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Effects of specific symbiotic supplements on anthropometric measurements, glycaemic control, and lipid profiles among individuals with type 2 diabetes mellitus in two teaching hospitals in Baghdad/Iraq: a double-blinded, randomised placebo-controlled trial

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Abstract

Background Experimental and clinical studies have suggested that symbiotics might effectively manage type 2 diabetes mellitus (T2DM) by modulating the intestinal microbiota. However, these studies' limited sources, small sample sizes, and varied study designs have led to inconsistent outcomes regarding glycaemic control. This study aimed to investigate the effects of symbiotics on the anthropometric measures, glycaemic control, and lipid profiles of patients with T2DM.

Methods A double-blind, placebo-controlled, parallel clinical trial was conducted at two diabetes outpatient clinics. The main researcher and participants were blinded to the capsule content throughout the study. Sixty-six patients with T2DM aged 30–75 years were randomly allocated, using even and odd numbers, into two equal groups. These groups received either symbiotic capsules containing 200 million colony-forming units plus fructo-oligosaccharide or a placebo for 12 weeks. The primary objective was a decrement in glycated haemoglobin [HbA1c]. The patients' anthropometric measures, fasting blood sugar, high-density lipoprotein [HDL], low-density lipoprotein [LDL], total serum cholesterol and serum triglyceride levels were also assessed at baseline and after 12 weeks of intervention. Non-parametric tests were used for statistical analyses.

Results Within-group analysis revealed significant decreases in body mass index (BMI) and waist circumference ($P=0.005$ and 0.023, respectively) and a significant increase in HDL levels in the symbiotic group ($P=0.04$). HbA1c levels significantly increased in the placebo group ($P=0.016$) but were not significantly reduced in the symbiotic group. The between-group analysis revealed significantly lower fasting blood sugar (FBS) levels in the symbiotic group, and higher in the placebo group ($P=0.02$). No significant changes existed in total serum cholesterol, LDL, and triglyceride levels in either the symbiotic or placebo group.

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Conclusions Symbiotics improve BMI, waist circumference, HDL, and FBS levels and prevent the worsening of HbA1c levels in patients with T2DM. Our preliminary results indicate the potential benefits of symbiotics in patients with T2DM, which may lead to better diabetes control. However, this evidence requires further assessment in larger trials.

Trial registration The trial was registered retrospectively at the International Standard Registered Clinical/Social Study Number Registry (ISRCTN34652973) on 05/01/2024.

Keywords Type 2 diabetes mellitus, Symbiotics, Anthropometric measurement, Glycaemic control, Lipid profiles

Background

Type 2 diabetes (T2D) is the most prevalent type of diabetes, accounting for approximately 90% of all cases worldwide. According to the International Diabetes Federation Diabetes Atlas ninth edition 2021, an estimated 537 million adults aged 20–79 years live with diabetes, representing 9.3% of the world's population. Iraq is one of the 21 countries and territories in the Middle East and North Africa region where one in six adults (73 million) live with diabetes [1].

The Food and Agriculture Organization of the United Nations and the World Health Organization (WHO) have defined probiotics as live microorganisms that, when administered in adequate amounts, confer health benefits to the host [2]. A prebiotic is a substrate selectively utilised by host microorganisms conferring health benefits [3]. The International Scientific Association of Probiotics and Prebiotics states that a symbiotic is a mixture comprising live microorganisms and substrate(s) selectively used by host microorganisms that confers a health benefit on the host [4].

Several studies have demonstrated a significant association between the composition of the gut microbiota and gut microbial metabolites in the development of obesity and diabetes [5]. Evidence from experimental and clinical studies supports the idea that modulation of the intestinal microbiota by probiotics may be effective in the prevention and management of type 1 diabetes and T2DM [6]. A systematic review and meta-analysis of 105 articles, representing 6,826 participants, revealed that probiotics induced improvements in body weight, body mass index (BMI), and waist circumference (WC) in individuals who were overweight and had T2DM. Furthermore, they reduced fasting blood glucose (FBG) and glycated haemoglobin (HbA1c) levels and homeostatic model of insulin resistance, with most improvements observed in mixtures containing bifidobacteria (*Bifidobacterium breve*, *Bifidobacterium longum*), *S. salivarius* subspecies, *Streptococcus thermophilus*, and lactobacilli (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbrueckii*), and influenced by trials conducted in one country [7].

However, the limited sources, small sample sizes, and differing designs of these studies have led to inconsistent outcomes regarding glycaemic control, pancreatic islet function, changes in gut microbiota composition, and other indicators. Therefore, future research should be conducted to provide favourable evidence for the use of probiotics [8].

Dietary fibre may influence gut microbiota composition and activity, thereby modulating the risk of metabolic diseases, obesity, insulin resistance and T2DM, considering its prebiotic effect on fibre-fermenting bacteria, which may increase the production of short-chain fatty acids, including butyrate, acetate and propionate, Butyrate is thought to be a major factor in preventing and treating diabetes owing to its ability to inhibit histone deacetylase [9].

Although cross-sectional studies have suggested an association between unfavourable disturbances in the gut microbiota and obesity or T2DM, NG49 guidelines from the National Institute for Health and Care Excellence (NICE) do not recommend probiotics as adjuvant therapy for T2DM and recommended more research [10]. However, NICE encourages adults with T2DM to consume high-fibre foods, such as fruits, vegetables, whole grains, and pulses (NG28), which are sources of prebiotics [11].

Symbiotic adjunction may help overcome some of the T2DM treatment's challenges such as chronic adverse effects, high cost of newer medications, and patients' low self-efficacy [12]. Probiotic, prebiotic, symbiotic supplementation improved glucose homeostasis in patients with diabetes [13], potentially decreasing the percentage of patients with uncontrolled T2DM.

