trimester, in terms of the obesogenic environment (17, 18). Once the microbiota of pregnant subjects in their third trimester was transmitted to germ-free mice, it triggered the mice to have apparent fat reposition, demonstrating that microbiota could disturb the host's metabolism (17). Hypertension is obviously related to fatness (19), predisposing overweight subjects to adverse cardiovascular consequences. In this case, the gut microbiome is changed, an event recognized as dysbiosis, which could lead to elevated blood pressure and its complications, potentially by low-grade inflammation enhancement (20).

More recently, the use of probiotics to improve metabolic profiles (21, 22), and reduce inflammatory factors (23-25) and oxidative stress biomarkers (26, 27) has been investigated. Microbes in the gut contribute to extraction of energy from food and should be considered an environmental factor involved in obesity and related disabilities, such as insulin resistance, diabetes, and cardiovascular disease (28-30).

The beneficial effects of the consumption of probiotics seem to be associated with the betterment of the gut microbiota situation, improvement of enterocyte's endurance to pathogenic products, reduction or near total removal of pathogenic germs inside the intestinal tract, improvement of nutritional intolerances, improvement of bioavailability of different nutrients, and decrease of the occurrence of allergies in vulnerable subjects (31).

It has been reported that supplementation with probiotics decreases blood pressure, demonstrating the significance of the gut microbiota in the arrangement of blood pressure in non-pregnant subjects (32). According to recent experimental and clinical studies, probiotic supplementation improves the gut microbiota and has the capability to help improve blood pressure in hypertensive situations (33-35). Likewise, in a 3-week randomized double-blind, placebo-controlled pilot trial on obese hypertensive subjects, probiotic cheese containing the probiotic Lactobacillus plantarum TENSIA along with a hypo-caloric diet decreased body mass index (BMI) and blood pressure (36).

On the other hand, other studies have revealed that supplementation with probiotics did not persuade any considerable changes in blood pressure (37, 38).

Subjects with GDM are at an additional hazard for high blood pressure-related disorders in pregnancy that can result in increased morbidity of the mother and fetus.

Despite the fact that many studies have been conducted on the use of probiotics during pregnancy, research about the effects of probiotics on the mother's metabolism is in its early stages, and studies regarding the effects of probiotics on the blood pressure of pregnant females with GDM are rare. For this reason and also because of conflicting results of previous studies and the importance of hypertension due to its high prevalence in females with GDM, it was decided to perform the present study. The present study investigated the effect of a probiotic supplement containing Lactobacillus acidophilus LA-5, Bifidobacterium BB-12, Streptococcus thermophilus STY-31, and Lactobacillus delbrueckii bulgaricus LBY-27 on systolic blood pressure (SBP) and diastolic blood pressure (DBP) of pregnant females diagnosed with GDM during pregnancy.

2. Methods

2.1. Participants

This study was a double-blind randomized clinical trial carried out to determine the effect of probiotic supplement on factors such as glucose metabolism, body weight changes, inflammatory markers, and oxidative stress among females with GDM at Al-Zahra referral University hospital in the city of Tabriz in Iran during the spring and summer of 2014. The target population included all nulliparous females diagnosed with GDM during 24th to 28th week of pregnancy, who referred to specialized gynecological and endocrine centers in Tabriz. The samples were selected from patients diagnosed with GDM, according to the inclusion and exclusion criteria and after mutual consent.

The inclusion criteria were nulliparity, a diagnosis of GDM at 24 weeks $\pm$ 0 days to 28 weeks $\pm$ 6 days of pregnancy through screening tests administered by experts using a one-step, 2-hour glucose tolerance test with 75 g of glucose (39). The mothers should be aged 18 to 45 years, have fasting blood glucose level of 92 to 126 mg/dl at the time of diagnosis, BMI of 18.5 or higher, no history of diabetes type 2, absence of chronic illness, be non-smoker and non-alcoholic, not having consumed probiotic food products for 2 weeks prior to the intervention, lack of antibiotics use 1 month or more prior to the intervention, absence of acute gastrointestinal problem 1 month or more prior to the intervention, and lack use of glucocorticoids or immunosuppressive drugs. The exclusion criteria included the need for and use of insulin and other glucose-lowering medications during the study, antibiotic use during the study, acute gastrointestinal problems during the

study and use of glucocorticoids and immunosuppressive drugs during the study.

