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ORIGINAL CONTRIBUTION

# 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

**Somayyeh Firouzi[1] · Hazreen Abdul Majid[2] · Amin Ismail[1] · Nor Azmi Kamaruddin[3] · Mohd-Yusof Barakatun-Nisak[1]**

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**Abstract**
*Aim* Evidence of a possible connection between gut microbiota and several physiological processes linked to type 2 diabetes is increasing. However, the effect of multi-strain probiotics in people with type 2 diabetes remains unclear. This study investigated the effect of multi-strain microbial cell preparation—also refers to multi-strain probiotics—on glycemic control and other diabetes-related outcomes in people with type 2 diabetes.
*Design* A randomized, double-blind, parallel-group, controlled clinical trial.
*Setting* Diabetes clinic of a teaching hospital in Kuala Lumpur, Malaysia.
*Participants* A total of 136 participants with type 2 diabetes, aged 30–70 years, were recruited and randomly assigned to receive either probiotics ($n = 68$) or placebo ($n = 68$) for 12 weeks.
*Outcomes* Primary outcomes were glycemic control-related parameters, and secondary outcomes were anthropomorphic variables, lipid profile, blood pressure and high-sensitivity C-reactive protein. The *Lactobacillus* and *Bifidobacterium* quantities were measured before and after intervention as an indicator of successful passage of the supplement through gastrointestinal tract.
*Statistical analysis* Intention-to-treat (ITT) analysis was performed on all participants, while per-protocol (PP) analysis was performed on those participants who had successfully completed the trial with good compliance rate.
*Results* With respect to primary outcomes, glycated hemoglobin decreased by 0.14 % in the probiotics and increased by 0.02 % in the placebo group in PP analysis ($p < 0.05$, small effect size of 0.050), while these changes were not significant in ITT analysis. Fasting insulin increased by 1.8 μU/mL in placebo group and decreased by 2.9 μU/mL in probiotics group in PP analysis. These changes were significant between groups at both analyses ($p < 0.05$, medium effect size of 0.062 in PP analysis and small effect size of 0.033 in ITT analysis). Secondary outcomes did not change significantly. Probiotics successfully passed through the gastrointestinal tract.
*Conclusion* Probiotics modestly improved HbA1c and fasting insulin in people with type 2 diabetes.

**Keywords** Glycated hemoglobin · Glycemic control · High-sensitivity C-reactive protein · Homeostasis model assessment-estimated insulin resistance · Probiotics · Type 2 diabetes mellitus · Blood pressure · Lipid profile

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✉ Mohd-Yusof Barakatun-Nisak
bnisak@upm.edu.my

[1] Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia

[2] Department of Social and Preventive Medicine, Faculty of Medicine, Centre for Population Health, University of Malaya, Kuala Lumpur, Malaysia

[3] Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

## Introduction

In Asia, type 2 diabetes (T2D) occurrence has been on the rise since the past 15 years [1] with the rate of increase showing no sign of slowing [1]. On a more local front, the prevalence of T2D in Malaysian adults aged 30 years and

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above was 22.9 % in 2013 [2] with majority of them being poorly controlled [3]. Medical nutrition therapy is the cornerstone of diabetes management; however, nutritional status and dietary adherence seemed to be far from the target treatment goal [3].

Diet is considered a primary determinant of a gut microbiota [4]. This is important in the current context because there are emerging evidences that gut microbiota can possibly influence several physiological functions that are linked to T2D [5]. Studies have shown some differences in the gut microbiota composition between people with and without T2D [6]. People with T2D are at risk to be in a low-grade chronic inflammation state, a condition that can be caused and/or aggravated by a compromised gut barrier function usually due to unhealthy gut microflora [7]. Hence, these findings suggest the possibility that modulating gut microbiota by probiotics supplementation may lead to favorable outcomes in people with T2D.

Probiotics are microbial cell preparations (MCP) or components of microbes that confer a health benefit on the host when administered in adequate amounts [8]. The beneficial role of probiotics in improving glycemic control has been discussed in the literature [9], and few numbers of clinical trials investigated the effect of probiotics in people with T2D. In three of the Iranian studies [10–12], two studies [10, 11] found positive effects on glycemic-related parameters after probiotics supplementation. The major limitations of these studies, however, were small sample size and inconsistent results. Besides, they were conducted among Iranian only and their results may not be extrapolated to Malaysian population due to the genetic differences, food consumption pattern, as well as environmental and cultural differences.

