Randomized control trials

Clinical application of probiotics in type 2 diabetes mellitus: A randomized, double-blind, placebo-controlled study

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S U M M A R Y
Background & aims: Type 2 diabetes has been associated with dysbiosis and one of the possible routes to restore a healthy gut microbiota is by the regular ingestion of probiotics. We aimed to investigate the effects of probiotics on glycemic control, lipid profile, inflammation, oxidative stress and short chain fatty acids in T2D.

Methods: In a double-blind, randomized, placebo-controlled trial, 50 volunteers consumed daily 120 g/d of fermented milk for 6 wk. Participants were assigned into two groups: probiotic group, consuming fermented milk containing Lactobacillus acidophilus La-5 and Bifidobacterium animalis subsp lactis BB-12 (10^9 colony-forming units/d, each) and control group, consuming conventional fermented milk. Anthropometric measurements, body composition, fasting blood and faecal samples were taken at baseline and after 6 wk.

Results: 45 subjects out of 50 (90%) completed follow-up. After 6 wk, there was a significant decrease in fructosamine levels (−9.91 mmol/L; p = 0.04) and hemoglobin A1c tended to be lower (−0.67%; p = 0.06) in probiotic group. TNF-α and resistin were significantly reduced in probiotic and control groups (−1.5 and −1.3 pg/mL, −1 and −2.8 ng/mL, respectively), while IL-10 was significantly reduced (−0.65 pg/mL; p < 0.001) only in the control group. Fecal acetic acid was increased in both groups (0.58 and 0.59% in probiotic and control groups, respectively; p < 0.01). There was a significant difference between groups concerning mean changes of HbA1c (+0.31 for control group vs −0.65 for probiotic group; p = 0.02), total cholesterol (+0.55 for control group vs −0.15 for probiotic group; p = 0.04) and LDL-cholesterol (+0.36 for control group vs −0.20 for probiotic group p = 0.03).

Conclusions: Probiotic consumption improved the glycemic control in T2D subjects, however, the intake of fermented milk seems to be involved with others metabolic changes, such as decrease in inflammatory cytokines (TNF-α and resistin) and increase in the acetic acid.

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1. Introduction

Diabetes mellitus is on the rise all over the world affecting more than 382 million people. Type 2 diabetes (T2D) accounts for 85–95% of all diabetes and is a complex chronic illness requiring multifactorial risk reduction strategies [1].

T2D is often associated with systemic inflammation [2] and increased oxidative stress [3]. Both molecular and metabolic mechanisms are links to β-cell dysfunction and/or insulin resistance [4]. However, the intake of probiotics has been demonstrated to reduce inflammation and oxidative stress markers, and to improve glycemic and insulin metabolism [5,6].

In addition, the gut microbiota also seems to be important to the pathophysiology of T2D [7]. Findings from two studies that used faecal samples suggested that compositional and functional changes in the gut microbiota might be directly linked to the development of T2D [8,9]. Various others mechanisms have been
proposed to explain the influence of the microbiota on insulin resistance and T2D, such as metabolic endotoxemia [10], modifications in the secretion of the incretins [11], and short-chain fatty acids (SCFA) production [12]. Multiple cytokines are involved in the development of T2D, and are fundamental signals in the intestinal immune system, able to subvert the physiological status of inflammation in the gut [13].

Alterations in the gut microbiota as a result of probiotics and prebiotics intake have been reported. Experimental studies have reported beneficial effects by strains of Lactobacillus on glycemic control, stress oxidative and/or inflammation in animal model of type 2 diabetes [5,14]. However, the effects of probiotics on diabetes endpoints remain unknown.

The objective of this study was to investigate the efficacy of the intake of fermented goat milk containing Lactobacillus acidophilus La-5 and Bifidobacterium animalis BB-12 on glycemic control, lipid profile, inflammation, oxidative stress and faecal SCFA in T2D subjects.