All 64 pregnant females were allocated using block randomization techniques to consume either a placebo capsule (n = 32) or a probiotic capsule (n = 32) for 8 weeks.

2.2. Sample Size

The required sample size in a 2-tailed test with $\alpha = 0.05$, power of 0.80, and based on systolic blood pressure and the study of Lauszus et al. (40) that was done on pregnant females, and using the following formula, was estimated as 27 patients in each group. Considering 20% follow up rate, 64 pregnant females were recruited in the study through convenience sampling and were randomly allocated using block randomization techniques, to take either placebo (n = 32) or probiotic (n = 32) capsules for 8 weeks:

$$n \geq \frac{\left(z\frac{\alpha}{2} + z_{1-\beta}\right)^2 + \left(s_1^2 + s_2^2\right)}{d^2} \quad (1)$$

2.3. Study Design

At baseline, pregnant females with GDM were randomly allocated to either the placebo or probiotic groups for 8 weeks. The probiotic 4Biocap capsule contained 180 mg (> 4*109 CFU) of a standard powder, including freeze-dried cultures of Lactobacillus acidophilus LA-5, Bifidobacterium BB-12, Streptococcus thermophilus STY-31 and Lactobacillus delbrueckii bulgaricus LBY-27 plus dextrose anhydride filler, and magnesium stearate lubricant. The capsules were produced by Chr. Hansen (Denmark) and packaged by Tehran Darou. The placebo capsule lacked the bacteria, yet, contained all other ingredients and characteristics of the probiotic capsule with the same design, shape, and color. To guarantee double blinding, a coder secretly labeled the capsule packages as "A" or "B" and they were then allocated by the counselor pursuant to the random sequence generated by a computer program (41).

During the 8 weeks of the study, the intervention group consumed a probiotic capsule and the control group a placebo capsule daily after meals.

Participants received a 2-week supply of supplements every 2 weeks and obedience was evaluated by phone conversations once a week. Throughout the study, to control participants in terms of capsule intake and to prevent attrition, telephone contact was established with each participant every week. Every 2 weeks, the mothers were requested to bring in the bubble packs of the supplements to count the number taken and establish their compliance and decide whether the subjects could remain in the study.

2.4. Assessment of Variables

During the initial interview, a general information questionnaire including age, gestational age, occurrence of diabetes mellitus in first-degree relatives, pre-pregnancy weight, level of education, occupation, physical activity, special dietary information, history of diseases, antibiotic use and supplement consumption, acute gastrointestinal disease during the past month, and the use of probiotic food products during the past 2 weeks was completed.

A 24-hour dietary recall questionnaire was completed on 3 nonconsecutive days of the week (2 weekdays and 1 in weekend) once at baseline, once at 4 weeks and once at the conclusion of the study. Nutritionist IV software (First Databank; USA) modified for Iranian foods was used to obtain the daily macro- and micro-nutrient intake of the subjects.

Blood pressures at baseline and at 2-week intervals and up to 8 weeks of treatment were measured using a calibrated standard mercury sphygmomanometer with recommended cuff size, (Riester; Diplomat) with ± 3 mmHg accuracy after 15 minutes of rest. The SBP and DBP were measured in the supine position after a 10-minute break with the arm at heart level (42, 43). Blood pressure was measured for the second time 2 minutes after the first measurement. The average blood pressure in mmHg was then recorded as the final blood pressure. The following values were eliminated, because of methodological artifacts: systolic BP > 250 mmHg or < 70 mmHg, diastolic BP > 130 mmHg or < 30 mmHg, and systolic differences > 60 mmHg or diastolic differences > 30 mmHg in comparison with the former or latter readings.