Probiotics have also demonstrated beneficial effects in other diabetes-related conditions including body weight, inflammatory markers and blood pressure. Study has shown that diversity of gut microbiota composition was strongly associated with body weight and fat mass [13]. In contrast, the meta-analysis of randomized controlled trials did not unravel any significant effect of probiotics on weight loss [14]. However, the total number of the studies included in the meta-analysis, total sample size and quality of the studies warranted future investigation [14].

The positive effect of probiotics on inflammatory markers has been demonstrated in animal models of T2D [15, 16]. Nevertheless, similar observation in human trials is limited and inconclusive due to the very small sample size [10–12]. Although two clinical trials have demonstrated promising improvements in blood pressure after being supplemented with probiotics among hypertensive participants [17] as well as healthy individuals [18], there are limited data regarding the blood pressure-lowering effect of probiotics among type 2 diabetics. With respect to potential lipid-lowering effect of probiotics, a meta-analysis by Guo et al. [19] demonstrated a positive effect of probiotics on lipid profile. However, the included studies had small sample size (32–78 participants) and small duration of intervention (4–10 weeks). Overall, the beneficial effects of probiotics on other diabetes-related parameters are inconclusive and scarce especially in the context of T2D.

To address the research gap, this randomized clinical trial was performed to investigate the effect of multi-strain probiotics supplementation on glycemic control and other diabetes-related outcomes in people with T2D. This study tested the hypotheses that supplementation with multi-strain probiotics will improve glycemic control as a primary outcome and other clinically important diabetes-related measures which include inflammatory marker, blood pressure, lipid profile and anthropometry status as secondary outcomes.

## Methodology

This 12-week randomized, double-blind, parallel-group, placebo-controlled trial was conducted at the diabetes clinic of a teaching hospital in Kuala Lumpur, Malaysia. The protocol was approved by the Clinical Research and Ethics Committee of the Universiti Kebangsaan Malaysia Medical Center, conformed to the contents of the 1975 Declaration of Helsinki as revised in 1983, and was registered at the US National Institutes of Health Web site (http://www.clinicaltrials.gov) #NCT01752803.

### Participants selection

All the patients attended the diabetes clinic from February 2012 to December 2013 were screened for the study. Potential participants were approached by three trained research assistants at the hospital or by making phone calls to briefly explain the study. Then, the main researcher described the study (procedure, risks/benefits/voluntary participation, no compensation) to those willing to join while they were given adequate time to consult with their doctors and families. Those participants who agreed to join the study signed a consent form. Eligible participants include those with established T2D for at least 6 months prior to the commencement of the study, not on insulin and antibiotics, aged 30–70 years, had glycated hemoglobin A1c (HbA1c) between 6.5 and 12 %, had fasting blood glucose (FBG) <15 mmol/L, had BMI between 18.5 and 40 kg/m$^2$, and on a stabilized dose of medication for at least 3 months before the study. The initial proposed range of age and HbA1c was extended due to the difficulties in fulfilling the sample size. The exclusion criteria were pregnancy, lactation, advanced diabetes complications (except

for hyperlipidemia and blood pressure) and having other chronic illnesses. The sample size calculation was based on effect of probiotics among T2D [10] in which a 0.4 % difference in HbA1c and a common standard deviation of 0.7 % were required to detect a significant improvement in primary outcomes with 80 % power. Anticipating 40 % dropout, withdrawal and non-compliance, a total of 68 participants were needed in each group.

### Intervention procedure

The random allocation sequence was generated by the main researcher with blocks of four and eight using a computer model (www.randomization.com). Using blocks of four and eight allowed having exact number of 68 participants in each group. The main researcher was in possession of allocation sequence and allocated the recruited participants accordingly. Sachets containing either probiotics—the term used throughout this study to refer to microbial cell preparations (MCP)—or a placebo were given to the participants according to their group assignment. Sachets were identical in terms of nutritional value, appearance, texture, weight and smell and were only differentiated by a code ("A" or "B") placed on them. All the procedures were performed by the main researcher. The main researcher and participants remain blinded to the contents of the sachets throughout the study procedure and the statistical analysis. An independent person who was not related to the study and the study product held the blinding codes. Code breaking was performed after analysis was completed and database was locked.

All the participants have received standard medical nutrition therapy according to the guidelines for T2D [20] as a standard clinical procedure in this hospital. They were also again received the same recommendation at baseline aiming to homogenize their food intake regardless of the assigned group. Energy requirement was prescribed based on quick method formula as follows: Energy requirement = weight (kg) × quick method factor. Values of the quick method factor were determined based on the participant's BMI and physical activity level [21]. The proportion of macronutrients was calculated accordingly with 45–60 % energy coming from carbohydrate, 15–20 % protein and 25–30 % fat [20]. The food exchange list was provided to all participants to allow a variety of food choices within each food group. Participants were asked to cease consumption of all foods and products known to contain probiotics bacteria 2 weeks before the study and throughout the study period. They were also informed not to change their routine intake of fiber content of their diet as an indication of prebiotic intake. The dietary advice was given by the main researcher.