2. Methods

2.1. Subjects

This randomized, double-blind, parallel-group, placebo-controlled trial was carried out in Ceará, Brazil, from June 2013 to February 2014. The study was performed on 50 volunteers with T2D according to ADA criteria [15], age 35–60 y, body mass index (BMI) lower than 35 kg/m² and type 2 diabetes diagnosed for at least one year, recruited from two endocrinology clinics. The appropriate sample size was calculated based on primary outcome of serum interleukin (IL)-6 levels obtained from Mazloom et al. [16], considering a alpha level of 0.05 and a power of 80%. The number of subjects required was 22.5 per group, but this number was increased to 25 per group to accommodate the withdrawals. The research team did recruitment within the clinics after indication of participation by endocrinologist.

We excluded participants with clinical evidence of chronic illness or gastrointestinal disorders; presence of renal, hepatic, haematological or immunodeficiency diseases; history of cancer or cardiovascular diseases; current treatment with insulin, DPP-4 inhibitor or GLP-1 analog; smoking; any intake of probiotics, supplements, antiobesity and anti-inflammatory drugs, and antibiotics in the three months preceding recruitment, and pregnancy or breast-feeding.

The study protocol was approved by the Federal University of Viçosa Ethics Committee. All patients provided written informed consent. This trial is registered with ensaiosclinicos.gov.br/rg/RBR-219644.

2.2. Randomisation and blinding

Participants were randomly assigned (1:1) by computer (Research Randomizer, version 4.0) to either probiotic or control group in block sizes of four. The randomization was stratified by gender. Volunteers, investigators, and trial staff were masked to treatment allocation. However, investigators analyzing study data were not masked. Blinding was done by a technical assistant, right after beverages production.

2.3. Study protocol

T2D subjects were randomly assigned to consume either 120 g/d of probiotic fermented goat milk containing 10⁸ UFCs of L. acidophilus La-5 and 10⁹ UFCs of Bifidobacterium animalis subsp. lactis BB-12 (probiotic group) or 120 g/d of conventional fermented goat milk contained Streptococcus thermophilus TA-40 (control group) for 6 wk, during the breakfast. The fermented milk (FM) was delivered every 2 wk and volunteers were instructed to keep it under refrigeration (4 °C).

The probiotic and conventional FM were developed in partnership with Embrapa Goat and Sheep (Ceará, Brazil). Pasteurized milk was cooled to 43 ± 2 °C for the addition of the starter culture (S. thermophilus TA-40; Danisco, Sassenage, France), and the probiotic cultures (L. acidophilus La-5 and B. animalis subsp. lactis BB-12; Chr. Hansen, Hoersholm, Denmark). The beverages were flavored with grape juice obtained from Embrapa Grape and Wine (Rio Grande do Sul, Brazil). Ingredients, chemical composition and antioxidants status of the probiotic FM are shown in Table supplementary 1 (see supplementary data). Both products had the same taste, color, and smell.

Fermented milk was sampled immediately after manufacture and delivered on the following day. Microbiological analysis was conducted every week during the trial. S. thermophilus enumeration was performed on M17 agar, containing lactose (Vetec, Duque de Caxias, Brazil, 5 g/L) and incubated aerobically at 37 °C for 48 h. Bifidobacterium lactis and L. acidophilus were determined in modified De Man Rogosa Sharpe (MRS) agar (Oxoid, Basingstoke, UK), followed by incubation at 37 °C for 72 h [17].

Microbiological analyses of the probiotic FM showed that the average colony counts of probiotic bacteria on days 1, 7 and 14 were 7.72 × 10⁷, 5.82 × 10⁷, and 1.62 × 10⁸ CFUs/g of L. acidophilus La-5 and 4.45 × 10⁸, 1.84 × 10⁹, and 1.56 × 10⁹ CFU/g of B. lactis BB-12, respectively. Both probiotic bacteria showed an appropriate survival rate during 14 days storage time (see Supplemental Table 2). Additionally, sensory evaluation was carried out in FM samples during the trial through acceptability tests, using the hybrid hedonic scale (1 = disliked extremely, 5 = neither liked nor disliked, 09 = liked extremely) [18]. The overall acceptability of the probiotic and conventional FM was 8.17 and 8.20, respectively (see Supplemental Table 3).

