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ORIGINAL ARTICLE

The effects of probiotics and synbiotic supplementation on glucose and insulin metabolism in adults with prediabetes: a double-blind randomized clinical trial

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Abstract

Aims Probiotics and/or prebiotics could be a promising approach to improve metabolic disorders by favorably modifying the gut microbial composition.

Objectives To assess the effects of probiotics and synbiotic on glycemic indices in prediabetic individuals who are at risk of type 2 diabetes and its complications.

Methods In a double-blind, randomized, placebo-controlled parallel-group clinical trial, 120 prediabetic adults participated and were randomly allocated to receive either probiotics or synbiotic or placebo supplements for 24 weeks. Anthropometric measurements, food record, physical activity and glycemic biomarkers including glycated hemoglobin (HbA1C), fasting plasma glucose (FPG), fasting insulin levels (FIL), homoeostasis model assessment for insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), and β-cell function (HOMA-B) were assessed at baseline and repeated at 12 and 24 weeks and compared within and between three groups using repeated measure ANOVA.

Results Compared with the placebo, synbiotic supplementation resulted in a higher significant reduction in FPG (-6.5 ± 1.6 vs. -0.82 ± 1.7 mg/dL, $P = 0.01$), FIL (-2.6 ± 0.9 vs. -0.8 ± 0.8 μIU/mL, $P = 0.028$), and HOMA-IR (-0.86 ± 0.3 vs. -0.16 ± 0.25, $P = 0.007$), and a significant elevation in the QUICKI ($+0.01 \pm 0.003$ vs. $+0.003 \pm 0.002$, $P = 0.006$). In addition, significant decreases in HbA1C was seen following the supplementation of probiotics and synbiotic compared with the placebo (-0.12 ± 0.06 and -0.14 ± 0.05 vs. $+0.07 \pm 0.06$%, $P = 0.005$ and 0.008, respectively). HOMA-B was not found to be different between or within the three groups.

Conclusion Glycemic improvement by probiotics and particularly synbiotic supplements in prediabetic individuals has been supported by current study. However, further studies are required for optimal recommendations in this important area of patient treatment.

Trial registration Iranian Registry of Clinical Trials: IRCT201511032321N2, Date registered February 27, 2016.

Keywords Probiotics · Synbiotic · Prediabetes · Clinical trial

Introduction

Type 2 diabetes mellitus (T2DM) and prediabetes are important public health problems which their incidence and prevalence have been raised dramatically [1]. They proceeded by a preclinical phase of impaired glucose regulation, in the fasting and/or in the postprandial state [2].

The prevalence of individuals with prediabetes in the world exceeds 25%, who are at high risk of developing type 2 diabetes and/or its complications [3]. It has been shown that the progressive alterations of insulin production and secretion from pancreas and insulin action in skeletal muscle, adipose tissue, and liver are the hallmarks of prediabetes and T2DM [2].

The evidence in the past decade has been shown that the intestinal microbiota composition can be associated with the development of insulin resistance and diabetes mellitus [4]

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and the normal gut microbiota may have a protective role against diabetes [5].

Therefore, it has been suggested that probiotics and/or prebiotics may be a useful strategy to improve metabolic health and prevent type 2 diabetes by changing gut microbiota and regulating insulin signaling [6, 7].

Probiotics are live microorganisms that confer health benefits when administered in adequate amounts. *Bifidobacterium* and *Lactobacillus* strains are the widest bacteria with exhibiting probiotic properties and they are included in many dietary supplements [8].

Prebiotics are food components like indigestible polysaccharides or fibers that confer health benefits with modulation of the microbiota [9]. Combination of prebiotics and probiotics is described as synbiotic, which the health benefit can be synergistic [10].

Despite preliminary results from animal studies showing the efficacy of probiotics, prebiotics, or synbiotics in treatment or prevention of diseases, the data in human remain ambiguous [11].

The actual effects of probiotics or prebiotics or synbiotics to affect intestinal ecology are still under debate, because there are numerous confounding elements, such as variety in product formulations, microbial strains, and concentrations of viable bacteria [12].

