Future Foods 12 (2025) 100679

Contents lists available at ScienceDirect

Future Foods

journal homepage: www.elsevier.com/locate/fufo

Fermented dairy product could improve glucose homeostasis in patients diagnosed with type 2 diabetes mellitus: A randomized controlled trial

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ARTICLE INFO

Keywords:
Type 2 diabetes mellitus
Fermented dairy
Probiotics
Bifidobacterium animalis ssp.lactis b420
glucose homeostasis

ABSTRACT

Type 2 diabetes mellitus (T2DM) is a global health issue linked to metabolic diseases and intestinal microbiota. Probiotic use through fermented dairy products is gaining attention for T2DM management, though clinical guideline adoption faces challenges in dosage, strain selection, and regulation. This study evaluated the impact of fermented dairy products both with conventional cultures and supplemented with *Bifidobacterium animalis* subsp. *lactis* B420 on glucose homeostasis in T2DM patients. Forty-six participants were randomized into three groups: ADA nutritional recommendations+300 g/day of a fermented dairy with *Bifidobacterium animalis* ssp.*lactis* B420 (PPY); ADA nutritional recommendations+conventional yoghurt (CY); ADA nutritional recommendations without fermented foods (NON). Anthropometric and biochemical data were collected at baseline and after 12 weeks. The PPY group showed an 11.4 % reduction in HbA1c ($p = 0.021$), while the CY group had an 8.5 % reduction ($p = 0.06$); only the PPY reduction was statistically significant in comparison to the NON group. Fasting glucose levels also decreased significantly in both PPY ($p = 0.016$) and CY ($p = 0.043$) groups. In conclusion, consuming 300 g/day of fermented dairy products (PPY and CY) as part of a healthy diet appears to be an effective strategy for managing T2DM. The addition of *Bifidobacterium animalis* ssp. *lactis* B420 may further enhance these effects.

1. Introduction

An estimated 537 million adults aged 20-79 years suffer from type 2 diabetes mellitus (T2DM), and according to the International Diabetes Federation (IDF), the global prevalence of this disease is set to increase to 783 million by 2045 (Federation, 2021), with high morbidity and mortality rates (Federation, 2021).

Adherence to healthy lifestyles is one of the main ways of managing T2DM, where diet is one of the key factors in prevention and treatment (Koenigsberg and Corliss, 2017). Thus, the consumption of healthy and/or functional foods has emerged as a potential tool to complement the effect of diet and drug therapy, leading to an improvement in clinical parameters associated with glucose homeostasis, or even achieving disease remission. In this context, the consumption of probiotics through fermented dairy products has gained increasing interest for preventing and managing T2DM. This is based on the hypothesis that specific probiotics may help modulate the intestinal microbiota, thereby regulating the biological processes linked to the disease (Chen et al., 2021;

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https://doi.org/10.1016/j.fufo.2025.100679
Received 6 February 2025; Received in revised form 30 May 2025; Accepted 8 June 2025
Available online 9 June 2025

Hills et al., 2019; Umirah et al., 2021). However, in conventional fermented dairy products, probiotic cultures are mainly incorporated for technological purposes, specifically for the fermentation process, rather than to provide direct health benefits to the consumer (Ranadheera et al., 2017). While some of these strains may exert functional effects, their inclusion is not guided by therapeutic intent (Sanders et al., 2019) and since the beneficial effects of probiotics are strain-specific and highly dependent on the host and clinical context (Kechagia et al., 2013), well-designed randomized controlled trials in populations with T2DM and with well-characterized probiotic strains are essential (Ejtahed et al., 2011; Marco et al., 2017). Likewise, regulatory authorities, such as the European Food Safety Authority (EFSA) and the US Food and Drug Administration (FDA), emphasize the need for strain characterization and clinical evidence to substantiate health claims for probiotics (Administration, 2020; EFSA on Dietetic Products, 2011).

One of the most promising strains for use in metabolic diseases is *Bifidobacterium animalis* ssp. *lactis* B420, which has been observed to induce an increase in *Akkermansia muciniphila* in obese and T2DM subjects, thus demonstrating its potential beneficial effect (Depommier et al., 2019; Stenman et al., 2015, 2014; Yang et al., 2021). Moreover, the effects of *B. animalis* ssp. *lactis* B420 have been investigated in both in vitro and in vivo models. In particular, studies conducted in animal models have demonstrated its beneficial impact on lipid metabolism, inflammation and gut microbiota profile (Amar et al., 2011; Stenman et al., 2015, 2014). In human studies, although *B. animalis* ssp. *lactis* B420 is an industrially available strain widely used in the development of consumer health products, its evaluation has been limited to encapsulated forms rather than when included in fermented dairy matrices. Very few studies have specifically targeted individuals with T2DM; nonetheless, a placebo-controlled, double-blind, randomized clinical trial demonstrated that a five-week intervention with *B. animalis* ssp. *lactis* B420 resulted in significant improvements in plasma lipid profiles among healthy subjects (Klein et al., 2008). These findings suggest that adding *B. animalis* ssp. *lactis* B420 to a fermented dairy matrix may offer potential benefits for glucose homeostasis, while also providing a more accessible, and adherence-friendly vehicle for individuals requiring dietary management of T2DM.