All anthropometric and biochemical measurements were conducted in a fasting state taken at baseline and after 6 wk intervention. A single experienced examiner performed all anthropometric measurements. Body weight and body composition were assessed by bio-impedance (Tanita TBF-300, Tanita Corporation, Tokyo, Japan). Waist circumference (measured midway between lowest rib and iliac crest) was measured using a non-stretchable measuring tape (Sanny, Sao Paulo, Brazil).

Dietary assessment was done using food records, which were conducted by the nutritionist in the first and the last week of intervention. Dietary intake was analyzed using Avanutri Revolução software (Rio de Janeiro, Brazil). Regular level of physical activity was defined as three times a week for at least 30 min.

Subjects were instructed to follow their usual diets, level of physical activity, and lifestyle, avoiding any changes in medication, and unusual or excessive food and alcohol drink consumption throughout the intervention period. We contacted the volunteers once a week to ask for adverse health events and to assess the adherence to treatment.

2.4. Biochemical measurements

For the biochemical measurements, blood samples were obtained after a 12 h overnight fast. Before the test day, the subjects were instructed not to consume alcohol and to refrain from heavy physical activity during 72 and 24 h, respectively. Samples were centrifuged at 1000 g for 10 min at 4 °C, aliquoted and analyzed or immediately stored at −80 °C for cytokines and oxidative stress analyses.
Blood samples were analyzed for fasting plasma glucose (FPG) and lipid profile by enzymatic colorimetric method (Bioclin, Minas Gerais, Brazil, and Beckman Coulter, California, USA). Insulin was measured through electrochemiluminescence (Modular Analytics DxI 800, Beckman Coulter, California, USA). Fructosamine was assayed by colorimetric method (BioSystems, Barcelona, Spain), and Hba1C with ion exchange high-performance liquid chromatography (Bio-rad kit, California, USA). The homeostasis model assessment index (HOMA-IR) was used as an indicator of insulin resistance and calculated as follows: HOMA-IR = fasting plasma insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5. Insulin resistance was diagnosed using a cut off value of 2.71 [19].

For determination of oxidative stress markers, plasma samples were collected in vacutainers containing sodium citrate. Colorimetric assay (Sigma–Aldrich antioxidant assay kit, Missouri, USA; interassay coefficients of variation 3%) was used to measure plasma total antioxidant status (TAS). Plasma total F2-isoprostane was measured by ELISA kit (Cayman s-isoprostane EIA kit, Michigan, USA; interassay coefficients of variation 9.5% at 80 pg/mL and 6.4% at 32 pg/mL). Briefly, plasma samples (400 μL) were hydrolyzed with (10M) NaOH (100 μL) at 45 °C for 2 h to measure both free and esterified isoprostane. After incubation, 100 μL of (10M) HCl was added, the samples were centrifuged at 1,500 g for 10 min to remove precipitated proteins.

Cytokine concentrations were determined using a multiplexed bead immunoassay. This is a bead-based suspension array using the LuminexxMAP technology in which fluorescent microspheres, known as microspheres, have cytokine capture antibodies on the bead surface to bind the proteins. The measures of 5 cytokines (IL-6, IL-10, TNF-α, adiponectin, and resistin) were measured using the human Cytokine/Adipokine magnetic bead kits (Millipore Corporation, Missouri, USA). Assays were performed in 96-well filter plates, as previously described [20]. The concentration of the samples was estimated from the standard curve using a fifth-order polynomial equation (Software xPonent/Analyst versión 4.2). Intra-assay coefficient of variation was 2.0, 1.6, 2.6, 4.0, and 3.0% for IL-6, IL-10, TNF-α, adiponectin, and resistin, respectively.