This study aimed to investigate the effects of symbiotic supplements in Iraqi patients with T2DM. The primary hypothesis predicted better management of T2DM with symbiotic supplementation, reflected by a decrement in HbA1c after 12 weeks of interventions as the primary objective, with secondary outcome reflected by improvement of anthropometric measures, FBS and lipid profiles.

Methods

Clinical trial design

A double-blind, placebo-controlled, parallel clinical trial was conducted. The manuscript was written according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines [14].

The sample size was defined using a specific formula [15] as follows: $n = [(Z\alpha/2 + Z\beta)^2 \times \{2(Ó^2\}]/(M1\text{-}M2)^2$, where n = sample size, M1 = mean change from baseline for drug, M2 = mean change from baseline for placebo and Ó = standard deviation from reference articles [16, 17]; HbA1c was the key variable used in determining sample size with a difference of 0.9%. The power of the study was set at 80%, and the level of significance was 5%. The sample size required per group was calculated to be 26. Considering a dropout rate of 20%, the total sample size required was 64 (32 in each group) (Fig. 1) [14]. We defined a clinically significant HbA1c decrease as an HbA1c difference of ≥ 0.5% between baseline and the last available HbA1c concentration, according to the NICE guideline, the analysis by *Lameijer* et al. and Tyndall et al. [17, 18].

Study setting

This study was conducted at two diabetes outpatient clinics, Al-Imameen Al-Kadhimin Medical City and Al-Yarmouk Teaching Hospital, both teaching hospitals in Al-Karkh District of Baghdad, Iraq. This study was advertised on social media platforms, such as Facebook and Instagram to recruit participants. The posts were published and shared to attract eligible participants. All patients who visited the aforementioned outpatient

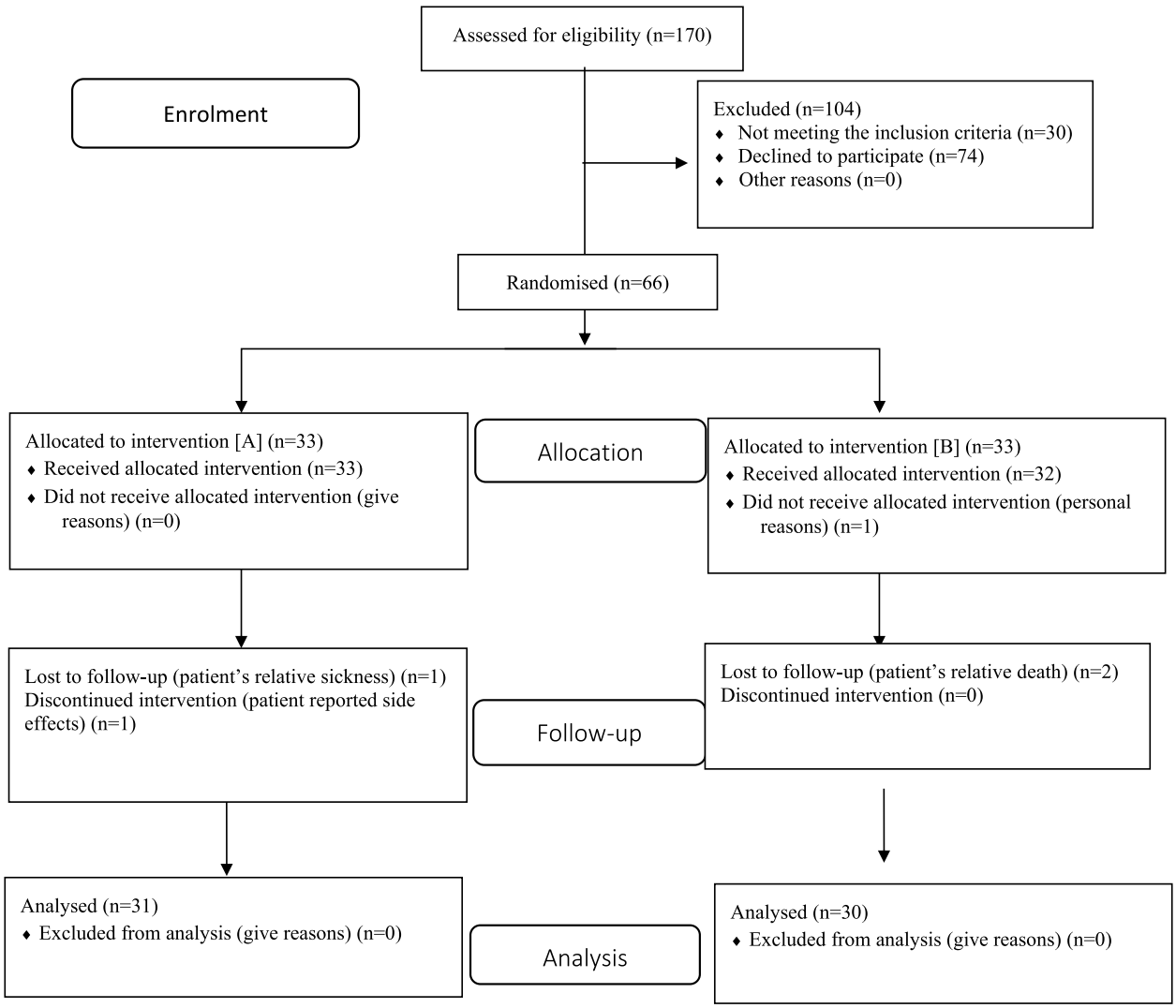

Fig. 1 Consolidated Standards of Reporting Trials 2010 flow diagram for the study

clinics and met the eligibility criteria were invited to participate during the recruitment period from 1 September 2021 to 30 June 2022.