On the other hand, there are contradictory effects of these products on metabolic diseases in the reported studies that might be related to the use of different strains and short duration of receiving interventions [8].

Therefore, regarding the gap of information, the present investigation was carried out to assess and compare the functional effects of probiotics and synbiotic products on fasting plasma glucose level, glycated hemoglobin, fasting insulin levels, insulin resistance, insulin sensitivity, and beta-cell function and to determine the best intervention period to improve the glycemic control by these supplements in prediabetic subjects.

Materials and methods

Study designs and participants

In this 24-week parallel-group, randomized double-blind, placebo-controlled clinical trial, prediabetic subjects confirmed by the American Diabetes Association (ADA) criteria [13] were selected between June 2016 and March 2017. The study was designed according to the CONSORT 2010 guideline [14]. They were recruited from the outpatient clinic of Isfahan Endocrine and Metabolism Research Center, Iran. Inclusion criteria were as follows: men and women 35–75 years old, fasting plasma glucose = 100–125 mg/dL or 2 h post-load serum glucose = 140–199 mg/dL according to oral glucose tolerance test (OGTT). The exclusion criteria were as follows: current smokers, suspected or definite history of alcohol or drug abuse, antibiotic use in the past 3 months or during the treatment period, using probiotic, prebiotic or synbiotic during the past 3 months, being pregnant, having gastrointestinal diseases, i.e., food allergies, celiac or irritable bowel disease, having liver, kidney, heart, or nervous system diseases, currently taking prescribed nonsteroidal anti-inflammatory drugs, antipsychotics, or nicotinic acid.

Sample size

The sample size calculation was based on a parallel-three group randomized clinical trial with repeated measurements of main outcomes at three timepoints. Using the prior published data [1], we estimated an effect size of 0.75 for main outcomes to have greater than 80% power to detect significant changes within and differences between groups by time interactions. Considering the type one error rate $\alpha = 0.05$ ($Z = 1.96$), and statistical power $1 - \beta = 0.80$ ($Z = 0.84$) for detecting a standardized effect size of at least $\Delta = 0.75$ about the effects of probiotic or synbiotic supplementation on improving glycemic variables in prediabetic individuals, 29 subjects were determined. For compensating possible attrition, 30% additional samples were recruited, in which a final 40 subjects in each study group or a total of 120 subjects were considered for study participation. Informed consent was obtained from all individual participants included in the study.

Randomization and blinding

After confirmation of eligibility criteria and obtaining written informed consent, the participants randomly allocated into the three equal groups using blocking stratified random assignment method (stratified based on gender and age). Computer-generated random numbers were used to implement the random allocation sequence. The randomization list was provided by a person not involved in the study. The participants were allocated to treatment with either probiotic, synbiotic, or placebo supplements for 6 months. Participants, laboratory staff, outcome assessors, and data analyst were blinded to the allocation of the supplements. The study pharmacist was responsible for delivery of the blinded supplements. The supplements which were assigned A, B, and C labels were otherwise identical. The blinding code was provided to the investigators after the statistical analyses.

Supplement administration

Participants were supplemented 6 g/day of either probiotic containing the freeze-dried *Lactobacillus acidophilus,*

Bifidobacterium lactis, Bifidobacterium bifidum, and Bifidobacterium longum (1×10^9 for each) with maltodextrin as filler, or synbiotic comprising the mentioned probiotics with an inulin-based prebiotic, or placebo including maltodextrin for 24 weeks. The supplements were stored in dried place under 20° centigrade. Subjects were instructed to ingest the supplement by mixing the powder into a cup of water after a main meal to minimize the killing of the probiotics by gastric acid. In addition, the participants were advised not to modify their dietary and/or physical activity habits during the study. To ensure that dietary consumption or physical activity had not been modified during the study, the participants were instructed to record 3-day food and physical activity diaries which were checked by a trained dietitian at the follow-ups.

The supplements were prepared in Tak Gen Zist Pharmaceutical Company, Tehran, Iran in sachet form. All of microbial and purity tests, solubility, and palatability of supplements were checked by two independent microbiologists.