2.5. Faecal analysis

Feces samples (5–10 g) were collected at enrollment and at the sixth week. Samples (blinded) were immediately placed at −80 °C and stored until analyzed. The extraction of SCFA (acetate, butyrate, and propionate) was based on the method of Smiricky-Tjardes et al. [21] and measured by GC (model GC-17A, Shimadzu, Maryland, USA). N2 was used as the carrier gas and the flux in the column was 1.0 mL/min. The temperatures of the injector and detector were set at 220 and 250 °C, respectively. Initial column temperature was 100 °C sustained for 5 min, rising at 108 °C/min until it reached 185 °C. Next, the samples were injected (1 mL) through a Hamilton syringe (10 mL) in split system 5. The results were represented as per 100 mg of faeces (% w/w).

2.6. Statistical analysis

Statistical analysis was done using SPSS Statistics version 20 (IBM, NY, USA). All data were checked for normal distribution using Shapiro–Wilk test and Skewness/Kurtosis. Data were reported as mean (SD), and median and interquartile interval (P25 and P75%), since some variables were not normally distributed (Hba1C, HOMA-IR, IL-6, and acetic acid). Comparison between probiotic and control groups at baseline was tested using unpaired Student’s t test or Mann–Whitney test. Paired Student’s t test or Wilcoxon matched-pairs signed-rank test were used to analyze differences between baseline and endpoint values. Pearson or Spearman’s correlation tests were performed to measure the degree of correlation between cytokine concentrations and glycemic control. A p value < 0.05 was considered statistically significant.

3. Results

A total of 45 (90%) subjects aged 35 to 60 y (mean 51.40 ± 6.80) concluded this study. Five patients were excluded according to reasons shown in Fig. 1. Abdominal discomfort was the only reported adverse effect. This subject (n = 1) belonged to the control group and was withdrawn from the study. Baseline characteristics are shown in Table 1. The main drugs used by the volunteers were metformin (94% in the probiotic group and 95.5% in the control group) and glibenclamide (44% in the probiotic group and 41% in the control group). At the baseline, no relevant differences could be detected in the general characteristics between control and probiotics group, except for Hba1C (p = 0.04), which was higher in the probiotic group. There were no statistically significant differences in anthropometric and body composition values between or within groups at the end of the study (data not shown). Similarly, at the beginning of the study, no significant differences were found between the two groups in terms of dietary intakes. Comparing the dietary intakes throughout the study separately in each group, we observed no significant differences within group (see Supplemental Table 4).

3.1. Impact of the intervention on glicemic control

Biochemical markers after probiotic treatment are show in Table 2. At 6-week follow-up, the consumption of probiotic fermented milk significantly decreased fructosamine levels (−9.91 mmol/L; p = 0.04) and Hba1C levels tended to be reduced (−0.67%; p = 0.06), while in the control group no significant effect was detected on glycemic control (p > 0.05). When the median changes in Hba1C were compared between groups, there was a significant difference (−0.31 for control group vs −0.65 for probiotic group; p = 0.02). The fasting plasma glucose (FPG), insulin concentrations, as well as insulin resistance, evaluated by the HOMA index, did not change significantly throughout the follow-up period in both groups (p > 0.05 for all).

3.2. Effect of probiotics on lipid profile

Within group comparisons of lipid profile revealed that consumption of probiotic fermented milk prevented a rise in total cholesterol (TC) and LDL-C, while in the control group we observed a significant increase in the TC and LDL-c (11.35 and 16.10% vs 0.01 and 0.04, respectively). Furthermore, when the mean changes were compared between the two groups, there was a statistically significant difference in the TC (−0.58 mmol/L, 95% CI: −1.13 to −0.03; p = 0.04) and LDL-C (−0.20 mmol/L, 95% CI: −1.02 to −0.03; p = 0.03). At the end of trial, no significant effect on HDL-C, VLDL, and triglycerides were found in both groups (p > 0.05 for all). The TC:HDLC ratio was significantly increased by 8.94% (0.28 mmol/L) in the control group during the study (p = 0.03), while no statistically significant changes (p = 0.75) was reported in probiotic group (Table 2).