Our aim, therefore, was to study the effect of the consumption of fermented dairy products with conventional probiotic cultures and supplemented with *Bifidobacterium animalis* ssp. *lactis* B420 on glucose homeostasis in patients diagnosed with T2DM, in order to generate knowledge to support the use of fermented dairy products for disease management in clinical practice.

2. Materials and methods

2.1. Participants

The present study included participants aged 25 to 65 years with T2DM diagnosed from 1 to 5 years before the beginning of the study with metformin and statins treatment, and without cardiovascular disease. The exclusion criteria included the use of any additional pharmacological treatments other than metformin and statins, as well as the presence of comorbid conditions, including kidney disease, liver disease, inflammatory bowel disease, thyroid disorder, and without the use of insulin, oestrogen or progesterone. Participants were also excluded if they had consumed probiotic supplements and/or antibiotics two months prior to the beginning of the study, smokers, or lactose intolerant. All information was obtained from patient records from the Endocrinology Service, where physicians also conducted a preliminary clinical evaluation to screen for inclusion and exclusion criteria. The recruitment of participants took place between November 2020 and January 2021, at the Endocrinology and Nutrition Service of the Clinics Hospital, Asunción, Paraguay.

Based on the only human clinical trial available at the time of study design that evaluated the effects of probiotic-enriched yoghurt in individuals diagnosed with T2DM (Mohamadshahi et al., 2014), a sample size of 15 participants per group was set to detect an estimated 14 % difference in HbA1c levels among the three intervention arms. This calculation was performed assuming a significance level of 0.05, and a statistical power of 80 %, assuming a 10 % drop-out rate. Of the 46 participants included after recruitment, 3 were excluded due to very poorly uncontrolled/decompensated diabetes, plus 4 others who dropped out for personal reasons during the follow-up period (Fig. 1). All the participants received guidelines and signed an informed consent form before starting the study. The protocol was approved, following the Helsinki declaration, by the Ethics Committee of the National University of Asunción (registered N° 89/2021 and P58/2020), also registered on ClinicalTrials.gov (NCT04988594).

2.2. Study design

Following the recruitment period, the participants were randomly allocated to one of three intervention groups using the Research Randomizer software (version 4.0). The randomization was performed also by the Endocrinology and Nutrition Service team. In the randomized controlled trial, the volunteers followed three dietary patterns for 12 weeks based on the American Diabetes Association (ADA) nutritional management recommendations established at the beginning of the intervention (American Diabetes, 2021; Evert et al., 2019). The three dietary interventions were: (1) Potential Probiotic Yoghurt (**PPY**): ADA nutritional recommendations + 300 g/day fermented dairy with a blend of selected strains as a starter culture (*Streptococcus thermophillus* + *Lactobacillus delbrueckii* subsp. *bulgaricus* 205/207), supplemented with *Bifidobacterium animalis* ssp. *lactis* B420 as a probiotic experimental strain. (2) Conventional Yoghurt (**CY**): ADA nutritional recommendations + 300 g/day fermented dairy with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* 205/207. (3) **NON**: ADA nutritional recommendations without any fermented food (Fig. 1). It should be noted that, regardless of the intervention group assigned, all 46 participants adhered to the medical nutrition guidelines for individuals with T2DM, as recommended by the ADA in 2019 and updated in 2021, throughout the 12-week intervention period. Furthermore, individual caloric intake was adjusted based on body composition data obtained using InBody Technology, in baseline and after 12 weeks of intervention, ensuring that the energy requirements were tailored to each participant's nutritional status.

2.3. Nutritional interventions

2.3.1. Product development

The yoghurt was developed exclusively for this study by Cooperativa Chortitzer Ltda (Lácteos Trebol, Paraguay). After preheating the skimmed-milk to 60° and homogenising at 180 bars, the base mixture was pasteurised at 90° for 5 min. The temperature was adjusted (41 to 43° C) and the lactic starter culture was added, allowed to ferment to a pH of 4.6, stirred and cooled to 15°. Once the mixture was homogenised, it was bottled and stored in a controlled chamber at 4 to 8°. No flavouring or colouring was added. The nutrient content was 3.8 % w/w protein, 0 % w/w milk fat, 0 % w/w added sugars (following the CXS 243–2018 Codex Alimentarius-International Food Standards for fermented milk denomination and the MERCOSUR resolution N° 01/12 establishing the criteria for the declaration of nutritional properties).

2.3.2. Potential probiotic yoghurt (*PPY*)

In the experimental product, the starter culture was composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* at 1×10^7 (CFU/g, total), and additionally the study strain *Bifidobacterium animalis* ssp. *lactis* B420™ (Danisco Dupont, Denmark) added at 1×10^9 (CFU/g, total).