The researcher was in weekly contact with participants and any concerns or side effects during the intervention were addressed. The participants were excluded from the study if there was any side effect or problem. Compliance was assessed based on returned tablet counts. If a participant found to have missed > 10% of supplement dose at follow-up phone call or clinic visits, it defined as noncompliance and he or she was excluded from the study.

An oral glucose tolerance test (OGTT) was performed to clarify glycemic status. Fasting plasma glucose level was measured using the glucose oxidase (GOD) method. Fasting insulin levels were measured using chemiluminescence (Siemens, Munich, Germany). Ion-exchange chromatography was used to test hemoglobin glycosylated (HbA1c).

HOMA-IR index

The homoeostasis model assessment for insulin resistance (HOMA-IR index) was used to determine the degree of insulin resistance using the following formula [16]:

$$\text{HOMA-IR} = \text{fasting blood glucose (mg/dL)} \times \text{fasting insulin (}\mu\text{IU/mL)}/405.$$

QUICKI index

Insulin sensitivity was assessed through quantitative insulin sensitivity check index (QUICKI) using the following formula [17]:

$$\text{QUICKI} = 1/[\log \text{insulin (}\mu\text{IU/mL)} + \log \text{glucose (mg/dL)}].$$

HOMA-B

Homeostatic model assessment of beta-cell function (HOMA-B) index was used to determine β-cell function via following formula [17]:

$$\text{HOMA-B} = [360 \times \text{insulin (}\mu\text{IU/mL)/fasting blood glucose (mg/dL)} - 63].$$

Anthropometric measurement

Height, weight, waist, and hip circumferences were measured as secondary outcomes at baseline and repeated at 12 and 24 weeks of intervention.

Body mass index (BMI) was used to determine body mass which calculated by weight (kg) divided into the square of height (m).

The waist-to-hip ratio (WHR) was calculated according to World Health Organization (WHO) recommendations [15]. All the measurements were taken by one person to decrease the error rate.

Clinical laboratory assessment

Participants who had met the inclusion criteria were instructed to arrive at Isfahan Endocrine and Metabolism Research Center between 7:00 and 9:00 AM after a 12-h overnight fast for laboratory testing at baseline and during each follow-up visits (12 and 24 weeks).

Assessment of physical activity and dietary intake

Food and physical activity records were assessed at baseline and at 12 and 24 weeks during the intervention. At each timepoint, participants were instructed to record their daily physical activities and daily food and beverage intakes for 3 days, including 12 weekdays and 1 weekend day. Participants were asked to write down the type and amount of eaten food and beverages. The portion sizes were converted to grams and every food and beverage item were subsequently coded and 3-day averages of energy and macronutrient intakes were analyzed using the Nutritionist 4 software [18]. Data entry was performed by a trained dietitian. Physical activity was assessed using the metabolic equivalent of task (MET) questionnaire. To measure the METs for each participant, the times (in hour per day) reported for each physical activity was multiplied by its related METs coefficient via standard tables [19].

Ethical approval

The study protocol was approved by the Ethics Committee of Isfahan University of Medical Sciences, Isfahan, Iran (approval number: IR.MUI.REC.1394.3.813). The trial has been registered at clinicaltrials.gov as IRCT201511032321N2.

Statistical analysis

Statistical analyses were based on the data which derived from per-protocol participants. The per-protocol analysis included only those participants who completed the intervention with > 90% product compliance and no antibiotic use or any complication. Recorded data including adverse events and outcome data were double-entered on SPSS software Version 15 (SPSS Inc., Chicago, IL, USA). Data were evaluated and managed for the presence of outliers, violations of normality, and missing data. Normality of continues data was evaluated using Kolmogorov–Smirnov test and Q–Q plot. Quantitative normally distributed data were presented as the mean ± standard deviation (SD) and categorical data as frequency (percentage). Participants' basic characteristics including age, gender, education levels, and anthropometric measures in the three groups were compared using one-way ANOVA and Chi square. To test our hypothesis that probiotic or synbiotic supplementations improve main outcomes in prediabetic participants, intra- and intergroup changes were compared by repeated measure analysis of variance (ANOVA) with Bonferroni post-hoc analysis where appropriate. Baseline values were used as covariates when they were significant in the model.