3.3. Effect of probiotics on markers of oxidative stress

At baseline, there was no difference between groups in TAS (0.01 mmM; 95% CI: −0.06 to 0.07; p = 0.86) and F2-isoprostane levels (11.66 pg/dL; 95% CI: −8.77 to 32.11; p = 0.25). No significant difference was detected in plasma TAS concentrations from baseline to post intervention in probiotic or control groups.
p = 0.40 and \( P = 0.23 \), respectively) (Table 2). Similarly, plasma F2-isoprostane concentrations did not change in the probiotic \( p = 0.76 \) or control \( p = 0.94 \) group over time.

### 3.4. Effect of probiotics on cytokines levels

Anti-inflammatory markers, such as IL-10 and adiponectin, decreased after intervention in control group \(-0.64\ \text{pg/mL} \ [95\% \text{ CI: } -0.47 \text{ to } -0.81; p = 0.001]\) for IL-10, \(-0.76\ \mu\text{g/mL} \ [95\% \text{ CI: } -0.75 \text{ to } -1.59; p = 0.07]\) for adiponectin, while no significant change was observed in the probiotic group \( p = 0.38 \) and \( p = 0.14 \), respectively). At the end of trial, the two groups exhibited reduction in TNF-\( \alpha \) \(-1.4\ \text{pg/mL} \ [95\% \text{ CI: } -0.01 \text{ to } 2.79; p = 0.04]\) for probiotic, \(-1.36\ \text{pg/mL} \ [95\% \text{ CI: } -0.24 \text{ to } -2.5; P = 0.02]\) for control group) and resistin \(-2.06\ \text{ng/mL} \ [95\% \text{ CI: } -0.66 \text{ to } -3.44; p = 0.006]\) for probiotic, \(-2.80\ \text{ng/mL} \ [95\% \text{ CI: } -1.45 \text{ to } -4.16; p = 0.001]\) for control group). No significant differences in IL-6 levels were observed in any groups post intervention. Reductions in resistin were positively correlated with reductions in HbA1c \( (r = 0.320; p = 0.03) \). No correlation was found between other cytokines and markers of glycemic control (data not shown). In Fig. 2 it is possible to compare the cytokines concentration between the two groups.

### 3.5. Effect of probiotics on faecal SCFA analysis

Data from faecal analysis showed a significant increase in the acetic acid in both groups \(0.58\% \ [95\% \text{ CI: } 0.97 \text{ to } 1.96; p = 0.005]\) for probiotic, \(0.59\% \ [95\% \text{ CI: } 0.98 \text{ to } 1.89; p = 0.006]\) for control group) at the end of trial. However, there were no significant differences in the butyric and propionic acids after intervention in control and probiotic groups, as shown in Fig. 3. Additionally, no significant difference was found between changes of the two groups in butyric, acetic and propionic acids \( p > 0.05 \), respectively. Interestingly, a higher proportion of propionic acid and a lower proportion of butyric acid were recorded in both groups. At end of trial, the proportion of propionic: acetic: butyric acids, taking into account the mean values, was also similar: 10: 8: 1 in the control group and 14: 10: 1 in the probiotic group.

### 4. Discussion

This is the first clinical trial to assess the impact of probiotic use on fructosamine, faecal SCFA, IL-10, resistin, adiponectin, and F2-isoprostane in T2D patients. It is speculated that improvement in the inflammatory markers, stress oxidative status, and SCFA contributes to diabetes control [14,22].