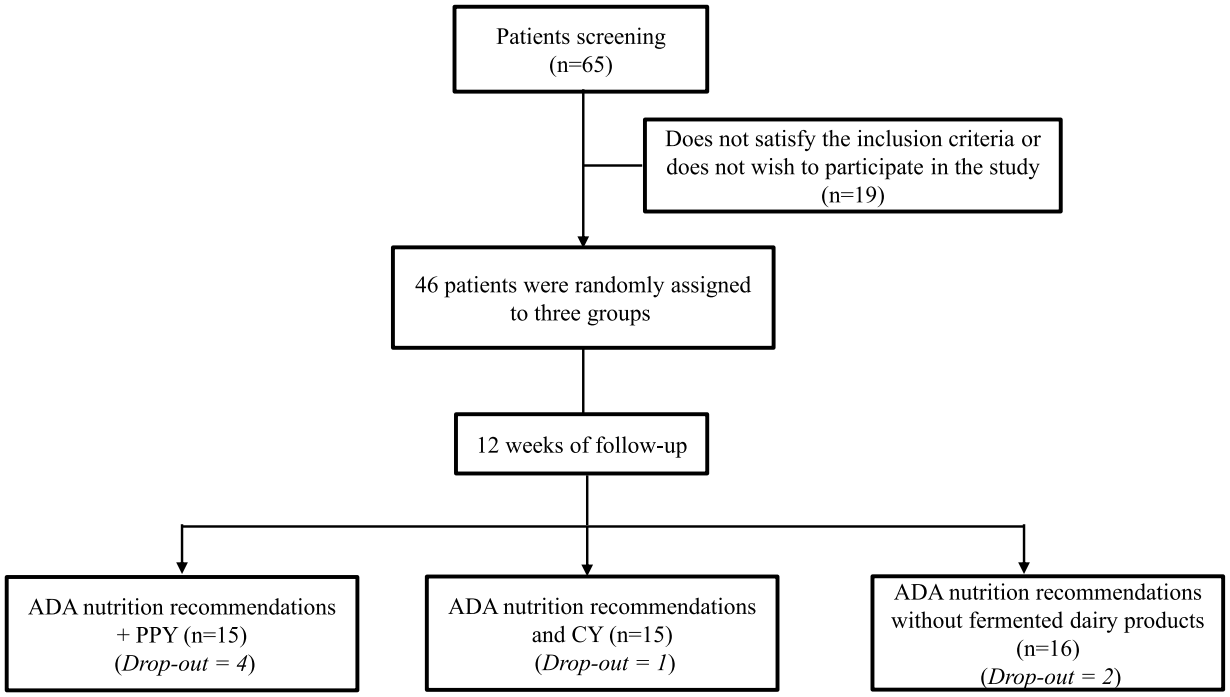

Fig. 1. Flowchart of the study. ADA: American diabetes association; PPY: potential probiotic yoghurt; CY: conventional yoghurt. Drop-outs include participants who were excluded at the beginning of the study due to mishandling of T2DM ($n = 3$), and participants who dropped out of the study during follow-up phase ($n = 4$).

2.3.3. *Conventional yoghurt (**CY**)*

In the conventional yoghurt, the starter culture was composed only of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* 205/207 at 1×10^7 (CFU/g, total).

2.3.4. *Consumption of ADA nutritional recommendations without any fermented food (**NON**)*

The guidelines of the ADA 2019 consensus were followed, where the macronutrient and micronutrient distribution was based on an individualized assessment based on current dietary patterns, preferences, and metabolic goals (after morpho-functional assessment and nutritional diagnosis)(Evert et al., 2019).

2.4. *Dietary intervention and nutritional follow-up*

All the participants received nutritional follow-up by a registered dietitian-nutritionist with the knowledge and skills to provide diabetes-specific medical nutrition therapy, as defined by the ADA consensus (Evert et al., 2019). The participants were visited every 15 days until the end of the trial. During the visit, a food frequency questionnaire (FFQ) was completed and the 24-h Dietary Recall food record was collected, ensuring adherence to the minimum score.

For the total caloric requirement, each participant was assessed using Inbody 570 bioimpedance/multifrequency equipment (Inbody®, Tokyo, Japan), showing the distribution of macro- and micronutrients and the dietary plan.

Emphasis was placed on the carbohydrates being high fibre (at least 14 g/1000 kcal), with at least half of grain consumption being whole intact grains from minimally-processed sources. For the protein, slightly higher levels of 20–25 % protein were included. Fats were included in the range of 20–25 % (with a particular emphasis on monounsaturated and polyunsaturated fats).

All the participants refrained from consuming yoghurt or any other fermented food from one month before the start of the intervention. The volunteers were instructed to maintain the cold chain of the yoghurt between 4 °C to 8 °C at all times, and received a weekly supply of yoghurt, together with a measuring cup for the 300 g/day ration. Adherence to yoghurt consumption was monitored by once-weekly telephone interviews.