Sphericity assumption in the framework of repeated measures ANOVA was evaluated using Muchly test and Huynh–Feldt or multivariate approach was considered when appropriate. A post-power analysis for determining the statistical power of the observed between groups differences was conducted. A $P < 0.05$ was considered statistically significant.

Code availability

The blinding code was provided to the investigators after the statistical analyses were completed. The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request and the supporting data are available.

Results

At the baseline, a total of 224 individuals (152 females and 72 male) with impaired glucose tolerance or impaired fasting glucose were invited to participate in the study. However, 104 subjects were excluded because of not fulfilling the inclusion criteria or decline to participate. From 120 individuals who agreed to participate in the study, 30 ones were dropped out from the study during the first 3 months and 5 ones between 12 and 24 weeks of the intervention period. The most reason for the attrition was using antibiotic during the study period (5 in the probiotic group, 6 in the synbiotic group, and 9 in the placebo group). In addition, low compliance was seen in six participants (two in the probiotic group, two in the synbiotic group, and two in the placebo group). The other reasons were disinclination, GI complication, and traveling.

Finally, the data of 85 randomly assigned participants who completed the 24-week intervention (27 in probiotic, 30 in synbiotic and 28 in placebo groups) were analyzed (see CONSORT flow diagram of participants' recruitment in Fig. 1). Because of more attrition rate than we had expected in the protocol, a post-power analysis was done.

At baseline, there were no statistically significant differences in age, gender, education, and anthropometric measures between three groups (Table 1). BMI, physical activity, total energy, and macronutrient intakes were also comparable at the baseline and did not change between the intervention groups during study period (Table 2).

A repeated measures ANOVA with a Greenhouse–Geisser correction and post-hoc test using the Bonferroni correction determined that HbA1C significantly decreased in synbiotic group ($P < 0.001$) and the differences in both the synbiotic and probiotic groups compared to placebo were significant ($P < 0.01$, observed power = 0.86). Mean fasting plasma glucose (FPG) concentration significantly decreased in the synbiotic ($P < 0.001$) and probiotic ($P < 0.01$) groups during the study period and a significant difference was observed in synbiotic group compared with placebo group ($P < 0.05$, observed power = 0.78).

The mean ± SD of fasting insulin levels (FIL) and insulin resistance index (HOMA-IR) decreased and insulin sensitivity index (QUICKI) increased in the synbiotic group through intervention period. The repeated measures ANOVA and the Bonferroni post-hoc test revealed that FIL ($P < 0.05$, observed power = 0.67), HOMA-IR ($P < 0.01$, observed power = 0.82), and QUICKI ($P < 0.01$, observed power = 0.83) had been significantly different between the synbiotic group compared to the placebo group.

However, HOMA-B was not found to be different between or within the three groups (Table 3).

On comparing the baseline with 12 and 24 weeks of the study results by post-hoc test using the Bonferroni correction, a significant improvement was seen in HbA1C, FPG, FIL, insulin resistance, and insulin sensitivity in both the study periods.