The present study showed that fermented milk consumption significantly decreased inflammatory cytokines (TNF-\( \alpha \) and...
resistin) in both treatments (control vs probiotic), as well as caused a significant increase in the acetic acid, but only the probiotic group showed improvement in glycemic control, as demonstrated by fructosamine and HbA1c. The main differences between the results were: significant decrease in IL-10, a tendency of decreasing in adiponectin levels, and significant increase in TC and LDL-C, which were observed only in control group. These results indicate that these last factors hinder the improvement in glycemic control of subjects in the control group. Furthermore, total phenolic content and antioxidant activity were significantly higher in the probiotic fermented milk (see Supplemental Table 1).

Most of the previous animal studies that evaluated the effect of probiotics on glycemic control in T2D, related that probiotics, especially Lactobacillus, can reduce FPG, HbA1c, and insulin, beyond inflammatory markers, such as IL-2, INF-g, IL-6, and TNF-a [5,23,24]. The intervention time ranged from 8 to 14 wk. Increase in GLUT4 mRNA expression levels in adipose tissue has also been reported [5,25].

Concerning clinical trials, previous studies including diabetic subjects have shown controversial results, especially regarding glycemic control and oxidative stress. Ejtahed et al. [26], reported reduced in HbA1c (p < 0.05) after 6 wk of yogurt intake containing 10^10 CFUs of L. acidophilus La-5 and B. lactis BB-12. On the other hand, L. acidophilus NCFM capsule (10^10 CFUs) intake during 4 wk, did not change the glycemic control, insulin, IL-1, IL-6, TNF-a, and protein C reactive (CRP) in T2D subjects [27]. Also, multispecies probiotic supplementation (L. acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus bulgaricus, B. breve, B. longum, and S. thermophiles – 10^10 CFUs each) associated with fructo-oligosaccharide (100 mg) during 8 wk did not result in significant reductions in FPG and a significant increase in the levels of insulin, HOMA-IR, and LDL-C was found in both groups [28]. Subsequently, this same research group reported that daily consumption of B. coagulans (10^7 CFUs) and 1 g of inulin in 124 diabetic patients [29]. On the order hand, after consumption of probiotic yogurt containing L. acidophilus La-5 and B. lactis BB-12 for 6 wk, there was an increase in erythrocyte superoxide dismutase (SOD) and GPx activities, and TAS (p < 0.05), although no significant changes were observed in HbA1c, insulin, and erythrocyte catalase activity [26].

We also hypothesized that the improvement in glycemic control after probiotic treatment could be mediated, in part, through immune-modulatory effects. To capture these interrelations, were selected a set of key inflammatory and anti-inflammatory cytokines, including adipokines produced primarily by adipocytes (adiponectin), macrophages (resistin and IL-6), or both (TNF-a), all related to glycemic control and insulin resistance [32,33].

Although other studies mention the relationship between inflammation and gut microbiota [10,34], few experimental and clinical studies investigated this association in the diabetes context [5,27,35]. Some experimental studies reported reductions in inflammatory cytokines, and/or improvements in glucose and insulin metabolisms after ingestion of Lactobacillus spp during 14 wk [5,35]. Two randomized trials did not observe effects on inflammatory cytokines after the intake during 4 or 6 wk of L. acidophilus NCFM (10^10 CFUs) or L. acidophilus, L. bulgaricus, Lactobacillus bifidum, and L. casei (CFUs and strain not reported), respectively [16,27]. In agreement with these previous studies, IL-6 did not change after intervention, however, TNF-a and resistin levels decreased in both treatments in our study. It has been shown that anti-inflammatory activities of goat milk oligosaccharides reside in their ability to function either as prebiotics or as a receptor for microorganisms (Daddaoua et al., 2006). Others studies shows the ability of bioactive peptides derived from food fermentation in the immune system regulation, including inhibition of NF-kB, a pro-inflammatory transcription factors [36,37].