2.5. *Biochemical measurements of metabolic parameters*

Venous blood from the participants was collected in tubes containing EDTA and to serum isolation, after a 12-h overnight fast. The EDTA tubes were placed in ice containers and kept in the dark, while the serum tubes were processed at room temperature. The samples were collected at baseline and after 12 weeks of intervention. Immediately after the blood extraction, the plasma was separated by centrifugation at 1500 x *g* for 15 min at 4 °C, while the serum samples were obtained after centrifugation at 1500 x *g* for 10 min at room temperature.

The lipid variables were assessed using a CB 350i chemical auto-analyzer (Wiener-Lab, Italy) with specific reagents. The levels of total cholesterol (TC), triglycerides and high-density lipoprotein (HDL-c) were measured using the homogeneous colorimetric method. Low-density lipoprotein (LDL-c) concentration was calculated by the Friedewald equation (Friedewald et al., 1972). VLDL particles were calculated using the formula TG/5, and glucose measurements were performed using the glucose oxidase, peroxidase method with Wiener-Lab reagents, while plasma insulin concentrations were measured on a Immulite 1000 Immunoassay Analyzer (Siemens, EURO/DPC, USA). Glycosylated haemoglobin (HbA1c) was measured using the High-Performance Liquid Chromatography (HPLC) method, with the fully automated d-10 Haemoglobin Testing System (Bio-Rad, France). The hs-CRP levels were measured following the Latex Immunoturbidimetric Method using Wiener-Lab reagents, while the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index was calculated using the using the formula previously described in (Matthews et al., 1985). All measurements were taken at the Institute of Health Sciences Research, National University of Asunción, Paraguay.

2.6. *Anthropometric measurements*

Body composition analysis was performed at the beginning and at the end of the intervention using an advanced BCA InBody 570®, and

the following variables were obtained: weight, height, BMI, musculoskeletal mass, body fat mass, protein, minerals, bone mineral content, body cell mass, total body water, intra and extracellular water, visceral fat, segmental fat and lean mass analysis, waist-to-hip ratio, basal metabolic rate and recommended caloric intake. The measurement technique was carried out according to the manufacturer's instructions.

2.7. Statistical analysis

The values given represent the mean and standard error. Comparison between baseline characteristics was carried out using ONE-WAY Anova. A comparison of the effects between baseline and post-intervention was performed using repeated measures analysis, by which the overall effect of the dietary intervention was evaluated (global ANOVA and p for diet), the effect of time (p for time), and the diet-time interaction (diet vs time). Sidak's test was used for multiple comparisons, and, independently for each dietary intervention, a *t*-test was conducted for related samples including each clinical variable from study. All the analyses were performed using SPSS software (now PASW Statistic for Windows, version 21) (IBM, Chicago, IL, USA), with $p < 0.05$ considered as significant.

3. Results

In the present study, 46 participants were randomized and assigned to the groups Potential Probiotic Yoghurt (PPY)($N = 15$), Conventional Yoghurt (CY) ($N = 15$), and ADA recommendations without any fermented foods (NON) ($N = 16$). During the follow-up period, 3 participants were excluded due to uncontrolled/decompensated diabetes and 4 others dropped out of the study for personal reasons (Fig. 1). No patients reported any adverse effect during the study related to yoghurt consumption, and both yoghurts were well-tolerated.

3.1. Effect of interventions on clinical variables included in the study

After 12 weeks of intervention, compared to baseline, an average weight reduction of 4.2 % was observed across all three groups (PPY: $p = 0.002$; CY: $p < 0.001$; NON: $p = 0.003$). This weight loss was accompanied by a mean decrease in BMI of 4.7 % (PPY: $p = 0.003$; CY: $p < 0.001$; NON: $p = 0.003$). Waist circumference was also significantly reduced in all groups, with an average decrease of 2.7 % ($p < 0.001$ for all groups). In the CY group, insulin levels increased by 34 % (from 6.7 ± 0.9 to 9.0 ± 1.2 μU/mL; $p = 0.014$), accompanied by a 26 % reduction in both triglycerides ($p = 0.009$) and VLDL cholesterol ($p = 0.009$). However, repeated measures analysis revealed a significant interaction between time and group only for waist circumference, indicating that the overall weight loss effect was similar across all groups, regardless of the specific intervention (Table 1). No other variables showed statistically significant time-by-group interactions.

3.2. Effect of conventional and potential probiotic yoghurt consumption on T2DM-related variables

Our results show an 11.4 % reduction in HbA1c levels in participants who consumed the Potential Probiotic Yoghurt (PPY), compared to those in the non-fermented dairy group (NON) ($p = 0.021$); and a reduction of 8.5 % in participants who consumed CY vs NON ($p = 0.060$) (Fig. 2**A**). In addition, absolute values of HbA1c reduced from baseline to 12 weeks in PPY: 7.5 ± 0.5 to 6.6 ± 0.4; CY= 7.2 ± 0.4 to 6.4 ± 0.3, and NON= 6.7 ± 0.4 to 6.7 ± 0.39 (Fig. 2**A**) (**Supplementary Figure 1**).