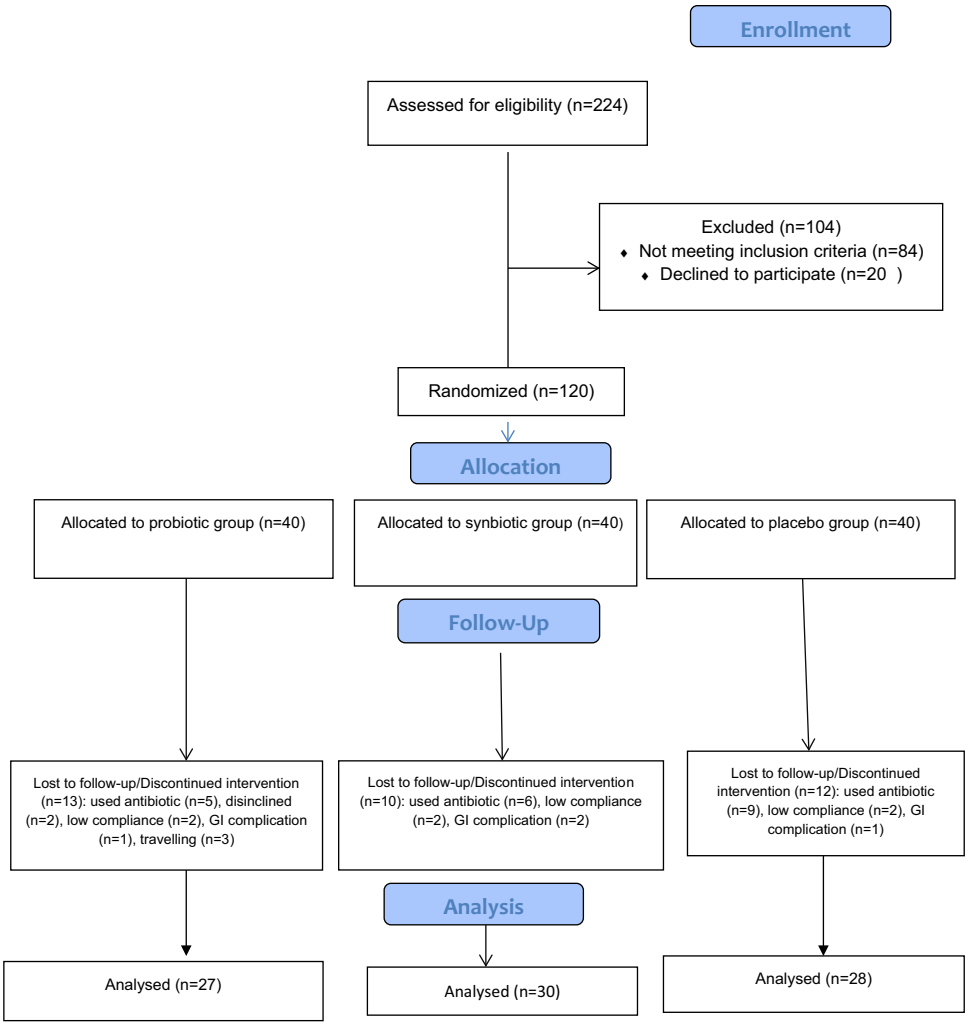

Fig. 1 Prediabetic participants' flow (diagram CONSORT 2010)

Table 1 Baseline characteristics presented by the three groups (mean values ± standard deviations and number with percentages)

	Total ($n=85$)	Probiotic group ($n=27$)	Synbiotic group ($n=30$)	Placebo group ($n=28$)	*P* value
Age (years)	52.95 ± 6.3	52.9 ± 6.3	52.97 ± 6.8	52.97 5.9	0.91
Gender					
Male *N* (%)	38 (45)	13 (48)	13 (43)	12 (43)	0.96
Female *N* (%)	47 (55)	14 (52)	23 (57)	16 (57)	
Education (years)	11.2 ± 3.6	11.8 ± 3.8	11.1 ± 3.8	10.5 ± 3.3	0.22
Weight (kg)	78.4 ± 11	77.3 ± 10.9	77.9 ± 11.8	79.8 10.8	0.54
Waist circumference (cm)	97.5 ± 9.3	96.2 ± 9.4	97.43 ± 9.6	98.7 8.8	0.47
Hip circumference (cm)	107.8 ± 6.6	106.9 ± 5.8	108 ± 6.8	108.4 7.1	0.61
WHR	0.9 ± 0.07	0.9 ± 0.07	0.9 ± 0.07	0.91 0.06	0.74

Adverse and beneficial events

During the study, no serious adverse events were registered. The only reported adverse event (14.1%) was mild gastrointestinal complications including flatulence, dysphagia, and dyspepsia (two in the probiotic group, five in the synbiotic group, and five in the placebo group). The beneficial events were reported in 44.7% of the subjects. Most of the beneficial events were reported in the probiotic group which were feel better in constipation, diarrhea, and dyspepsia (17 in the probiotic group, 8 in the synbiotic group, and 2 in the placebo group) and feel more energetic and health (5 in

Table 2 BMI, dietary intake, and physical activity at the baseline and after 12 and 24 weeks of intervention in the three groups