Both measurements were performed by a trained nurse. She was blinded to the participants' assignments and study outcome for limiting bias. Trial registration code: IRCT201405181597N3

2.5. Statistical Analysis

Statistical analysis was carried out using the SPSS version 16 software. Quantitative data were recorded as mean ± standard deviation (SD), and qualitative data as frequency and percentage (%). A P value of < 0.05 was considered statistically significant.

The aim of the present study was to determine the effect of a probiotic diet on SBP and DBP over time. Mixed analysis of variance (ANOVA) was utilized to investigate changes in the mean core of the dependent variables (SBP and DBP) at 5 checkpoints (baseline, week 2, week 4, week 6, and week 8) between groups (A and B).

The data was first tested for accuracy of analysis using mixed ANOVA. The Shapiro-Wilk test determined that the

dependent variables were normally distributed. Levene's test determined that variance homogeneity was not significant. Mauchly's sphericity test indicated that the assumption of sphericity had been violated. To overcome this problem, corrections were applied to the degrees of freedom using Greenhouse-Geisser corrections. The pairwise comparisons were corrected using Bonferroni adjustments. The mixed model was used to investigate possible differences in dependent variables by weight changes.

3. Results

Eighty-four pregnant females with GDM were assessed for eligibility and finally 64 nulliparous females were enrolled in the study. Of these, 8 participants left the trial for personal reasons and finally, 56 subjects were analyzed (see CONSORT flow diagram in Figure 1).

At baseline, the 2 groups were similar with regards to all demographic characteristics (Table 1).

Also, there was no statistically significant difference between the groups in terms of classification of blood glucose at baseline.

The study did not find any statistically significant difference between the 2 groups in terms of energy dietary intakes, macronutrients, and micronutrients at baseline. Total daily energy intake from baseline to 4 weeks and then to 8 weeks after the study showed significant decrease in both intervention and control groups, which was due to the same dietary recommendations (therapeutic lifestyle changes and low glycemic load diet) provided in the groups because of their diagnosis of GDM. Energy intake reduction was equal in both groups and it was not significantly different in between the 2 groups at the beginning, during, and at the end of the study.

To control the confounding effect of energy intake, intake of macronutrients, and micronutrients were adjusted through regression analysis model (residual model) relative to total energy intake (44).

Daily modulated intake to energy carbohydrate, total fat, protein, dietary fiber, calcium, phosphorus, iron, and magnesium, from baseline to 4 weeks after study and up to 8 weeks after study, didn't show any statistically significant differences between the intervention and control groups. Daily modulated intake of saturated fat did not differ significantly in intervention and control groups at baseline (28.30 ± 0.64 gr versus 26.72 ± 0.66 gr ($P = 0.093$), respectively), yet, there were significant differences in the intakes of saturated fat at 4 weeks and 8 weeks of study between the 2 groups (22.70 ± 0.50 gr versus 20.59 ± 0.52 gr at 4 weeks ($P = 0.005$) and 18.92 ± 0.53 gr versus 15.74 ± 0.54 gr at 8 weeks ($P < 0.001$) of study in the probiotic and placebo groups, respectively).

Changes in systolic and diastolic BP over 5 time checkpoints between the 2 groups are summarized in Tables 2 and 3, respectively, where significant time * group interaction demonstrated that post hoc tests for pairwise comparison were needed. Time-based Post hoc pairwise comparisons revealed that SBP and DBP did not differ significantly between study groups up to week 4. In addition, the probiotic group scores for SBP and DBP were significantly lower than the placebo group during week 6 and week 8 of the participant's pregnancy (Table 4).

The SBP did not differ significantly in the probiotic group at any time point, yet, it increased significantly during week 8 in the placebo group (Table 5). In present study, there was a clear tendency toward lower DBP in the probiotic group after the second week and higher DBP in the placebo group after week 6. The DBP tended to be significantly superior to placebo at all-time checkpoints after week 6 (Figures 2 and 3).

4. Discussion

The present investigation produced promising results. This was the first study to evaluate the effects of supplementation with probiotic capsules containing *L. acidophilus* LA-5, Bifidobacterium BB-12, *S. thermophilus* STY-31, and *L. delbrueckii bulgaricus* LBY-27 on SBP and DBP in pregnant females diagnosed with GDM. The results showed that probiotic supplementation effectively prevented an increase in SBP as the pregnancy progressed and significantly decreased DBP after 2 weeks of consumption.