Participants were asked to fast for at least 10–12 h prior to each time point of assessment. A fasting blood sample was taken and processed for further analysis. The measurements were taken at baseline and week 12 with an interim analysis at week 6.

Participants were also asked to report any adverse effect through phone calls or during follow-up visits. Adverse effects were also assessed in follow-up visits through face-to-face interviews and recorded in the case report form. In the case of minor and tolerable adverse effects, participants were given the option either to continue or to stop taking the supplement. However, they were still asked to complete the follow-up visits even after they stopped taking the supplements. An expected common adverse effect includes minor gastrointestinal disturbances. Nevertheless, participants who experienced unexpected serious events were asked to stop taking the supplements and were encouraged completing the study.

### Supplementation and compliance

Supplementation was provided by Hexbio® B-Crobes Laboratory Sdn. Bhd. (Ipoh, Malaysia). Each sachet provided a $3 \times 10^{10}$ dose of six viable microbial cell preparation strains: three strains from the genus *Lactobacillus, Firmicutes* phyla (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus lactis*) and three strains from the genus *Bifidobacterium* and *Actinobacteria* phyla (*Bifidobacterium bifidum*, *Bifidobacterium longum and Bifidobacterium infantis*). The daily dose of each strain was $10^{10}$ CFUs. Participants were asked to keep the sachets at a dry place below 25 °C and away from direct sunlight. They were also asked to mix the contents of the sachets with one glass of water (approximately 250 mL) and drink twice per day (morning and evening) before or after meal. Participants were asked to bring the remaining sachets to determine the compliance to supplementation with acceptable means of compliance between 85 and 100 % [22]. The acute toxicity test of supplement showed that this supplement was not considered as hazardous, and compatibility test of the products showed that all six strains are compatible with each other.

### Outcome measurements

All the measurements were taken at baseline, week 6 and week 12. Primary outcome was glycemic control, and secondary outcomes include other diabetes-related variables including anthropometry and BMI, lipid profile, blood pressure and high-sensitivity C-reactive protein (hs-CRP). Fecal sample was analyzed in order to quantify the amount of *Lactobacillus* and *Bifidobacterium* subspecies plural (spp.) before and after the supplementation.

Body weight was measured using a digital scale (SECA; London British Indicators, London, UK) to the nearest 0.1 kg. Height was measured using the height attachment on the same scale to the nearest 0.1 cm. Body mass index (BMI) was calculated and categorized according to the World Health Organization classification [23]. Waist circumference was measured to the nearest 0.1 cm using a flexible tape. Blood pressure was measured with the use of an automatic blood pressure monitor (Omron M4-I; Omron Healthcare Europe BV, Hoofdorp, Netherlands) with participants resting in a sitting position for 5–10 min prior to measurement.

Blood samples were collected after 10–12 h overnight fasting. Blood was centrifuged for 15 min at $2555 \times g$, and serum was stored frozen at −20 °C until they were analyzed except for FBG and HbA1c which were analyzed within 2 h after blood collection. FBG were measured using the enzymatic method and hs-CRP using particle-enhanced immunoturbidimetric assay. Triglycerides (TG) and total cholesterol (TC) were measured by specific enzymatic assay. The high-density lipoprotein (HDL) level was detected by homogeneous assay. The Cobas® 8000 modular analyzer, series 702 (Roche Diagnostics, Mannheim, Germany) was used for analysis of hs-CRP, FBG, TG, HDL and TC. HbA1c was measured by ion-exchange high-performance liquid chromatography using the Adams A1c HA-8160 automated HbA1c analyzer (Arkray, Inc., Kyoto, Japan) in the diabetes mode. Serum insulin was determined using solid-phase, two-site chemiluminescent enzyme-labeled immunometric assay using the Immulite (R) 2000 Analyser (Siemens, Gwynedd, UK). The homeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated using Matthews et al.'s equation [24]. The quantitative insulin sensitivity check index (QUICKI) was calculated using Katz et al.'s equation [25]. The levels of low-density lipoprotein (LDL) were calculated using the Friedewald et al.'s equation [26].