Additionally, a significant reduction in fasting glucose levels was observed from baseline to week 12 in participants who consumed PPY and CY, with p-values of 0.016 and 0.043, respectively (Fig. 2**B**). Glucose levels showed no significant changes in the group that did not consume fermented dairy products, and no significant differences were observed in the body composition and in the dietary energy intake after diet vs. time interaction analyses (**Supplementary Table 1**).

4. Discussion

In this 12-week intervention study, we aimed to analyse the effect of the consumption of a yoghurt with probiotic potential (PPY), a conventional yoghurt (CY) and the absence of any type of fermented dairy product (NON) on parameters associated with glucose homeostasis in patients diagnosed with type 2 diabetes mellitus (T2DM). We observed a significant reduction in HbA1c in the PPY group compared to the NON group (11.4 % decrease, $p = 0.021$), and a positive but non-significant trend in the CY group compared to the NON group (8.5 % decrease, *p*

Table 1
Effect of interventions on clinical variables included in the study. Values expressed as mean ± standard error. HDL-c, High density lipoprotein; LDL-c, Low density lipoprotein; VLDL, very low-density lipoprotein; hs-CRP, High sensitivity C-reactive protein; HOMA-IR, homeostatic model assessment; BMI, Body Mass Index. PPY: potential probiotic yoghurt; CY: conventional yoghurt; NON: ADA recommendations without any fermented food consumption. ***(a)*** Variables were calculated using *t*-test for related measures. ***(b)*** The interaction between group and time was assessed by ANOVA for repeated measures. All analyses were performed through PASW (now SPSS Statistics for Windows (version 21.0)) (IBM. Chicago. Illinois). p-values < 0.05 were considered statistically significant.

	PPY			***CY***			***Non-***			
Parameter	***Baseline***	***12 weeks***	***p value (a)***	***Baseline***	***12 weeks***	***p value (a)***	***Baseline***	***12 weeks***	***p value (a)***	***p value interaction time vs diet (b)***
Weight	94.2 ± 5.5	89.3 ± 5.6	0.002	85.0 ± 5.2	81.5 ± 5.0	< 0.001	75.4 ± 2.9	72.6 ± 2.9	0.003	0.222
Height	1.7 ± 0.03		n/a	1.6 ± 0.03		n/a	1.6 ± 0.03		n/a	n/a
BMI	33.8 ± 1.4	32.0 ± 1.4	0.003	31.6 ± 1.7	30.0 ± 1.5	< 0.001	28.3 ± 0.7	27.2 ± 0.7	0.003	0.381
Waist perimeter	110.3 ± 4.3	106.4 ± 4.0	< 0.001	105.2 ± 4.0	102.4 ± 3.8	< 0.001	97.5 ± 2.2	95.5 ± 2.2	< 0.001	0.038
Insulin	9.6 ± 1.9	9.0 ± 1.2	0.655	6.7 ± 0.9	9.0 ± 1.2	0.014	9.2 ± 2.1	15.4 ± 5.7	0.152	0.220
HOMA-IR	3.1 ± 0.6	2.4 ± 0.3	0.186	2.1 ± 0.3	2.3 ± 0.3	0.491	2.4 ± 0.4	4.2 ± 1.5	0.139	0.085
Triglycerides	159.1 ± 16.4	144.2 ± 16.5	0.441	145.1 ± 17.0	108.1 ± 12.5	0.009	129.9 ± 14.7	117.2 ± 17.9	0.329	0.396
Total cholesterol	194.7 ± 11.7	182.3 ± 11.3	0.436	173.6 ± 8.7	166.4 ± 8.9	0.392	184.4 ± 10.2	193.9 ± 13.2	0.184	0.278
HDL-c	46.0 ± 2.5	45.18 ± 2.7	0.580	44.9 ± 1.6	44.5 ± 1.9	0.653	50.4 ± 3.0	53.8 ± 3.9	0.08	0.081
LDL-c	116.9 ± 10.0	108.2 ± 8.8	0.517	99.6 ± 7.6	100.3 ± 8.0	0.927	108 ± 8.6	116.6 ± 10.8	0.171	0.389
VLDL	35.7 ± 5.4	28.9 ± 3.3	0.248	29.0 ± 3.4	21.6 ± 2.5	0.009	30.2 ± 5.0	23.4 ± 3.6	0.159	0.990
Non-HDL	148.7 ± 10.8	137.0 ± 10.1	0.448	128.6 ± 8.0	121.8 ± 8.3	0.410	134 ± 10.2	140.1 ± 11.8	0.329	0.404
hs-CRP	4.4 ± 1.2	3.6 ± 1.6	0.643	8.6 ± 2.3	6.9 ± 2.9	0.484	3.3 ± 1.0	2.8 ± 0.8	0.139	0.524