	Groups	At baseline	At 12 weeks	At 24 weeks	*P* value[a] time effect	*P* value[a] between groups
BMI (kg/m^2)	Probiotic ($n=27$)	29.6 ± 3.5	29.64 ± 3.7	29.5 ± 3.6	0.31	0.26
	Synbiotic ($n=30$)	29.1 ± 2.9	29.2 ± 3.0	29 ± 2.9	0.4	
	Placebo ($n=28$)	30.4 ± 3.2	30.5 ± 3.3	30.6 ± 3.4	0.32	
	P value	0.66	0.31	0.21		
Total energy (Kcal/day)	Probiotic ($n=27$)	2004.8 ± 410	2030.6 ± 422	2004 ± 379	0.73	0.76
	Synbiotic ($n=30$)	1967.7 ± 480	2105 ± 523.5	2094.5 ± 515	0.52	
	Placebo ($n=28$)	2025.6 ± 384	2013.5 ± 381	1999 ± 529	0.55	
	P value	0.81	0.72	0.69		
Carbohydrate (g/day)	Probiotic ($n=27$)	273.5 ± 64	270 ± 55	268 ± 52.5	0.81	0.67
	Synbiotic ($n=30$)	271 ± 59	283 ± 65	286 ± 60	0.67	
	Placebo ($n=28$)	271 ± 53	273 ± 53	278 ± 54	0.83	
	P value	0.98	0.1	0.43		
Protein (g/day)	Probiotic ($n=27$)	77 ± 19	76.5 ± 18	77 ± 20	0.79	0.83
	Synbiotic ($n=30$)	69 ± 19.7	74 ± 23	76 ± 24	0.37	
	Placebo ($n=28$)	73 ± 18.8	76 ± 19	76 ± 18	0.32	
	P value	0.21	0.84	0.97		
Fat (g/day)	Probiotic ($n=27$)	67.6 ± 17	67.3 ± 15.6	68.8 ± 15.7	0.45	0.24
	Synbiotic ($n=30$)	68 ± 14	68.3 ± 15.6	67.2 ± 18	0.84	
	Placebo ($n=28$)	71 ± 13	72 ± 10.6	73.8 ± 11.4	0.62	
	P value	0.54	0.42	0.24		
Physical activity (Kcal/day)	Probiotic ($n=27$)	2501 ± 328	2394 ± 31.8	2406 ± 297	0.22	0.22
	Synbiotic ($n=30$)	2523 ± 425.6	2422 ± 358.7	2353 ± 324	0.41	
	Placebo ($n=28$)	2534 ± 419	2532 ± 445.6	2537.6 ± 446	0.74	
	P value	0.94	0.35	0.15		

[a]Resulted from repeated measures ANOVA

the probiotic group, 4 in the synbiotic group, and 2 in the placebo group).

Discussion

The major finding of the present study was that synbiotic treatment, improved fasting plasma glucose, fasting insulin levels, glycated hemoglobin, insulin resistance, and insulin sensitivity compared with placebo. Moreover, probiotics could affect glycated hemoglobin improvement compared with placebo. The findings of our study are in agreement with the other studies, suggesting that a combination of probiotic and prebiotic in the synbiotic supplement is more effective than probiotics alone in glycemic control [10]. In the previous studies, it has been demonstrated that some kinds of probiotics had beneficial effects on insulin resistant syndrome and synbiotic resulted in an improvement in FPG, insulin, HOMA-IR, HOMA-B, and QUICKI [8, 20]. However, pooled results on effects of probiotics or synbiotics on glycemic profile were either non-significant or highly heterogeneous [20].

Some of the studies have demonstrated that *Lactobacillus* and *Bifidobacterium* have beneficial effects by promoting weight loss and visceral adiposity reduction [21]. The previous studies have also shown that synbiotics reduces appetite, food intake, and, therefore, body weight [22–24]. However, in our study, improvements in glucose and insulin metabolism occurred during supplementation with probiotic/synbiotic without any changes in food ingestion or weight.