Dietary intake assessment was carried out using 3-day food records in which participants were asked to record their daily intake (2 weekdays and 1 weekend) before each visit to ensure that their food intakes were homogenous throughout the study period. The accuracy of the recorded foods including portion size, preparation, cooking method and food brand were checked and reviewed with the participants at each visit by main researcher. Nutrient analysis was performed using a computerized dietary analysis program (Nutritionist Pro, version 2.0; First Data Bank, Hearst Corp., NY, USA) by a trained dietitian. The results were presented as mean daily intakes of energy, macronutrients, fiber, sodium and cholesterol. Physical activity level was assessed by short form of the Malay version of the International Physical Activity Questionnaire (IPAQ), whose validity has been previously verified [27].

## Fecal analysis

The quantities of *Lactobacillus* and *Bifidobacterium* spp. in fecal samples were measured at baseline and week 12 to demonstrate the successful passage of the supplements from gastrointestinal tract. In accordance with similar previous studies [6, 28], a subsample of participants (the last 20 participants from each group) was selected for determination of the number of colony-forming units (CFUs) of the *Lactobacillus* and *Bifidobacterium* spp. before and after the intervention using plate-counting method. Fecal samples were transformed to the laboratory in ice box within 3 h upon collection. De Man Rogosa and Sharpe agar (Difco, USA) supplemented with L-Cystein was opted as a selective media for culturing *Bifidobacterium* subspecies plural (spp.) [29]. Rogosa S/L agar was chosen as a selective media for *Lactobacillus* spp. [30]. Media were prepared according to the manufacturers' instructions.

After performing serial dilution of fecal samples, 0.1 mL of dilutions of $10^{-3}$–$10^{-6}$ were cultured under anaerobic conditions produced by gas pack (Oxoid Ltd, Basingstoke, UK) and placed in an incubator for 48 h. The number of colonies was then counted under a colony-counting machine (Stuart Scientific, Staffordshire, UK). Before colony counting, several colonies from each subject were selected for morphology and gram staining. The accuracy of the counted colonies was confirmed using polymerase chain reaction (PCR) (See supplementary materials).

## Statistical analysis

Statistical analyses were performed using SPSS software version 22 (SPSS Inc., Chicago, USA). The significance level for the differences between and within groups was set at $p < 0.05$. Data were normally distributed unless otherwise stated. In this case, data were log-transformed to improve the skewness of the data into a normalized distribution. Baseline characteristics between the two groups were analyzed using independent sample *t* test or Chi-square test where necessary.

Sensitivity analyses included intention to treat (ITT) and per protocol (PP). ITT analysis was performed on a complete set of data with those missing data imputed according to the last observation carried forward (LOCF) method. On the other hand, PP analysis was performed on those participants who had successfully completed the 12-week intervention with more than 85 % compliance rate. In this article, the results presented are related to PP analysis unless otherwise stated, while the results of ITT analysis are reflected by the *p* values.

General linear model analysis of variance (GLM ANOVA) was performed separately for each group to obtain the results of within-group analysis. Changes

between the groups as an indicator of interaction were analyzed using the same test considering grouping as the between-factor level. The partial eta-squared was calculated to determine the magnitude of change, with an effect size of 0.01–0.05 considered small, 0.06–0.13 medium and ≥0.14 large. Variables with significant changes were further analyzed using within-subject contrast test to determine at which time point the changes were significant. Subanalysis was also performed to determine the effect of probiotics in normal weight and overweight and obese (OW/OB) participants using the GLM ANOVA.

## Results

### Recruitment and subject flow

A total of 6976 patients were screened, of which 456 were identified eligible and have an updated contact in the hospital system. A total of 136 (29.8 %) patients were then agreed to participate. The attrition rate at the end of the 12-week intervention was 20.6 % without consideration of non-compliance and 25.7 % with their consideration. The compliance rate was comparable between groups (placebo = 89.05 %, probiotics = 86.15 %, $p = 0.319$). For the purpose of PP analyses, 48 participants in probiotics and 53 participants in placebo group completed the intervention successfully with compliance rate of >85 % (Fig. 1). None of the participants routinely consumed probiotics supplements or food sources that are rich in probiotics.

### Demographic characteristics, compliance and adverse effects

A total of 136 participants (52.2 % male) were randomly allocated to the probiotics ($n = 68$) or placebo ($n = 68$) group. The two groups were comparable at baseline (Table 1). Majority of the participants were treated with oral anti-diabetic (OAD) agents. Only one (1.5 %) from the placebo and 6 (8.8 %) from Probiotic group were on diet alone. The proportion of participants on OAD was comparable between the groups ($p = 0.144$). The proportion of participants on lipid-lowering drugs and antihypertensive drugs were also comparable between groups. Majority of the participants were OW/OB [placebo 14 (21 %), probiotics 18 (26 %)], and the BMI of the participants was comparable between groups ($p = 0.419$).