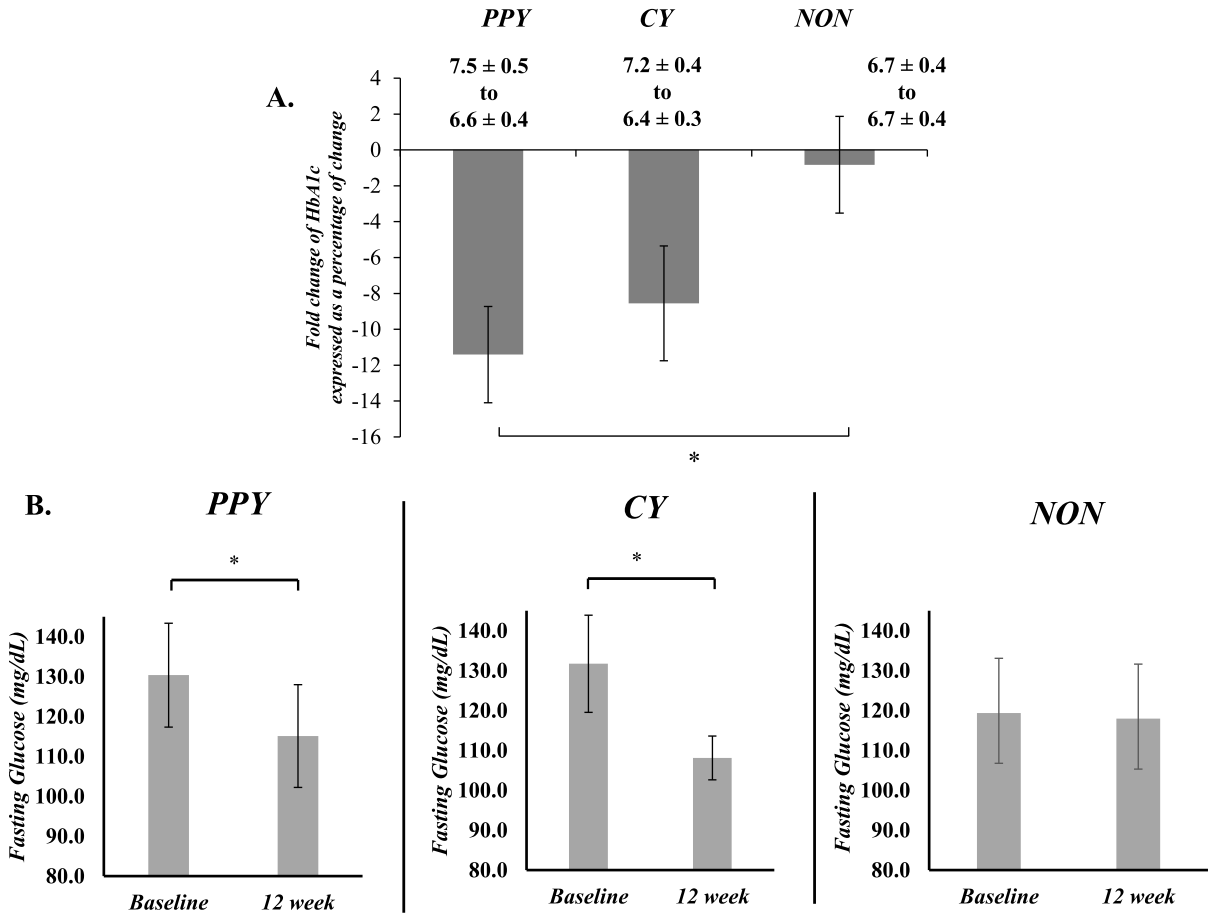

Fig. 2. Effect of the fermented dairy product on glucose homeostasis. A. Glycated haemoglobin levels after the interventions. Data shows the fold change between week 12 and baseline ± *S*.E.M. The fold change was calculated using the equation ((HbA1c 12 weeks - HbA1c baseline)/HbA1c baseline) x 100; and is expressed as a percentage change. The analysis corresponds with the one-way ANOVA of the fold change according to the intervention. Post hoc multivariable analysis was performed using the DMS test, * $p < 0.05$. HbA1c measurement was carried out by High Performance Liquid Chromatography (HPLC) using the d-10 Haemoglobin Testing System (Bio-Rad). B. Effect of the interventions on fasting glucose levels. The bars represent the mean ± typical error. The analysis corresponds to a *t*-test for related samples carried out independently for each intervention group. * Significance (bilateral) < 0.05. All analyses were performed using SPSS software (now PASW Statistic for Windows, version 21) (IBM, Chicago, IL, USA). PPY: potential probiotic yoghurt; CY: conventional yoghurt; NON: ADA recommendations without any fermented food consumption.

= 0.060). Regarding fasting blood glucose, both the PPY ($p = 0.016$) and CY ($p = 0.043$) groups showed a significant decrease after the intervention.