Compared to those without diabetes, pregnant females with GDM in the absence of intervention are at higher risk of adverse maternal and fetal complications (45-47). Gestational hypertension and preeclampsia are major complications of GDM and place the mother at risk of serious complications such as HELLP syndrome, nervous disorders, and kidney dysfunction (48), which sometimes lead to early termination of pregnancy. It appears that the severity of the effects of GDM are much higher in developing countries than in developed countries (49). In such countries, pregnant females at high risk for hypertensive disorders require exact and careful management to avert maternal and fetal complications (50).

It has been shown that probiotics and food products that contain them could improve blood pressure by mechanisms such as reducing total cholesterol, low-density lipoprotein (LDL) (51-53), blood glucose and insulin resistance (54, 55), and stabilizing the renin-angiotensin system (56, 57). Probiotics can reduce the effects of pregnancy complications associated with inflammation and high blood pressure (58, 59).

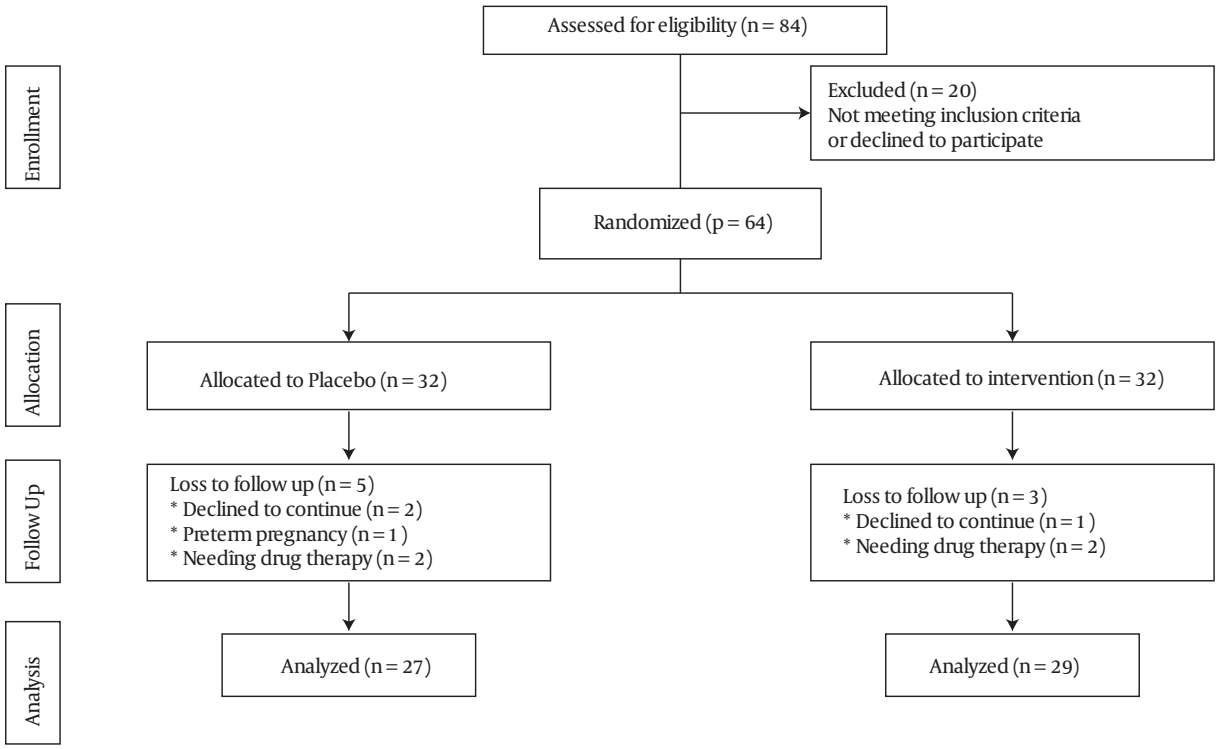

Figure 1. 2010 Consort Flow Chart of Subjects

Table 1. Demographic Data of Study Participants[a]