It has been suggested that probiotics/synbiotics intake may improve markers of insulin metabolism by reducing cytokines and suppressing the nuclear factor kappa-light-chain-activator-enhancer of the B-cell pathway [25]. The results of a recent meta-analysis revealed that synbiotic supplementation can significantly reduce HOMA-B in diabetic patients [26]. However, in our study, homeostatic model assessment-B-cell function (HOMA-B) remained unchanged during probiotic or synbiotic supplementation. This result is consistent with the prior observation of Triplot et al. [27] that have reported that 12 weeks of supplementation with

Table 3 Biochemical characteristics at the baseline, after 12 and 24 weeks of intervention in probiotic group, synbiotic group, and placebo group

	Groups	At baseline	At 12 weeks	At 24 weeks	*P* value time effect	*P* value time × group	*P* value between groups	Observed power	Post-hoc group[a]
HbA1C (%)	Probiotic	5.68 ± 0.4	5.53 ± 0.3	5.56 ± 0.3	0.068	0.046	0.004	0.86	G1/G3
	Synbiotic	5.72 ± 0.4	5.59 ± 0.4	5.57 ± 0.4	< 0.001				G2/G3
	Placebo	5.70 ± 0.4	5.69 ± 0.4	5.77 ± 0.5	0.46				
Fasting plasma glucose (mg/dL)	Probiotic	107.19 ± 7.6	104.15 ± 6.9	100.70 ± 7.7	0.003	0.07	0.01	0.78	G2/G3
	Synbiotic	107.93 ± 8.5	99.81 ± 10.3	101.36 ± 8.9	< 0.001				
	Placebo	104.56 ± 8.2	104.12 ± 7.8	103.68 ± 8.9	0.89				
Fasting insulin levels (μIU/mL)	Probiotic	14.98 ± 8	13.90 ± 7.1	13.11 ± 6.2	0.24	0.76	0.028	0.67	G2/G3
	Synbiotic	14.55 ± 6.7	12.01 ± 5.4	11.90 ± 5.6	0.009				
	Placebo	15.28 ± 6.4	15.31 ± 7	14.42 ± 6.5	0.24				
QUICKI	Probiotic	0.320 ± 0.02	0.321 ± 0.02	0.332 ± 0.02	0.2	0.5	0.006	0.83	G2/G3
	Synbiotic	0.319 ± 0.02	0.328 ± 0.02	0.331 ± 0.02	< 0.001				
	Placebo	0.316 ± 0.01	0.316 ± 0.02	0.320 ± 0.02	0.34				
HOMA-IR	Probiotic	3.85 ± 1.9	3.49 ± 1.7	3.28 ± 1.6	0.085	0.45	0.007	0.82	G2/G3
	Synbiotic	3.81 ± 1.7	3.10 ± 1.5	3.02 ± 1.7	0.002				
	Placebo	3.80 ± 1.3	4.11 ± 1.9	3.71 ± 1.8	0.38				
HOMA-B	Probiotic	118.76 ± 62	123.6 ± 74	126.9 ± 60	0.88	0.25	0.8	–	–
	Synbiotic	120.76 ± 49	122.7 ± 49	112.5 ± 43	0.3				
	Placebo	132.27 ± 61	135.3 ± 53.6	131 ± 59	0.4				

p value < 0.05 is considered as significant

[a] *G1* probiotic group ($n = 27$), *G2* synbiotic group ($n = 30$), *G3* placebo group ($n = 28$); all presented *P* values are based on repeated measures ANOVA

probiotics failed to enhance β-cell function in subjects with the metabolic syndrome. With this in mind, it is reasonable to suggest that probiotics/synbiotics could be used more effectively in the prevention, rather than in the treatment, of glycemic disorders in prediabetic subjects.

The accurate mechanisms of how probiotics/synbiotics exert their stimulatory effects on glycemic metabolism remain still unclear. We know that probiotics and synbiotics can improve the composition of gut microbiota and it has been demonstrated that gut microbiome imbalances affect a number of organs including adipose tissue, skeletal muscle, and the liver which further exacerbates insulin resistance and glycemic imbalances. Insulin resistance can promote progression of metabolic syndrome and eventually type 2 diabetes mellitus, and it represents a factor contributing to hyperglycemia [28].