Interventions

To ensure allocation concealment and blinding, the placebo and symbiotic supplements were identically packaged, and the main researchers and participants were blinded to the capsule content throughout the study procedure and final analysis. The symbiotic and placebo packs were identical in appearance and differentiated only by the code (A or B) placed on them. Simple randomisation was used for equal allocation to two parallel groups, with odd and even numbers used for allocation. Patients who visited the outpatient clinics on even-numbers interview dates received packages labelled A, and vice versa. The allocation ratio was set to 1:1.

This study was conducted among 66 individuals with diabetes who were randomly allocated into two groups of 33 each, to receive either a daily dose of Protexin® BALANCE capsule which contains symbiotics in the form of 200 million CFUs of *L. casei* PXN 37, *Lactobacillus rhamnosus* PXN 54, *S. thermophilus* PXN66, *L. acidophilus* PXN 35, *B. breve* PXN 30, *B. longum* PXN 30, *Lactobacillus bulgaricus* PXN 39 and fructo-oligosaccharide (FOS) ($n=33$) or placebo ($n=33$) for 12 weeks. A systematic review and meta-analysis found significant differences in the interspecies probiotic doses assayed, where the daily minimum and maximum doses varied from 1×10^8 colony-forming unit (CFU)/day to 1.35×10^{15} CFU/day, respectively, and the time of administration ranges from 4 to 24 weeks. Despite this variation, at least one or several key clinical data points (BMI, lipid parameters) were modulated, thereby discretely improving the outcomes pursued in participants with metabolic diseases [19].

The American Diabetes Association (ADA) recommends that HbA1C and lipid profile be measured every three months to assess whether patients' glycaemic targets have been reached and maintained [20]; therefore, a 12-week duration was set to observe the effect of the intervention on the variables.

The placebo was prepared locally with the help of a pharmacist by adding approximately 200 mg of starch to empty capsules, which were then properly sealed and packaged, similar to symbiotic capsules. All participants were instructed to take two capsules orally daily with lunch and not alter their routine physical activity and usual diet.

Methods

Anthropometric measures

The patients' heights were measured using a non-stretchable tape, with 0.1 cm accuracy, whereas body weights were measured using a digital floor scale without shoes and with minimum clothing [21]. BMI was determined by dividing body weight in kilograms by height squared in metres [22], and WC was assessed at baseline and after 12 weeks of intervention [21, 23].

Biochemical analysis

Glycated haemoglobin [HbA1c] and fasting blood samples (fasting blood sugar [FBS], high-density lipoprotein [HDL], low-density lipoprotein [LDL], total cholesterol [TC] and serum triglyceride [TG]) were collected at baseline and after 12 weeks of intervention [24, 25].

Compliance was assessed through weekly phone interviews or, more frequently, if required, and patients were asked about any side effects. Face-to-face interviews were conducted at the end of 12 weeks, and patients were instructed to bring their capsule packages for the pill count.

Participants

The eligibility criteria were as follows:

1. Individuals with T2DM, according to the criteria of the American Diabetes Association [26] for at least 10 months before study initiation
2. Individuals aged 30–75 years
3. Individuals with HbA1c level $\geq$ 6.5%
4. Individuals able to provide informed consent

The exclusion criteria were as follows:

1. Individuals who were current smokers.
2. Individuals with immunocompromised conditions
3. Individuals with diabetes controlled by insulin
4. Individuals who were pregnant or breastfeeding
5. Individuals with inflammatory bowel disease; pancreatitis; chronic kidney, hepatic, and pulmonary diseases; severe anaemia; and cancer
6. Individuals using nutritional supplements, laxatives, or nonsteroidal anti-inflammatory drugs in the past 3 weeks
7. Individuals using antibiotics within the past 3 months before the study initiation

Ethical consideration

This study was approved by the Arab Board Committee and the Iraqi Ministry of Health and Environment/Baghdad Health Directorate Al-Karkh/Training and Human Development Center Research Committee (research protocol number: 2021034 on 25 July 2021, decision number: 34, www.khdb.gov.iq). This study was conducted based on the ethical guidelines of the Helsinki Declaration.

Written consent was obtained from each participant after the main researcher described the study procedures. The WHO template for informed consent was translated into Arabic and personalised for our study [27].

We affirm that this manuscript is an honest, accurate, and transparent account of this study.

Statistical analyses

The collected data were imported into Microsoft Excel 2010 and analysed using the Statistical Package for the Social Sciences version 26. Non-parametric tests, specifically the Mann–Whitney and Wilcoxon signed-rank tests, were used in lieu of the independent two-sample t-test and paired t-test, respectively, for non-normally distributed data. A p-value of < 0.05 was considered as a discrimination point for significance.

Table 1 Age, diabetes mellitus duration, and biochemical and anthropometric distributions of studied groups before treatment

Variable	Placebo (30) Mean ± SD	Symbiotic (31) Mean ± SD	P^* value
Age/year	59.06 ± 9.75	59.77 ± 10.61	0.53
Duration/year	8.26 ± 6.76	9.51 ± 5.77	0.25
BMI (kg/m^2)	29.24 ± 3.89	30.18 ± 4.10	0.24
WC (cm)	105.70 ± 11.98	104.87 ± 10.23	0.94
HbA1c (mg/dl)	7.67 ± 1.35	7.62 ± 1.45	0.83
FBS (mg/dl)	133.77 ± 24.98	144.44 ± 66.55	0.82
LDL (mg/dl)	109.87 ± 35.02	114.30 ± 45.06	0.97
HDL (mg/dl)	44.38 ± 12.96	41.08 ± 9.46	0.45
TC (mg/dl)	179.28 ± 44.95	189.18 ± 47.89	0.51
TG (mg/dl)	165.32 ± 80.42	209.50 ± 104.86	0.046

WC waist circumference, *TC* total cholesterol, *TG* total triglyceride, *SD* standard deviation

FBS fasting blood sugar, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *BMI* body mass index

* Mann–Whitney U test

Results

This study enrolled 66 patients with T2DM (34 females and 32 males). Five patients withdrew from this study for various reasons, leaving a final cohort of 61 patients (30 in the placebo group and 31 in the symbiotic group). Only one patient reported significant abdominal pain and flatulence after symbiotic use (Fig. 1) [14].