Variables		Probiotic Group, n = 29	Placebo Group, n = 27	P Value
Age, y		28.14 ± 1.16	26.48 ± 1.01	0.289
Gestational Age, d		182.17 ± 6.18	181.30 ± 7.61	0.308
BMI, kg/m²		31.41 ± 0.73	29.86 ± 0.65	0.120
Family history	Yes	16 (55.17)	12 (44.44)	0.422
	No	13 (44.83)	15 (55.56)	
Education	Under Diploma	4 (13.79)	5 (18.52)	0.891
	Diploma	17 (58.62)	15 (55.56)	
	Academic education	8 (27.59)	7(25.93)	
Job	Clerk	10 (34.48)	9 (33.33)	0.198
	Housewife	19 (65.52)	18 (66.67)	
Hesitancy	City	12 (41.38)	12 (44.44)	0.817
	Town	17 (58.62)	15 (55.56)	
Physical activity	Low	22 (75.86)	17 (62.96)	0.294
	Average	7 (24.14)	10 (37.04)	

Abbreviation: BMI, body mass index.
[a]Values are expressed as mean ± SD or No. (%).

Table 2. The Effects of Probiotics on Systolic Blood Pressure[a]

Variables	Probiotic Group, (n = 29)	Placebo Group, (n = 27)	Test Statistics Mixed ANOVA
At the baseline	108.9 (2.05)	105. 6 (2.13)	F (Time) (3.39, 183.5) = 1.71; P Value = 0.159
Week 2	107.1 (2.07)	107.9 (2.14)	
Week 4	107.4 (2.03)	109.6 (2.10)	
Week 6	104.8 (2.05)	112.9 (2.13)	
Week 8	106.5 (1.85)	115.2 (1.93)	
Test Statistics Mixed ANOVA	F (Group) (1, 54) = 2.37; P Value = 0.129		F (Group*Time) (3.39, 183.5) = 5.44; P Value = 0.001

Abbreviation: SBP, systolic blood pressure.
[a]Values are expressed as mean (s.e.).

Table 3. The Effects of Probiotics on Diastolic Blood Pressure[a]

Variables	Probiotic Group, (n = 29)	Placebo Group, (n = 27)	Test Statistics Mixed ANOVA
Baseline	68.79 (1.31)	65.00 (1.36)	F (Time) (3.23, 174.7) = 1.06; P Value = 0.371
Week 2	66.03 (1.32)	65.56 (1.37)	
Week 4	66.21 (1.37)	69.44 (1.42)	
Week 6	62.41 (1.35)	70.74 (1.40)	
Week 8	60.69 (1.54)	71.30 (1.60)	
Test Statistics Mixed ANOVA	F(Group)(1, 54) = 6.44; P Value = 0.014		F (Group*Time) (3.23, 174.7) = 14.51; P Value < 0.001

Abbreviation: DBP, diastolic blood pressure.
[a]Values are expressed as mean (s.e.).