The supplement was generally acceptable by the participants, and they were satisfied with the taste and smell of it. In terms of the adverse effects, few of them had minor gastric disturbances while two unexpected events were observed in the probiotic group that was unlikely due to the intervention (sexual impotency and carbuncle). The incidence of adverse effects was comparable between groups ($p = 0.284$).

### Changes in dietary intake and physical activity levels

Dietary intake and physical activity levels did not change significantly between the groups over the course of the study (Table 2). Fiber intake as a source of prebiotic was also comparable between two groups throughout the study (6.9 ± 4.8 in probiotic group and 6.6 ± 3.8 in placebo group at baseline vs. 6.4 ± 4.4 in probiotic group and 7.0 ± 5.0 in placebo group at week 12, $p = 0.874$). As these variables were comparable between the groups at baseline and throughout the study period, they did not require adjustment in the analysis of the data.

### Changes in primary outcomes

The HbA1c has marginally increased in placebo group, while it decreased 0.14 ± 0.41 % in probiotics group. These changes was significant between groups ($p < 0.05$, effect size 0.050; Table 3), but not significantly different within each group. According to within-subject contrast test, these changes were significant between baseline and week 6 and between baseline and week 12 ($p < 0.05$) (Fig. 2). ITT analysis revealed that HbA1c remained unchanged in the placebo group while marginally decreased in the probiotics group, although not to a statistically significant extent.

Fasting insulin decreased significantly between the groups ($p < 0.05$, effect size 0.062) (Table 3). Within-subject contrast test of PP analysis indicated that these changes were only significant between baseline and week 12 ($p < 0.05$) (Fig. 2). In ITT analysis, they were significant between baseline and both week 6 and week 12 ($p < 0.05$, effect size: 0.033). Other glycemic control parameters did not change significantly between groups throughout the study. It was also noted that the HOMA-IR value significantly increased by 23 % in the placebo group (0.4 ± 2.0, $p < 0.05$, paired $t$ test between baseline values and week 12) and significantly decreased by 13 % in the probiotics group (0.4 ± 1.7, $p < 0.05$, paired $t$ test between baseline values and week 12).

### Changes in secondary outcomes

In both ITT and PP analyses, anthropometric parameters, BMI, hs-CRP, lipid profile and blood pressure did not change significantly between groups (Table 3). The TG levels improved in probiotics group as compared to placebo group, but not significant.