The age ranges of the participants in the placebo and symbiotic groups were 39–70 and 30–75 years, respectively. No significant differences were found in the mean age between the two groups ($P = 0.53$). The mean and standard deviation of diabetes duration were 8.26 ± 6.76 years (minimum duration: 11 months) and 9.51 ± 5.77 (minimum duration: 10 months) in the placebo and symbiotic groups, respectively (Table 1).

No significant differences in sex, treatment, fasting status, and diabetes duration were found between the placebo and symbiotic groups ($p > 0.05$, Table 2).

Based on the biochemical findings between the two studied groups before treatment, no significant differences were found between the groups, except for the TG level, which was significantly higher in the placebo group compared with the symbiotic group ($p = 0.046$). After treatment, no significant differences were found between the groups, except for the FBS level, which significantly decreased in the symbiotic group but increased in the placebo group ($p = 0.02$, Table 3).

Table 2 Demographic distribution of studied groups

Demographic variables		Groups				P
		Placebo		Symbiotic		
		N	%	N	%	
Sex	Male	15	50.0	14	45.2	0.80**
	Female	15	50.0	17	54.8	
Treatment	Monotherapy	14	46.7	9	29.0	0.15*
	Dual therapy	9	30.0	17	54.8	
	Triple therapy	7	23.3	5	16.1	
Fasting status	Yes	26	86.7	28	90.3	0.71**
	No	4	13.3	3	9.7	
Duration	≤ 10 years	21	70.0	19	61.3	0.59**
	> 10 years	9	30.0	12	38.7	
Total		30	100.0	31	100.0	

*Chi-squared2 test

**Fisher's exact test

Table 3 Mean biochemical and anthropometric distributions of the studied groups after treatment

Variables	Placebo group		Symbiotic group		*P* value*
	Mean	SD	Mean	SD	
BMI (kg/m^2)	28.24	5.82	28.73	6.81	0.31
WC (cm)	105.60	11.88	103.11	9.66	0.50
HbA1c (mg/dl)	7.93	1.35	7.24	1.50	0.52
FBS (mg/dl)	135.55	25.45	128.10	44.43	0.02
LDL (mg/dl)	105.78	35.19	109.32	46.89	0.09
HDL (mg/dl)	43.46	12.93	44.82	9.64	0.99
TC (hg/dl)	179.70	46.91	192.96	53.96	0.29
TG (mg/dl)	163.92	69.28	193.39	108.65	0.52
Test	***Mann–Whitney U test**				

WC waist circumference, *TC* total cholesterol, *TG* total triglyceride, *FBS* fasting blood sugar, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *BMI* body mass index

Table 4 shows the changes in biochemical and anthropometric measures before and after placebo and symbiotic treatment (within-group analysis). HbA1c levels significantly increased after placebo treatment ($p=0.016$). However, BMI, WC, LDL, HDL, and TG levels decreased after placebo treatment, and the differences were not significant ($p>0.05$).

FBS and TC concentrations increased in the placebo group, but the changes were not significant ($p>0.05$).

BMI and WC significantly decreased after symbiotic treatment ($p=0.005$ and $p=0.023$, respectively), whereas HDL levels significantly increased after symbiotic treatment ($p=0.04$).

TC concentrations increased in the symbiotic group, but the increase was not significant ($p=0.44$).

Although HbA1c, FBS, LDL, and TG levels decreased after symbiotic treatment, the differences were not statistically significant ($p>0.05$).

After testing the effect of certain variables such as sex, the mode of therapy patients were following, whether they were fasting during the trial duration, and the duration of their T2DM. No significant differences were observed in sex, type of treatment, fasting status, diabetes duration, or HbA1c levels after treatment with symbiotics (Table 5).

We tested the effect size of the study's significant results in the placebo and symbiotic groups (Table 6). The WC decrement after symbiotic treatment and the HbA1c increment after placebo treatment were negligible, even though they were statistically significant, as the difference between the two means was < 0.2 SD.

Discussion

This study aimed to assess the effects of symbiotic supplements consisting of seven bacterial strains at a dose of 2×10^8 CFU/day and a prebiotic FOS in one capsule for 12 weeks. This study revealed that after symbiotic treatment, there was a significant decrease in BMI,

Table 4 Comparison of biochemical and anthropometric means of the symbiotic and placebo groups before and after treatment