T2DM is a metabolic disease that affects blood glucose levels and acts as a starting point for a variety of microvascular and macrovascular complications (Organization, 2023). Its incidence is increasing, mainly due to the current global obesity pandemic, reflecting the limiting complications experienced by people with diabetes during their lifetime.

For people living with diabetes, low-cost treatments and new strategies are urgently needed in which diet and a healthy lifestyle are key tools in the prevention and management of the disease (Mohammadi et al., 2021). Previous studies have shown that adherence to a healthy dietary pattern promotes disease management and could even lead to remission (Basterra-Gortari et al., 2019; Penn et al., 2009). In addition, other studies have demonstrated the link between gut microbiota and metabolic diseases (Hadi et al., 2021; Tian et al., 2023), suggesting the possibility of using probiotic agents as a tool for disease management (Li et al., 2016; Sanders et al., 2010). In this context, the consumption of probiotics through fermented dairy products like yoghurt has been the subject of growing interest in the prevention and management of T2DM. Studies have shown that regular yoghurt consumption, especially in its low-fat and sugar-free form, is associated with a lower risk of developing type 2 diabetes and with improvements in glycemic control in diagnosed patients (O'Connor et al., 2014; Zhong et al., 2024). In this sense, mechanistically, gut microbiota modulates the inflammatory cascade by decreasing TLR4 receptor activation in response to low levels of lipopolysaccharide (LPS), thereby decreasing the synthesis of inflammatory cytokines and reactive oxygen species. Additionally, these microorganisms have the ability to synthesize the short-chain fatty acids (SCFA) which promote the synthesis of glucagon (GLP-1) and peptide YY (PPY). These factors, among others, also contribute to improved glucose metabolism and homeostasis (Al Bander et al., 2020; Ghosh et al., 2021; Gurung et al., 2020; Yaribeygi et al., 2020).

However, the inclusion of conventional yoghurt in clinical nutrition protocols remains limited by the fact that its probiotic strains are added primarily for technological purposes - i.e. to ferment milk - and not to provide specific health benefits. Importantly, the effects of probiotics are strain-specific, and no single strain provides the full spectrum of benefits commonly attributed to probiotics (Hill et al., 2014). Regulatory authorities, such as the European Food Safety Authority (EFSA) and the US Food and Drug Administration (FDA), emphasize the need for strain characterization and clinical evidence to substantiate health claims for probiotics (Administration, 2020; EFSA on Dietetic Products, 2011).

In this context, the incorporation of well-characterized strains with demonstrated efficacy in metabolic disorders—such as *Bifidobacterium animalis* subsp. *lactis* B420—may enhance the clinical value of fermented

dairy products. B420 has shown promising effects in reducing body fat mass, improving glycemic control, and modulating metabolic parameters in both preclinical and clinical studies (Korpela et al., 2014; Stenman et al., 2015). Formulating yoghurts with such strains not only aligns with international regulatory frameworks for probiotic health claims but also paves the way for their inclusion in evidence-based guidelines for medical nutrition therapy in T2DM management.

Thus, in our study we evaluated the consumption of fermented dairy products with conventional cultures *(Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* 205/207) and with the addition of *Bifidobacterium animalis* ssp. *lactis* B420 in patients diagnosed with T2DM. We observed that, after 12 weeks of intervention, HbA1c and glucose levels decreased in both yoghurts, suggesting an improvement in glucose homeostasis, possibly following the mechanism described above. This is in line with a previous study that showed a decrease in HbA1c of 9.2 % in a group that consumed a yoghurt containing *Lactobacillus acidophilus* and *Bifidobacterium lactis* for 12 weeks compared to a placebo group (Mirjalili et al., 2023). However, in contrast to this study, our intervention strictly adhered to international food regulations established by the Codex Alimentarius, FDA and EU for fermented dairy products. These regulatory frameworks define "yoghurt" as a fermented dairy product obtained exclusively through the action of *Streptococcus thermophilus and Lactobacillus delbrueckii* subsp. *bulgaricus*. While these guidelines allow for the inclusion of additional microbial strains, such as probiotics, must not alter the standard identity of yoghurt. So, in accordance with these stipulations, we incorporated the probiotic strain *Bifidobacterium animalis* subsp. *lactis* B420 into the yoghurt matrix. Similarly, previous intervention studies in people with T2DM reported a reduction in HbA1c following an 8-week intervention with a fermented dairy product containing *Bifidobacterium animalis* subsp. *lactis* Bb12 and *Lactobacillus acidophilus* La5, compared with a traditional fermented product (Ejtahed et al., 2011; Mohamadshahi et al., 2014). However, the intervention duration in those studies was shorter than in the present trial, and notably, the products used did not include *Streptococcus thermophilus or Lactobacillus delbrueckii* subsp. *bulgaricus* as starter cultures. This is a key distinction, as mentioned above, the use of these two bacterial species is required by food regulations for a product to be classified and labeled as "yoghurt." Therefore, while the findings of these studies are relevant, the absence of these cultures may limit the comparability and generalizability of their results to officially recognized yoghurt-based interventions.