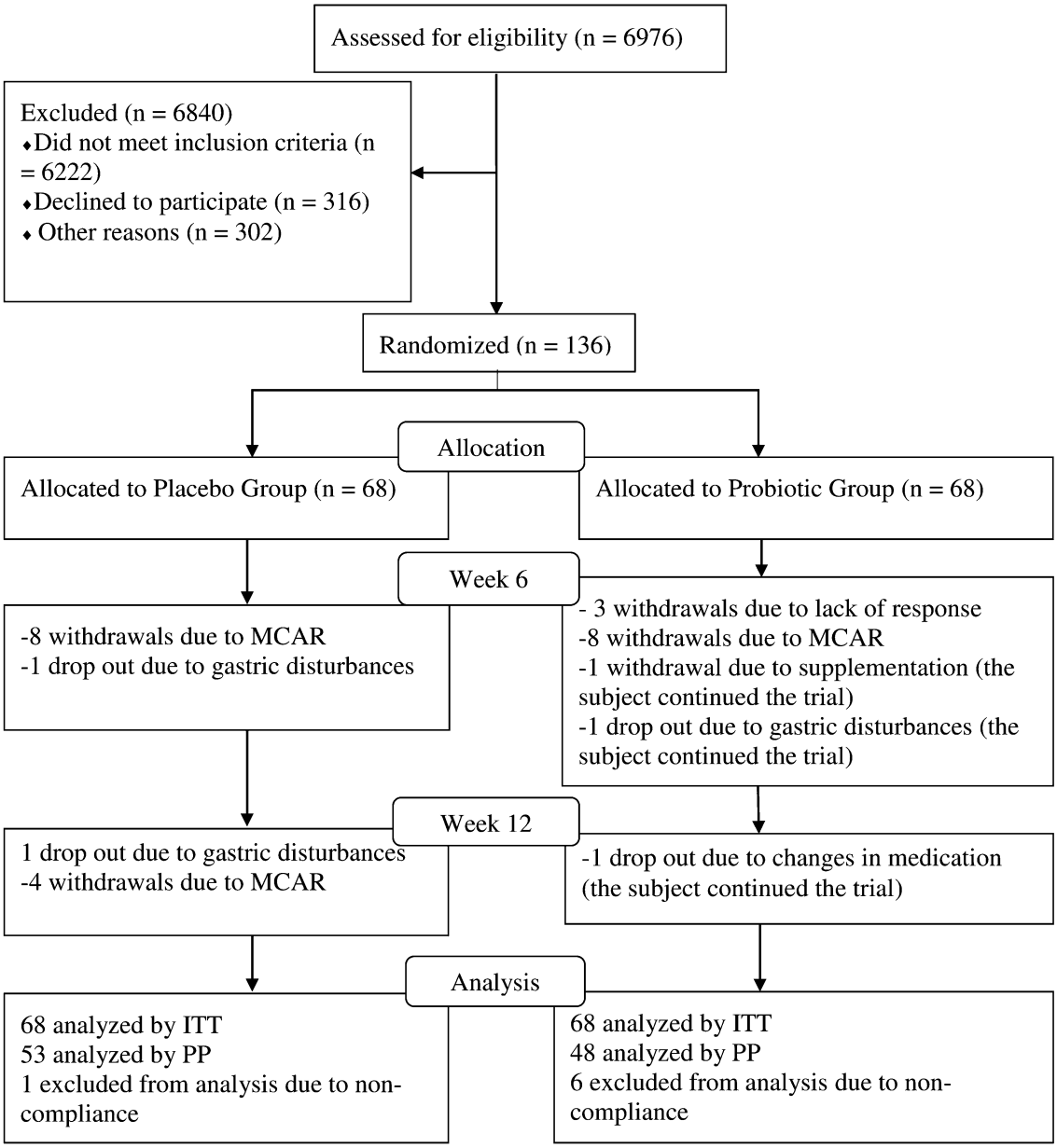


**Fig. 1** CONSORT flowchart

### Changes in quantities of *Lactobacillus* and *Bifidobacterium* in fecal sample

For the fecal subsampling analyses, there were two participants from each group withdrew from the study. Hence, in total 18 participants in each group completed the fecal sampling procedures. While the colony-forming units (CFUs) of *Bifidobacterium* spp. increased marginally (almost twofold) in the placebo group, the CFUs have increased significantly (up to 4.5-fold) in the probiotic group (Table 3). The quantities of *Lactobacillus* spp. increased in both groups. The increment was not significant in placebo group, but the twofold increase in probiotics group was significant in PP analysis.

### Changes in subgroups

In the subgroup PP analyses, participants were classified according to the BMI categories [normal weight ($n = 28$) and OW/OB ($n = 73$)]. Analysis of glycemic control