Variables	Symbiotic (mean ± SD)				Placebo (mean ± SD)			
	Before	After	Z score	*P* value	Before	After	Z score	*P* value
BMI (kg/m$^{2)}$	30.18 ± 4.10	28.73 ± 6.81	−2.84	0.005	29.24 ± 3.89	28.24 ± 5.82	−0.88	0.38
WC (cm)	104.87 ± 10.23	103.11 ± 9.66	−2.29	0.023	105.70 ± 11.98	105.60 ± 11.87	−1.73	0.08
HbA1c (mg/dl)	7.62 ± 1.45	7.24 ± 1.50	−1.69	0.09	7.67 ± 1.35	7.93 ± 1.35	−2.41	0.016
FBS (mg/dl)	144.44 ± 66.55	128.10 ± 44.43	−1.71	0.088	133.77 ± 24.98	135.55 ± 25.45	−0.23	0.82
LDL (mg/dl)	114.30 ± 45.06	109.32 ± 46.89	−0.92	0.92	109.87 ± 35.02	105.78 ± 35.19	−0.46	0.65
HDL (mg/dl)	41.08 ± 9.46	44.82 ± 9.64	−2.06	0.04	44.38 ± 12.96	43.46 ± 12.93	−1.22	0.22
TC (mg/dl)	189.18 ± 47.89	192.96 ± 53.96	−0.44	0.44	179.28 ± 44.95	179.70 ± 46.91	−0.64	0.52
TG (mg/dl)	209.50 ± 104.86	193.39 ± 108.65	−0.45	0.45	165.32 ± 80.42	163.92 ± 69.28	−1.44	0.15

WC waist circumference, *TC* total cholesterol, *TG* total triglyceride, *SD* standard deviation

FBS fasting blood sugar, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *BMI* body mass index

* Wilcoxon signed-rank test

Table 5 Effects of sex, Mode of treatment, fasting status, and diabetes duration on HbA1c in the symbiotic group

Variables		HbA1c (mg/dl)		P*
		Mean	Standard deviation	
Sex	Male	7.48	1.83	0.63*
	Female	7.04	1.17	
Mode of treatment	Monotherapy	6.82	1.98	0.23**
	Dual therapy	7.39	1.29	
	Triple therapy	7.50	1.31	
Fasting status	Yes	7.33	1.55	0.32*
	No	6.43	.45	
Duration/year	≤ 10 years	7.56	1.56	0.09*
	> 10 years	6.74	1.31	

HbA1c, glycated haemoglobin

* Mann–Whitney U test

** Kruskal–Wallis test

Table 6 Cohen's d effect size of the difference between two means before and after intervention

Variables	Effect size
FBS	0.2
BMI	0.26
WC	0.18
HDL	0.4
HbA1c	0.03

Pooled standard deviation was used

HbA1c glycated haemoglobin, *FBS* fasting blood sugar, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *BMI* body mass index, *WC* waist circumference

waist circumference, and FBG levels, whereas HDL levels significantly increased. Conversely, HbA1c levels significantly increased in the placebo group. Before the intervention, HbA1c levels in the placebo group ranged from 6.5% to 11.47%, with 12 (40%) of patients having uncontrolled diabetes (HbA1c > 7.5%). After the intervention, HbA1c levels in the placebo group ranged from 6.5% to 11%, with 13 (43.3%) of patients still having uncontrolled diabetes. Similarly, before the intervention, HbA1c levels in the symbiotic group ranged from 6.5% to 12.73%, with 11 (35%) patients having uncontrolled diabetes. After the intervention, HbA1c levels ranged from 5% to 11.8%; however, the same number [11] and percentage (35%) remained uncontrolled which may explain the negligible effect size.

Cholesterol synthesis and absorption mainly occur in the intestine; therefore, the intestinal microflora profoundly affect lipid metabolism. Several mechanisms have been suggested for cholesterol reduction by probiotics, including the removal of cholesterol by assimilation during growth, the binding of cholesterol to the cellular surface by non-growing or dead *Lactococcus* cells, and the deconjugation of bile acids [28]. Additionally, the gut microbiome promotes energy absorption by enhancing the synthesis of triacylglycerols and inhibiting the oxidation of fatty acids, potentially affecting the energy balance of the human body, and leading to insulin resistance. The intestinal microflora produces several inflammatory mediators, such as lipopolysaccharides and branched-chain amino acids (BCAAs). BCAAs activate the body's immune response, whereas inflammatory mediators activate Toll-like receptor 4, reducing sensitivity to insulin [29].

A systematic review found fair evidence that interventions with prebiotics, especially oligofructose-enriched inulin, may improve metabolic and inflammatory biomarkers related to T2DM and reported improvements in glycaemia, and body weight [30].

Another review provided evidence from various studies on the ability of prebiotic consumption to alter gut microbial profile, improve gut microbial metabolism and function, and improve host physiology to alleviate diabetes and obesity. FOS shows great potential due to its prebiotic activity and low caloric value. Additionally, a diet supplemented with FOS promotes the production of butyrate, which influences lipid metabolism in humans [31].

Similar findings were reported in a meta-analysis by Dixon et al., which found statistically significant pooled effects of probiotics in reducing BMI and serum glucose levels and increasing HDL levels. However, in contrast to our findings, this study reported significant reductions in HbA1c and TC levels. Subgroup analysis revealed that the reduction was not significant when symbiotics were administered in capsule formulations and at doses $< 1.0 \times 10^9$ CFU, which is consistent with the approach used in our study. The reduction in LDL levels was apparent in predominantly female patient groups and those receiving higher dosages ($> 1.0 \times 10^9$ CFU) [32].

In contrast to our findings, Mo R et al. found that probiotic interventions reduced TC and LDL levels but exhibited no significant effects on HDL levels. The study reported that the effects of probiotics on decreasing TC and LDL levels were greater in younger patients (age < 50 years) and in single-strain probiotics, mainly *Lactobacillus plantarum*, whereas a mixture of *L. acidophilus* and *Bifidobacterium* spp. showed no significant beneficial effects. Notably, 83.9% of the participants in our study were aged ≥ 50 years, and our probiotic mixture did not contain this strain. Furthermore, compared with the consumption of probiotic capsules, the consumption of probiotics in fermented milk products resulted in a

more significant reduction in LDL levels [33]. Consistent with our findings, the two aforementioned meta-analyses did not demonstrate significant changes in the TG levels [31, 32].