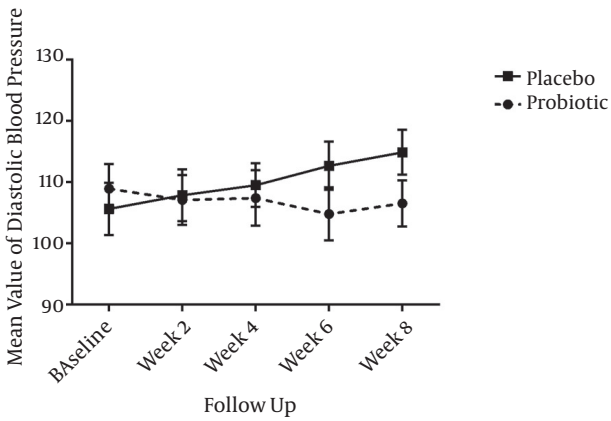

Figure 2. Trend of Changes in Systolic Blood Pressure Throughout the Study

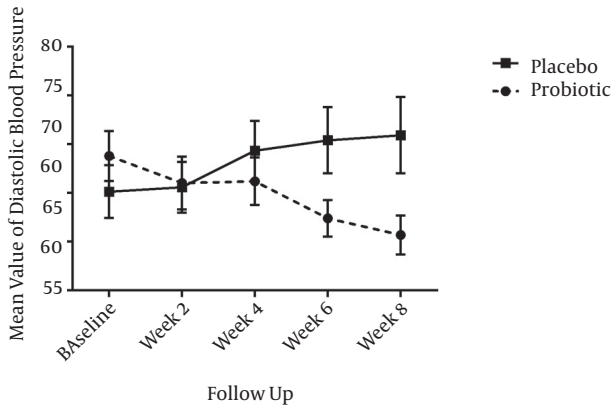

Figure 3. Trend of Changes in Diastolic Blood Pressure Throughout the Study

It has been shown that probiotics can modulate the expression of human genes in intestinal cells similarly to drugs used for high blood pressure (60). Meta-analysis on the effects of probiotics on serum lipid levels reported a significant decrease of 6.4 mg/dL for total cholesterol, 4.9 mg/dL for LDL, and 3.9 mg/dL for serum triglycerides (53).

An imbalance in intestinal micro-flora can reduce the ratio of gram-positive and gram-negative bacteria. The level of access to LPS pro-inflammatory molecules and their transit to the blood circulation might then increase, leading to an increase in cytokine secretion and the activity of macrophages, eventually causing inflammation (21, 61).

Affordable probiotics are clearly mentioned in the scientific literature and probiotic supplements are well-accepted by patients (62, 63).

The impact of supplements and probiotic yogurt intake on blood pressure in type 2 diabetes has been examined. Ejtahed et al. (64) studied the effect of a daily intake of 300 g probiotic yoghurt containing *L. acidophilus* and *Bifidobacterium lactis* for 6 weeks on blood pressure in patients with type 2 diabetes, and found that probiotic yogurt influenced blood pressure. This result was likely achieved because the researcher did not allow sufficient time for the effects of probiotic yoghurt to unfold and because there was an insufficient number of live probiotics per gram in the yogurt.

An examination of the effect of low-calorie diet with 50 g of probiotic cheese containing *L. plantarum tensia* for 3 weeks on blood pressure among obese patients showed that the supplement significantly decreased DBP (P = 0.026) and somewhat decreased SBP (P = 0.064) (36). The researcher attributed this effect to vascular relaxation that was caused by reduction of oxygen-free radicals (65, 66).

Table 4. Pair-Wise Comparisons of Systolic Blood Pressure and Diastolic Blood Pressure Values Based on Time[a]

	Baseline	Week 2	Week 4	Week 6	Week 8
SBP	0.253	0.774	0.451	0.008	0.002
DBP	0.059	0.802	0.107	< 0.001	< 0.001

Abbreviations: DBP, diastolic blood pressure; SBP, systolic blood pressure.
[a]Values are expressed as P value.

Table 5. Pair wise Comparisons of Systolic Blood Pressure and Diastolic Blood Pressure Values Based on Group[a]

Pair	SBP		DBP	
	Probiotic Group (N = 29)	Placebo Group (N = 27)	Probiotic Group (N = 29)	Placebo Group (N = 27)
Baseline*week 2	> 0.999	> 0.999	0.037	> 0.999
Baseline*week 4	0.709	0.830	> 0.999	0.096
Baseline*week 6	0.678	0.071	0.005	0.023
Baseline*week 8	0.556	0.001	< 0.001	0.003
Week2*week 4	> 0.999	> 0.999	> 0.999	0.208
Week2*week 6	> 0.999	> 0.999	0.234	0.021
Week2*week 8	0.499	0.012	0.010	0.007
Week4*week 6	> 0.999	> 0.999	0.271	> 0.999
Week4*week 8	> 0.999	> 0.999	0.023	> 0.999

Abbreviations: DBP, diastolic blood pressure; SBP, systolic blood pressure.
[a]Values are expressed as P value.