**Table 1** Baseline characteristics of participants in each group

| Baseline parameter | Placebo group ($n = 68$) Mean ± SD | Probiotics group ($n = 68$) Mean ± SD | $p$ value |
|---|---|---|---|
| **Age (years)** | 54.2 ± 8.3 | 52.9 ± 9.2 | 0.362 |
| **Anthropometric characteristics** | | | |
| Weight (kg) | 76.6 ± 15.6 | 74.6 ± 15.1 | 0.514 |
| Height (cm) | 161.8 ± 9.4 | 160.0 ± 8.4 | 0.285 |
| Waist circumference[b] | | | |
| Male (cm) | 102.0 ± 13.7 | 100.4 ± 13.5 | 0.429 |
| Female (cm) | 96.7 ± 9.5 | 97.3 ± 14.6 | 0.618 |
| BMI ($kg/m^2$) | 29.3 ± 5.3 | 29.2 ± 5.6 | 0.837 |
| **Dietary intake** | | | |
| Energy (kcal) | 1508 ± 503 | 1473 ± 402 | 0.485 |
| % of calories from carbohydrates | 53.9 ± 8.2 | 54.1 ± 7.9 | 0.917 |
| % of calories from protein | 14.7 ± 3.7 | 16.8 ± 3.9 | 0.612 |
| % of calories from fat | 28.6 ± 5.4 | 29 ± 2 | 0.917 |
| Fiber (g) | 6.5 ± 3.8 | 6.9 ± 4.7 | 0.283[a] |
| Sodium (mg) | 1840 ± 919 | 1522 ± 639 | 0.065 |
| Cholesterol (mg) | 153 ± 107 | 145 ± 96 | 0.683[a] |
| **Physical activity levels** | | | |
| Total physical activity score (MET_min/wk) | 1989 ± 1869 | 1784 ± 2100 | 0.570 |
| Sedentary activity (hours/day) | 5.5 ± 3.0 | 6.2 ± 3.3 | 0.212 |
| **Glycemic control parameters** | | | |
| FBG (mmol/L) | 8.0 ± 2.0 | 8.1 ± 2.2 | 0.600[a] |
| HbA1c (%) | 7.65 ± 1.31 | 7.58 ± 1.3 | 0.795[a] |
| Fasting insulin (μU/mL) | 14.4 ± 10.1 | 11.6 ± 7.3 | 0.569[a] |
| HOMA-IR (units) | 4.2 ± 2.4 | 3.2 ± 1.8 | 0.419[a] |
| QUICKI (units) | 0.23 ± 0.04 | 0.24 ± 0.00 | 0.118 |
| **Inflammatory markers** | | | |
| hs-CRP (mg/L) | 3.31 ± 3.15 | 2.75 ± 3.72 | 0.975[a] |
| **Lipid profile** | | | |
| TG (mmol/L) | 1.45 ± 0.60 | 1.48 ± 0.57 | 0.721[a] |
| TC (mmol/L) | 4.62 ± 0.82 | 4.93 ± 1.12 | 0.504 |
| HDL (mmol/L) | 1.28 ± 0.27 | 1.32 ± 0.32 | 0.389 |
| LDL (mmol/L) | 2.75 ± 0.76 | 2.83 ± 0.89 | 0.670 |
| Mean blood pressure | | | |
| Systolic (mmHg) | 138.8 ± 16.9 | 142.5 ± 20.7 | 0.339 |
| Diastolic (mmHg) | 79.8 ± 10.5 | 81.8 ± 10.0 | 0.966 |
| ***Lactobacillus* and *Bifidobacterium* load in stool** | | | |
| *Lactobacillus* spp. (CFU/g) | $2.0 \times 10^7 \pm 0.7 \times 10^7$ | $2.1 \times 10^6 \pm 4.9 \times 10^6$ | 0.092 |
| *Bifidobacterium* spp. (CFU/g) | $2.6 \times 10^6 \pm 0.9 \times 10^7$ | $0.3 \times 10^6 \pm 8.2 \times 10^6$ | 0.322 |

*BMI* body mass index, *FBG* fasting blood glucose, *HbA1c* glycated hemoglobin, *HOMA-IR* homeostasis model assessment-estimated insulin resistance, *hs-CRP* high-sensitivity C-reactive protein, *MET_min/wk* metabolic equivalent of task_minute/week, *QUICKI* quantitative insulin sensitivity check index, *SD* standard deviation, *spp* subspecies plural

[a] Log-transformed independent sample *t* test

[b] Male: $n = 34$ in placebo group, $n = 31$ in probiotics group, female: $n = 34$ in placebo group, $n = 37$ in probiotics group