The gut microbiota interacts with host metabolism leading to insulin resistance and type 2 diabetes through several probable mechanisms including alteration energy homeostasis, induction glucose metabolism, and low-grade inflammation. Although the underlying mechanisms for the insulin resistance development remain unclear, a popular theory is that bacterial lipopolysaccharides (LPSs) derived from the outer membranes of Gram-negative bacteria have been known to induce metabolic endotoxemia by promoting secretion of pro-inflammatory cytokines [29]. LPSs bind to and activate the TLR4/CD14 complex, which activates pro-inflammatory pathways and resistance to insulin [30].

The gut microbiota may exert effects on glucose metabolism through an altered intestinal integrity of the gut epithelial wall which reduces LPS levels. It has been observed that selectively increasing the abundance of *Bifidobacterium* spp., through supplementation, reduces intestinal permeability in animal models [31, 32].

Gut microbiota also influences energy and glucose metabolism through the production of short-chain fatty acids (SCFAs). Prior articles have reported that inclusion of prebiotic substances like inulin can stimulate the growth or metabolic activity of some bacterial groups including *lactobacillus* or *Bifidobacterium* and might increase production of SCFA in the colon [33–36].

These findings point to beneficial effects of SCFAs on metabolism and propose novel targets of using synbiotics for prevention and treatment of type 2 diabetes [37].

Another probable pathway mediating the crosstalk between the gut microbiota and glucose homeostasis is that *Lactobacillus* and *Bifidobacterium* can transform primary bile acids into secondary bile acids which could be activated GLP1 secretion from the intestinal L cells [37, 38].

An interesting observation in our study was that synbiotic and probiotic supplementation was associated with improvement in HbA1C, fasting plasma glucose, fasting insulin

levels, HOMA-IR, and QUICKY score among participants with prediabetes in both 12 and 24 weeks of intervention periods.

Up to now, knowledge about the long-term efficacy of probiotic/synbiotic administration has been still lacking and the intervention period of most of the previous trials to observe the effects of these supplements on metabolic disorders has been less than 3 months [21].

In a systematic review, it has been demonstrated that probiotics and synbiotics may be suggested as supplements to improve metabolic disorders when administered for a period ≥ 8 weeks [26].

Our study has been shown that 12-week period is sufficient for probiotic or synbiotic administration to observe their effects on glycemic improvements in prediabetic subjects.

Strengths

Some of the important strengths of the present study include its relatively long duration, its randomized double-blind design, comparison of prebiotic and synbiotic and the inclusion of prediabetic subjects who are at increased risk of metabolic diseases.

In addition, discrepancies in the composition of the three groups in this study were minimized because of the good definition of the population by the inclusion and exclusion criteria.

Synthesis of the synbiotic was carried out for the first time in Iran during this study and the probiotics were derived from national dairy products.

The present study was the first to test the effects of our synthetic synbiotic, consisting of *L. acidophilus, B. lactis, B. bifidum*, and *B. longum* plus Inulin in humans. Overall, this novel synbiotic was well tolerated without any serious side effect by the volunteers.

Limitation

Despite our weekly follow-up by the phone call and clinic visits, the dietary and physical activity assessment in this study relied only on subjective reports which are not as accurate as objective methods for measuring their compliance.

Conclusion

Oral intake of probiotics and especially synbiotics as co-adjuvants for the glycemic control in individuals with prediabetes is partially supported by the data shown in the present study. However, further studies are required to understand the precise mechanism of how probiotics and synbiotics affect these metabolic disorders.

Finally, it should be recognized that more clinical trials addressing optimal nutraceutical compositions aimed at preventing or minimizing clinical consequences of hyperglycemia and insulin resistance are needed for optimal recommendations in this important area of patient treatment and prevention.

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Author contributions MA, AA, AF, and NK conceptualized the study and are the writing group. NK, MTE, and PJ were main researchers. AF and NK developed the statistical design and analysis. All of the authors have seen and approved the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no other competing interests.

Ethical approval This study has been reviewed by the ethics committee of Isfahan University of Medical Sciences and was in accordance with the ethical standards in the last version of Declaration of Helsinky.

Informed consent All persons gave their informed consent prior to inclusion in the study.

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