In regard to anti-inflammatory cytokines (IL-10 and adiponectin), no changes were observed in the probiotic group, unlike

### Table 2

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control (n = 22)</th>
<th>Probiotic (n = 23)</th>
<th>Intervention effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 6</td>
<td>Comparisons</td>
</tr>
<tr>
<td>FPG, mmol/L</td>
<td>7.38 (2.37)</td>
<td>7.54 (2.59)</td>
<td>p&lt;0.16</td>
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<td>Fru, mmol/L</td>
<td>295.5 (62.23)</td>
<td>296.90 (39.64)</td>
<td>1.36</td>
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<tr>
<td>HbA1c, %</td>
<td>5.35 (49.6–1.1)</td>
<td>5.66 (49.6–1.1)</td>
<td>0.01</td>
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<tr>
<td>Insulin, μU/mL</td>
<td>8.3 (5.0–10.1)</td>
<td>6.56 (5.2–1.65)</td>
<td>−11.4</td>
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<td>HOMA-IR</td>
<td>2.15 (1.71)</td>
<td>2.3 (1.55)</td>
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<tr>
<td>TC, mmol/L</td>
<td>4.85 (1.32)</td>
<td>5.30 (1.19)</td>
<td>0.55</td>
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<tr>
<td>LDL-C, mmol/L</td>
<td>2.24 (1.24)</td>
<td>2.60 (1.11)</td>
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<tr>
<td>HDL-C, mmol/L</td>
<td>1.51 (0.26)</td>
<td>1.53 (0.34)</td>
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<td>L</td>
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<td>TC-HDL-C</td>
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<td>3.48 (0.97)</td>
<td>0.28</td>
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<td>LDL-C-HDL-C</td>
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<td>TAG, mmol/L</td>
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<td>1.99 (0.91)</td>
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<td>TAS, (mM)</td>
<td>0.31 (0.12)</td>
<td>0.33 (0.08)</td>
<td>0.02</td>
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<tr>
<td>F2-isoc (pg/mL)</td>
<td>62.99 (33.67)</td>
<td>63.42 (32.35)</td>
<td>−0.43</td>
</tr>
</tbody>
</table>

Data are means (SD) or median (P25–P75). FPG – fasting blood glucose; Fru – fructosamine; TC – total cholesterol; TAG – triglyceride; TAS – total antioxidant status; F2-isoc – F2-isoprostane.

a Difference between baseline and endpoint. p value obtained from paired t test/Wilcoxon matched-pairs signed-rank test for the within-group comparisons.
b Obtained from unpaired Student’s t test or Mann–Whitney test, as statistical difference between changes (control vs probiotic).
Fig. 2. Effects of the fermented milks intake on cytokine levels. Data are shown as mean (SD). *p < 0.05, †p = 0.001 from paired t test or for Wilcoxon matched-pairs signed-rank test, as statistical within group differences (baseline vs endpoint). IL = interleukin; TNF-α = tumor necrosis factor alpha.

Fig. 3. Effects of the fermented milks intake on faecal short-chain fatty acid concentrations. Data are shown as mean (SD). †p ≤ 0.01 from Wilcoxon matched-pairs signed-rank test, as statistical within group differences (baseline vs endpoint).
the control group, which showed a significant reduction at the end of trial. Thus, our results suggest that the reduction in these cytokines reflected on lower glycemic control observed in the control group. Recently, Mohamadshahi et al. [38], also reported that the consumption of probiotic yogurt enriched with *L. acidophilus* La-5 and *B. lactis* BB-12 (10^6 CFUs) for 8 wk caused significant decrease in HbA1c and TNF-α levels in the intervention group, but no changes were observed in IL-6 and hs-CRP levels. In mice, *L. rhamnosus* GG orally administrated for 13 wk improves insulin sensitivity by stimulating adiponectin secretion and consequent activation of AMPK [39], highlighting the important role of adiponectin in glycemic control. Some contradictory results may be explained by complex regulation of cytokines, and we point out that the production of IL-6 and IL-10 is stimulated by TNF-α, which was reduced in the present study.