In agreement with our study, the meta-analysis by Rittiphairoj et al. stated that probiotics reduced FBG more than the placebo or no-intervention groups and that there was some evidence of a reduction in HbA1c levels in the probiotic group, although this did not reach statistical significance. Their subgroup analysis found that the reduction in FBG levels was more pronounced in participants with FBG levels > 130 mg/dL than in their counterparts [34]. Notably, 60% of our participants had FBS levels ≤ 130 mg/dL, which may explain the non-significant reduction within the group and the significant reduction between the groups in our analysis. Another meta-analysis involving 237 and 235 participants in the treatment (probiotic yoghurt) and control (mostly conventional yoghurt) groups, respectively, found no effects of probiotic yoghurt on FBG and HbA1c levels in T2DM [35].

Razmpoosh et al. demonstrated a significant increase in the levels of HDL in the probiotic group, but no significant alterations were observed in TG and TC levels. They also observed a significant decrease in fasting plasma glucose (FPG) levels, consistent with our findings. However, they found no significant changes in anthropometric measurements, including weight, WC, and BMI [36]. Importantly, their study lasted for only 6 weeks, and they used Familact probiotics, which had different combinations and doses of probiotic strains than the probiotics we used.

Khalili et al. found that a daily capsule containing a minimum of 108 CFU of *L. casei* 01 for 8 weeks significantly decreased weight, BMI, WC, and FBS levels in the intervention group compared with the placebo group. These findings are in line with our results, despite the differences in supplementation [37]. A meta-analysis revealed that probiotic intake resulted in a significant improvement in serum levels of FBS and a non-significant improvement in HbA1c levels [38], which is congruent with our study.

Another meta-analysis included 13 randomised controlled trials involving 818 participants in eight countries in 2020. It revealed that participants who received multiple species of probiotics had a statistically significant reduction in FBS and TG levels. No significant differences were observed in HbA1c, LDL, HDL, and TC levels between the probiotic and control groups [39]. Subgroup analysis revealed that the effect of probiotic supplementation on TG was significant when participants' ages were ≤ 55 years and revealed that participants coming from eastern regions had higher HDL concentrations than those from the western regions after probiotic supplementation [39].

In 2016, Firouzi et al. examined the effects of multistrain probiotics in 136 Malaysian adults with T2DM. Sixty-eight participants consumed a probiotic powder sachet containing six viable strains (*L. acidophilus*, *L. casei*, *Lactococcus lactis*, *Bifidobacterium bifidum*, *B. longum*, and *Bifidobacterium infantis*) at a twice-daily dose of 30 billion CFUs. In contrast, the other 68 participants in the control group consumed a placebo powder sachet for 12 weeks. However, the patients were required to follow a prescribed diet, which may not reflect real-life consumption. They found that the HbA1c level decreased by 0.14% in the probiotic group and increased by 0.02% in the placebo group in the per-protocol analysis, whereas these changes were not significant in the intention-to-treat analysis [40]. This finding partly agrees with ours, as the HbA1c level decreased by 0.38 from the mean in the probiotic group and increased by 0.26 from the mean in the placebo group; however, these changes were only significant in the placebo group.

They also reported that the participants in the probiotic group experienced a decline in FPG levels, whereas those in the control group experienced an increase in FPG levels from baseline. However, these findings were not statistically significant, consistent with the within-group analysis [38]. Conversely, Asemi et al. found that both the probiotic and placebo groups experienced increased FPG levels from baseline [41].

Limitations of study

The dosage of FOS in the symbiotic supplement was not known in the intervention group.

This study was self-funded, limiting our ability to recruit a larger sample size or extend the study duration beyond 12 weeks.

Furthermore, this study was conducted in only two teaching hospitals on one side of Baghdad, potentially limiting the generalisability of the results.

Conclusions

Symbiotics improve BMI, WC, HDL and FBG levels and prevent the worsening of HbA1c levels in patients with T2DM. Our preliminary results indicate the potential benefits of using symbiotics as an adjuvant therapy in patients with T2DM who are on oral hypoglycaemic drugs, which may lead to better diabetes control. However, this requires further evaluation in larger clinical trials.

Abbreviations

BCAAs	Branched-chain amino acids
BMI	Body mass index
CFU	Colony-forming unit
FBG	Fasting blood glucose
HbA1c	Glycated haemoglobin
FBS	Fasting blood sugar
FPG	Fasting plasma glucose
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
NICE	National Institute for Health and Care Excellence
T2DM	Type 2 diabetes mellitus
WC	Waist circumference
WHO	World Health Organization