Nevertheless, our study has certain limitations, one of which is the number of participants included in each group ($n = 15$). The size of the groups in our study was limited due to the difficulty of finding participants who met the inclusion criteria, such as recently diagnosed diabetes, no consumption of fermented dairy products, no recent antibiotic treatment and no use of insulin, oestrogens or progesterone. A clinical trial with a larger sample size would be necessary to consolidate our findings.

Based on these results, our study suggests that the use of fermented dairy products (both PPY and CY) could contribute to the management of T2DM, representing a low-cost and easy-to-adhere alternative. Regarding the inclusion of the B420, it is important to highlight that it is not intended to replace traditional starter cultures (*Streptococcus thermophilus and Lactobacillus delbrueckii* subsp. *bulgaricus*), which are required under international food regulations, but rather to enrich the formulation with a strain with potential clinical relevance in T2DM. This approach preserves the regulatory definition and identity of yoghurt while introducing potential therapeutic functionality.

5. Conclusion

The consumption of 300 g/day of fermented dairy products such as conventional yoghurt and supplemented with *Bifidobacterium animalis* ssp. *lactis* B420, within the context of a healthy diet, appears to be an effective tool for T2DM management by improving glucose homeostasis. Thus, the addition of a strain such as *Bifidobacterium animalis* ssp. *lactis* B420 could enhance or improve these effects and would facilitate its inclusion in clinical practice guidelines.

Funding

Universidad Nacional de Asunción CP 1812/2019, CP 2668/2021, Grant to ER-DN by the Fundacion Carolina, Agencia Española de Cooperación Internacional para el Desarrollo, Gobierno de España. Cooperativa Chortitzer Ltda - Lácteos Trebol, Paraguay. Cooperativa Paraguaya de Levaduras (COPALSA), Paraguay.

Ethical statement

The authors of the manuscript entitled "Fermented dairy product could improve glucose homeostasis in patients diagnosed with type 2 diabetes mellitus: A randomized controlled trial" declare that all the procedures performed in the framework of the present work were carried out in accordance with the Helsinki declaration and have been approved by the Ethics Committee of the Faculty of Medical Sciences and the Health Sciences Research Institute of the National University of Asunción, Paraguay (registered N° 89/2021 and P58/2020, respectively).

We also declare that the privacy rights of the subjects included in the study have been respected and that informed consent for experimentation with human subjects has been obtained.

CRediT authorship contribution statement

Eugenia Ruiz-Díaz Narváez: Writing – original draft, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Rosa Vega Medina:** Methodology, Investigation, Conceptualization. **Helena García-Fernández:** Methodology, Investigation. **Ana Iris Ramirez:** Methodology, Investigation. **Sussam Benitez:** Methodology, Investigation. **Gloria Echagüe:** Methodology, Investigation. **Liliana Sosa:** Methodology, Investigation. **Noelia Alvarenga:** Methodology, Investigation. **Ana Ayala Lugo:** Methodology, Investigation. **José López-Miranda:** Writing – review & editing. **Pablo Pérez-Martínez:** Writing – review & editing, Validation, Supervision, Resources, Investigation, Formal analysis, Conceptualization. **Oriol Alberto Rangel-Zuñiga:** Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by Universidad Nacional de Asunción (Instituto de Investigaciones en Ciencias de la Salud), Paraguay; Cooperativa Paraguaya de Levaduras (COPALSA), Paraguay; and Junta de Andalucía (Consejería de Salud, Consejería de Innovación, Ciencia y Empresa). Oriol Alberto Rangel Zúñiga is supported by an ISCIII research contract (Programa Miguel-Servet CP19/00142) funded by Instituto de Salud Carlos III, Spain (co-funded by Fondo Social Europeo - "*El FSE invierte en tu futuro*"-) and Eugenia Ruiz-Diaz Narvaez by Fundacion Carolina (Agencia Española de Cooperación Internacional para el Desarrollo, Gobierno de España) and Facultad de Ciencias Medicas, Universidad Nacional de Asunción, Paraguay. We would like to thank Cooperativa Chortitzer (Lacteos Trebol), Mariano R. Alonso (Paraguay) and Danisco (Dupont, Danmark) for the development of the product for this research. We would also like to express our gratitude to all the

participants who participated in the study and the Clinic Hospital (Facultad de Ciencias Medicas, Universidad Nacional de Asuncion, Paraguay) for carrying out the recruitment and randomization process in this study. This study was registered a clinical trial on Clinicaltrials.gov (NCT04988594). The CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN) is an initiative of the Instituto de Salud Carlos III, Madrid, Spain. Thanks also go to Paloma Arrellaga Sosa, Jose Andres Morales Martinez and Rosario Carreras for providing technical assistance.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2025.100679.

Data availability

Data will be made available on request.

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