Additionally, microbiota components account for the production of SCFA, which are linked to anti-inflammatory mechanisms (inhibition of NF-kB) and also exerting a protective function in favor of intestinal epithelium [40]. However, reports on the ability of lactic acid bacteria to modulate SCFA at the intestinal level are limited. In our literature review [41], we didn’t find experimental or clinical trials that evaluated the effect of probiotics intake on the SCFA in T2D context. Interestingly, growing evidence suggests a cross talk between SCFA and probiotics in glycemic control, especially butyric and propionic acids via the G protein coupled receptors FFA2 and FFA3 [42,43]. However, we found a significant increase in acetic acid after intervention in both groups, which has little effect on incretins (GLP-1, PYY, and GIP) secretion [42] compared to butyric and propionic acids. This increase in the acetic acid in both treatments may be attributed to phenolic compounds present in FM [44].

With regard to lipid profile, contradictory results have also been shown in the literature. *L. acidophilus* La-5 and *B. lactis* BB-12 (10^6 CFUs) used contributed to the decrease of TC (4.5%) and LDL-C (7.5%) levels (p < 0.05) after 6 wk in T2D subjects [45]. In a different study, the same probiotics taken for 8 wk, caused reductions in LDL-C/HDL-C (p = 0.01) and increase HDL-C levels [46]. However, other studies using different species of *Lactobacillus* or *B. coagulans* and inulin for 6 wk did not observe effects on the lipid profile in T2D subjects [16,29]. Our study demonstrated that consumption of probiotic FM prevented a rise in TC and LDL-C compared to the control group, which can be attributed, in part, by the higher total phenolic concentrations and antioxidant capacity in probiotic FM, however it was not enough to cause significant reductions in these parameters in the probiotic group.

Finally, probiotics as functional foods offer great potential to improve health and/or help prevent certain diseases when taken as part of a balanced diet and healthy lifestyle. Our results showed low intake of micronutrients and dietary fiber, and high intake of saturated fatty acids (see Supplemental Table 4). In conclusion, this trial suggests that probiotic consumption improves the glycemic control in T2D subjects, however, the intake of fermented goat’s milk seems to be involved with changes in inflammatory markers (TNF-α and resistin) and in the acetic acid concentrations. The major findings of this study are the following: 

(i) *L. acidophilus* and *B. lactis* intake seem to be associated with a decrease of the fructosamine and HbA1c levels; (ii) fermented goat’s milk consumption appears to be related to acetic acid content in faecal samples; (iii) the role of probiotic in averting an increase of CT and LDL-C and a reduction of anti-inflammatory cytokines. In our study, the study design was designed to be a short-term trial, our results need to be confirmed in long-term trials testing the hypothesis that probiotic supplementation is effective in improving glycemic control, regulating SCFA levels and decreasing oxidative stress in T2D subjects.

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### Clinical trial registry

This trial is registered with ensaioclínicos.gov.br/rgr/RBR-219644.

### Conflict of interest

None.

### Authors contributions

LBT, KMOS, and HSDM designed the trial. LBT and KMOS were responsible for the analysis and production of fermented milk. LBT and HSDM conducted the research—were responsible for study recruitment, screening, and delivery of interventions. LBT and HSDM did the statistical analysis. LBT and LLO analyzed and interpreted stress oxidative data. All authors participated in data interpretation. LBT wrote the first draft of the report, and all other authors commented on the draft and approved the final version. Authorship order reflects overall contribution to the work presented. None of the authors had a conflict of interest.

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### Appendix A. Supplementary data

Supplementary data related to this article can be found at [http://dx.doi.org/10.1016/j.clnu.2015.11.011](http://dx.doi.org/10.1016/j.clnu.2015.11.011).

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