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Authors' contributions
SZ: main researcher, conceptualized the study's design, collected, analysed and interpreted patient data and contributed to writing the manuscript. BA: serve as the main supervisor and conceptualized the initial idea of the study. KF: contributed to the study design and participated in writing the manuscript, including revising and finalizing the content. ZZ: assisted in the study design, prepared and provided the placebo, and was responsible of blinding processp. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was approved by the Arab Board Committee and Iraqi Ministry of Health and Environment/Baghdad Health Directorate Al-Karkh/Training and Human Development Center Research Committee (research protocol number: 2021034 on 25 July 2021, decision number: 34, www.khdb.gov.iq). Written consent was obtained from each participant after the main researcher described the study procedures.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. IDF Diabetes Atlas 2021 | IDF Diabetes Atlas. https://diabetesatlas.org/atlas/tenth-edition. Accessed 14 AUG 2022.
2. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat rev Gastroenterol hepatol. 2014;11:506–14.
3. Gibson GR, Hutkins RW, Sanders ME, Prescott SL, Reimer RA, Gibson GR, et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Med Bio Commo. https://digitalcommons.unl.edu/foodsciefacpub. Accessed 15 Aug 2022.
4. Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley EMM, et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. Nat Rev Gastroenterol Hepatol. 2021;18:649–67 https://www.nature.com/articles/s41575-021-00440-6.
5. Meijnikman AS, Gerdes VE, Nieuwdorp M, Herrema H. Evaluating causality of gut microbiota in obesity and diabetes in humans. Endocr Rev. 2018;39:133–53 https://academic.oup.com/edrv/article/39/2/133/4772276. Accessed 15 Aug 2022.
6. Gomes AC, Bueno AA, De Souza RGMH, Mota JF. Gut microbiota, probiotics and diabetes. Nutr J. 2014;13:1–13 https://nutritionj.biomedcentral.com/articles/10.1186/1475-2891-13-60. Accessed 15 Aug 2022.
7. Koutnikova H, Genser B, Monteiro-Sepulveda M, Faurie JM, Rizkalla S, Schrezenmeir J, et al. Impact of bacterial probiotics on obesity, diabetes and non-alcoholic fatty liver disease related variables: a systematic review and meta-analysis of randomised controlled trials. BMJ Open. 2019;9:e017995 https://bmjopen.bmj.com/content/9/3/e017995. Accessed 15 Aug 2022.
8. Wang X, Yang J, Qiu X, Wen Q, Liu M, Zhou D, et al. probiotics, pre-biotics and synbiotics in the treatment of pre-diabetes: a systematic review of randomized controlled trials. Front Public Health. 2021;26: 645035.
9. Prasad KN, Bondy SC. Dietary fibers and their fermented short-chain fatty acids in prevention of human diseases. Mech Ageing Dev. 2018. https://pubmed.ncbi.nlm.nih.gov/30336163. Accessed 15 Aug 2022.
10. Recommendations for research | Non-alcoholic fatty liver disease (NAFLD): assessment and management | Guidance | NICE. https://www.nice.org.uk/guidance/ng49/chapter/Recommendations-for-research#probiotic-and-prebiotic-supplements. Accessed 15 Aug 2022.
11. Recommendations | Type 2 diabetes in adults: management | Guidance | NICE. https://www.nice.org.uk/guidance/ng28/chapter/Recommendations#dietary-advice-and-bariatric-surgery. Accessed 15 Aug 2022.
12. Sleath B, Carpenter DM, Blalock SJ, Davis SA, Hickson RP, Lee C, et al. Development of a new diabetes medication self-efficacy scale and its association with both reported problems in using diabetes medications and self-reported adherence. Patient Prefer Adher. 2016;7:1003–10 https://www.researchgate.net/publication/303849969_Development_of_a_new_diabetes_medication_self-efficacy_scale_and_its_association_with_both_reported_problems_in_using_diabetes_medications_and_self-reported_adherence.
13. Paul P, Kaul R, Harfouche M, Arabi M, Al-Najjar Y, Sarkar A, et al. The effect of microbiome-modulating probiotics, prebiotics and synbiotics on glucose homeostasis in type 2 diabetes: A systematic review, meta-analysis, and meta-regression of clinical trials. Pharmacol Res. 2022;185:106520.
14. Moher D, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, et al. CONSORT 2010 explanation and elaboration: Updated guidelines for reporting parallel group randomised trials. Int J Surg. 2012;10:28–55.
15. Sakpal TV. Sample size estimation in clinical trial. Perspect Clin Res. 2010;1:67 /pmc/articles/PMC3148614.
16. Madempudi RS, Ahire JJ, Neelamraju J, Tripathi A, Nanal S. Efficacy of UB0316, a multi-strain probiotic formulation in patients with type 2 diabetes mellitus: A double blind, randomized, placebo controlled study. PLoS One. 2019;14. https://pubmed.ncbi.nlm.nih.gov/31721790/. Accessed 29 Nov 2023.
17. Lameijer A, Fokkert MJ, Edens MA, Slingerland RJ, Bilo HJG, van Dijk PR. Determinants of HbA1c reduction with FreeStyle Libre flash glucose monitoring (FLARE-NL 5). J Clin Transl Endocrinol. 2020;22:100237 /pmc/articles/PMC7578738.
18. Tyndall V, Stimson RH, Zammitt NN, Ritchie SA, McKnight JA, Dover AR, et al. Marked improvement in HbA1c following commencement of flash glucose monitoring in people with type 1 diabetes. Diabetologia. 2019;62:1349–56 https://pubmed.ncbi.nlm.nih.gov/31177314.
19. López-Moreno A, Suárez A, Avanzi C, Monteoliva-Sánchez M, Aguilera M. Probiotic strains and intervention total doses for modulating obesity-related microbiota dysbiosis: a systematic review and meta-analysis. Nutrients. 2020;12:1921 https://www.mdpi.com/2072-6643/12/7/1921/htm.
20. Association AD. Standards of medical care in diabetes—2022 abridged for primary care providers. Clin Diabetes. 2022;40:10–38. https://doi.org/10.2337/cd22-as01.