An increasing number of clinical trials have found that cardiovascular-associated hypotension can be improved by probiotics.

Berve and Lancome studied the effect of probiotic supplements containing *L. casei*, *L. acidophilus*, *L. rhamnosus*, *Bifidobacteria*, and *S. thermophilus* for 8 weeks on blood pressure among 60 pre-diabetic patients. The supplements reduced SBP and DBP, however the decrease was not statistically significant. In the placebo group, SBP and DBP increased slightly, yet, the change was not significant. The percentage of change in SBP in the probiotic group was statistically different from the placebo group (-3.10 $\pm$ 2.22 vs. 3.24 $\pm$ 1.96); however, it was not significant after adjusting for confounder variables (37).

Lactobacillus helveticus LBK-16H lowered blood pressure in 94 hypertensive patients receiving twice-daily 150 mL servings of milk fermented by the probiotic or a control for 10 weeks. The results showed a significant reduction in SBP and DBP at the end of the intervention (mean difference of -4.1 $\pm$ 0.9 mmHg in SBP (P = 0.001) and a -1.8 $\pm$ 0.7 mmHg in DBP (P = 0.048)) (67).

The results of these studies varied according to the probiotic strains used and the duration of consumption.

Findings from a previous study suggest that the effect of probiotic supplement on the prevention of hypertension in pregnancy could be an indirect result of the effect of controlling weight gain by probiotics (68, 69).

Several studies suggest a link between high blood pressure and weight gain in pregnant females (70-72) and indicate that limiting weight gain during pregnancy could prevent hypertension (73). On the other hand, inflammatory markers such as C-reactive protein (CRP) and tumor necrotizing factor (TNF)-α increase in obese pregnant females and this inflammation could cause endothelial dysfunction (74).

The results of a previous study by the author found that probiotic supplementation controlled excessive weight in females in the probiotic group compared to the placebo (75).

In the current study, the SBP results were influenced by weight changes during the study, yet these did not affect longitudinal changes in DBP. After taking into account weight change in the probiotic and placebo groups, probiotic supplementation was still successful in preventing an increase of SBP and a decrease of DBP in pregnant females with GDM. Further clinical trials are needed to confirm that the clinical use of probiotic supplementation by patients with GDM can prevent complications such as high blood

pressure.

The strength of this study was its design as a randomized double-blind clinical trial with an adequate sample size. In addition, dietary compliance was monitored and strengthened by nutritional consultation in weekly phone calls and face-to-face follow-up every 2 weeks.

The limitations of the study included the absence of fecal sample testing of participants in the probiotic group to demonstrate transit of the specific microbiota through the gastrointestinal tract. Another limitation was that the use of food products containing probiotics could have promoted acceptance among the study respondents and more objective results.

4.1. Conclusions

The results of this study point to new horizons in the treatment and management of GDM. The results indicate that pregnant females with GDM can benefit from daily consumption of probiotic supplements for at least 6 weeks. This appears to be an acceptable strategy for prevention of an increase in systolic and diastolic hypertension and related complications.

Ethics: The study was approved by the ethics committee of Shahid Beheshti University of Medical Sciences (No. 116/449, date 1393/02/28) and was registered in clinical trial of world health organization (IRCT201405181597N3 code). Written informed consent was obtained from all patients to perform the study and to publish the results and report individual patient data. The study protocol conformed to the ethical guideline of the declaration of Helsinki, 1975.

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Footnotes

Authors' Contribution: All authors critically revised the manuscript and approved its final submitted version. Neda Dolatkhah contributed to all parts of the research. Majid Hajifaraji contributed to all parts of the research. Fatemeh Abbasalizadeh, Naser Aghamohammadzadeh, and Fatemeh Jahanjou contributed to clinical examinations and data collection as well as interpretation of the results. All authors contributed to drafting or reviewing the manuscript. All authors read and approved the final manuscript.

Competing Interests: There were no conflicts of interest associated with this study and there was no significant financial funding that could have influenced its outcomes.

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