21. Fryar CD, Gu Q, Ogden CL, Flegal KM. Anthropometric reference data for children and adults; United States, 2011–2014. 2016. https://stacks.cdc.gov/view/cdc/40572. Accessed 29 Nov 2023.
22. Calculator: Body mass index (BMI) for adults (Metric, Patient education) _ UpToDate. https://www.uptodate.com/contents/calculator-body-mass-index-bmi-for-adults-metric-patient-education. Accessed 29 Nov 2023.
23. Determining body composition in adults - UpToDate. https://www.uptodate.com/contents/determining-body-composition-in-adults. Accessed 29 Nov 2023.
24. Cardiovascular disease: risk assessment and reduction, including lipid modification. NICE guidline NG238. 2023.
25. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Lernmark Å, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clinical chemistry. 2011;1;57:el-47. https://diabetesjournals.figshare.com/articles/figure/Guidelines_and_Recommendations_for_Laboratory_Analysis_in_the_Diagnosis_and_Management_of_Diabetes_Mellitus/23519148. Accessed 17 Nov 2024.
26. American Diabetes Association. 2 Classification and diagnosis of diabetes. Diabetes Care. 2015;38:S8–16 https://diabetesjournals.org/care/article/38/Supplement_1/S8/37298/2-Classification-and-Diagnosis-of-Diabetes.
27. Research Ethics Review Committee. https://www.who.int/groups/research-ethics-review-committee/guidelines-on-submitting-research-proposals-for-ethics-review/templates-for-informed-consent-forms. Accessed 11 July 2021.
28. Lye HS, Kuan CY, Ewe JA, Fung WY, Liong MT. The improvement of hypertension by probiotics: effects on cholesterol, diabetes, renin, and phytoestrogens. Int J of Mol Sci. 2009;10:3755–75 https://www.mdpi.com/1422-0067/10/9/3755/htm.
29. He FF, Li YM. Role of gut microbiota in the development of insulin resistance and the mechanism underlying polycystic ovary syndrome: a review. J Ovarian Res. 2020;13:1–13 https://link.springer.com/articles/10.1186/s13048-020-00670-3.
30. Colantonio AG, Werner SL, Brown M. The Effects of Prebiotics and substances with prebiotic properties on metabolic and inflammatory biomarkers in individuals with type 2 diabetes mellitus: a systematic review. J Acad Nutr Diet. 2020;120:587–607.e2.
31. Megur A, Daliri EBM, Baltriukienė D, Burokas A. Prebiotics as a tool for the prevention and treatment of obesity and diabetes: classification and ability to modulate the gut microbiota. Int J Mol Sci. 2022;23:6097 https://www.mdpi.com/1422-0067/23/11/6097/htm.
32. Dixon A, Robertson K, Yung A, Que M, Randall H, Wellalagodage D, et al. Efficacy of probiotics in patients of cardiovascular disease risk: a systematic review and meta-analysis. Curr Hypertens Rep. 2020;22. https://pubmed.ncbi.nlm.nih.gov/32860083. Accessed 15 Aug 2022.
33. Mo R, Zhang X, Yang Y. Effect of probiotics on lipid profiles in hypercholesterolaemic adults: a meta-analysis of randomized controlled trials. Medicina Clínica (English Edition). 2019;152:473–81.
34. Rittiphairoj T, Pongpirul K, Janchot K, Mueller NT, Li T. Probiotics contribute to glycemic control in patients with type 2 diabetes mellitus: a systematic review and meta-analysis. Adv Nutr. 2021;12:722–34 https://academic.oup.com/advances/article/12/3/722/5944126.
35. Barengolts E, Smith ED, Reutrakul S, Tonucci L, Anothaisintawee T. The effect of probiotic yogurt on glycemic control in type 2 diabetes or obesity: a meta-analysis of nine randomized controlled trials. Nutrients. 2019;11:671 https://www.mdpi.com/2072-6643/11/3/671/htm.
36. Razmpoosh E, Javadi A, Ejtahed HS, Mirmiran P, Javadi M, Yousefinejad A. The effect of probiotic supplementation on glycemic control and lipid profile in patients with type 2 diabetes: A randomized placebo controlled trial. Diabetes Metabo Syndr. 2019;13:175–82.
37. Khalili L, Alipour B, Asghari Jafarabadi M, Hassanalilou T, Mesgari Abbasi M, Faraji I. Probiotic assisted weight management as a main factor for glycemic control in patients with type 2 diabetes: A randomized controlled trial. Diabetol Metab Syndr. 2019;11:1–9 https://dmsjournal.biomedcentral.com/articles/10.1186/s13098-019-0400-7.
38. Ardeshirlarijani E, Tabatabaei-Malazy O, Mohseni S, Qorbani M, Larijani B, Baradar Jalili R. Effect of probiotics supplementation on glucose and oxidative stress in type 2 diabetes mellitus: a meta-analysis of randomized trials. DARU J Pharm Sci. 2019;27:827–37 https://link.springer.com/article/10.1007/s40199-019-00302-2.
39. Liang T, Wu L, Xi Y, Li Y, Xie X, Fan C, et al. Probiotics supplementation improves hyperglycemia, hypercholesterolemia, and hypertension in type 2 diabetes mellitus: an update of meta-analysis. Crit Rev Food Sci Nutr. 2020;61:1670–88 https://www.tandfonline.com/doi/abs/10.1080/10408398.2020.1764488.
40. Firouzi S, Majid HA, Ismail A, Kamaruddin NA, Barakatun-Nisak MY. Effect of multi-strain probiotics (multi-strain microbial cell preparation) on glycemic control and other diabetes-related outcomes in people with type 2 diabetes: a randomized controlled trial. Eur J Nut. 2016;56:1535–50. Available from: https://link.springer.com/article/10.1007/s00394-016-1199-8.
41. Asemi Z, Zare Z, Shakeri H, Sabihi SS, Esmail Zadeh A. Effect of multispecies probiotic supplements on metabolic profiles, hs-CRP, and oxidative stress in patients with type 2 diabetes. Ann Nutr Metab. 2013;63:1–9 https://www.karger.com/Article/FullText/349922.

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