**Table 2** Dietary intake throughout the study in probiotics and placebo group

| | *n* | Mean ± SD | | | Within-group *p* value | Week 6 − baseline | Week 12 − baseline | Interaction *p* value | |
|---|---|---|---|---|---|---|---|---|---|
| | | Baseline | Week 6 | Week 12 | | | | PP | ITT |
| Energy (kcal) | | | | | | | | 0.592 | 0.582 |
| Probiotics group | 48 | 1473 ± 402 | 1448 ± 366 | 1443 ± 440 | 0.267 | −25 ± 500 | −30 ± 424 | | |
| Placebo group | 53 | 1508 ± 503 | 1389 ± 425 | 1494 ± 347 | 0.973 | −119 ± 375 | −14 ± 495 | | |
| % of energy from carbohydrate | | | | | | | | 0.739 | 0.780 |
| Probiotics group | 48 | 54.1 ± 7.9 | 57.2 ± 7.5 | 57.5 ± 8.5 | 0.337 | 3.1 ± 6.9 | 3.4 ± 6.8 | | |
| Placebo group | 53 | 53.9 ± 8.2 | 55.9 ± 8.1 | 53.8 ± 8.5 | 0.543 | 2.0 ± 7.9 | −0.1 ± 8.1 | | |
| % of energy from protein | | | | | | | | 0.412 | 0.169 |
| Probiotics group | 48 | 17.1 ± 3.7 | 16.5 ± 2.9 | 17.5 ± 4.0 | 0.866 | −0.6 ± 4.4 | −0.4 ± 4.8 | | |
| Placebo group | 53 | 16.3 ± 3.9 | 16.0 ± 3.9 | 16.0 ± 4.0 | 0.122 | −0.3 ± 3.3 | 0.3 ± 3.8 | | |
| % of energy from fat | | | | | | | | 0.094 | 0.128 |
| Probiotics group | 48 | 29.2 ± 5.8 | 27.0 ± 6.8 | 26.6 ± 4.2 | 0.021 | −2.2 ± 6.0 | −2.6 ± 5.5 | | |
| Placebo group | 53 | 28.6 ± 5.4 | 28.8 ± 6.5 | 29.1 ± 5.9 | 0.957 | 0.2 ± 5.9 | 0.5 ± 6.0 | | |
| Fiber (g) | | | | | | | | 0.354 | 0.287 |
| Probiotics group | 48 | 6.9 ± 4.8 | 8.8 ± 11.2 | 6.4 ± 4.4 | 0.221 | 1.9 ± 4.3 | −0.5 ± 4.2 | | |
| Placebo group | 53 | 6.6 ± 3.8 | 6.5 ± 4.6 | 7.0 ± 5.0 | 0.766 | −0.1 ± 4.0 | 0.4 ± 4.4 | | |
| Sodium (mg) | | | | | | | | 0.051 | 0.058 |
| Probiotics group | 48 | 1583 ± 687 | 1738 ± 749 | 1776 ± 820 | 0.157 | 155 ± 524 | 193 ± 801 | | |
| Placebo group | 53 | 2036 ± 913 | 1843 ± 1074 | 1777 ± 823 | 0.200 | −193 ± 960 | −259 ± 992 | | |
| Cholesterol (mg) | | | | | | | | 0.298 | 0.290 |
| Probiotics group | 48 | 153 ± 107 | 130 ± 97 | 139 ± 102 | 0.379 | −23 ± 87 | −14 ± 114 | | |
| Placebo group | 53 | 153 ± 107 | 139 ± 89 | 163 ± 101 | 0.445 | −14 ± 105 | 10 ± 106 | | |
| Total physical activity score (MET_min/wk) | | | | | | | | | |
| Probiotics group | 48 | 1848 ± 2086 | 2166 ± 2262 | 2251 ± 3047 | 0.442 | 318 ± 1860 | 403 ± 1725 | 0.225 | 0.136 |
| Placebo group | 53 | 2068 ± 1848 | 1745 ± 2166 | 1817 ± 2251 | 0.466 | −323 ± 1902 | 72 ± 1808 | | |
| Sedentary activity (hours/day) | | | | | | | | 0.283 | 0.339 |
| Probiotics group | 48 | 6.2 ± 3.3 | 5.8 ± 3.2 | 5.8 ± 3.1 | 0.647 | −0.4 ± 3.0 | −0.4 ± 2.5 | | |
| Placebo group | 53 | 5.5 ± 3.0 | 5.6 ± 2.8 | 5.3 ± 2.6 | 0.224 | 0.1 ± 1.9 | −0.2 ± 2.5 | | |

*n* number of participants, *SD* standard deviation, *ITT* intention to treat, *PP* per protocol

parameters among normal weight and OW/OB revealed that normal weight participants had better improvements in terms of glycemic control parameters compared to OW/OB as indicated by the effect size (Table 4). However, this significant difference was only observed in HbA1c levels (Fig. 3). TG level was also improved significantly in normal weight participants in ITT analysis (marginally significant in PP analysis) while did not changed significantly among OW/OB participants (Fig. 4). Other diabetes-related parameters, however, did not show any trend of differences among normal weight or OW/OB participants between the two groups.

## Discussion

This study found that probiotics supplementation significantly improved HbA1c in PP analysis and fasting insulin in both ITT and PP analyses in the probiotic group compared to those in the placebo group. In a subsequent analysis based on the BMI category, participants in the normal weight category from the probiotic group have significantly decreased HbA1c levels in both ITT and PP analyses and TG levels in ITT analysis. However, it does not significantly affect FBG, insulin resistance, insulin sensitivity, weight, waist circumference, BMI, hs-CRP, TC, HDL or LDL profile or blood pressure.

### Effect of probiotic on glycemic control as a primary outcome measure

Improvements in glycemic control as seen in this study were in accordance with several studies reported previously [10, 12, 31, 32]. However, there were also 3 studies [11, 33, 34] that did not find any improvement in glycemic control after probiotics supplementation. Two

**Table 3** Changes of variables throughout the study

| | *n* | Mean ± SD | | | Within-group *p* value | Week 6 − baseline | Week 12 − baseline | Interaction *p* values | |
|---|---|---|---|---|---|---|---|---|---|
| | | Baseline | Week 6 | Week 12 | | | | PP | ITT |
| Glycemic control parameters and inflammatory marker | | | | | | | | | |
| FBG (mmol/L)[a] | | | | | | | | 0.667 | 0.955 |
| Probiotics group | 48 | 7.3 ± 1.1 | 7.0 ± 1.2 | 7.2 ± 1.2 | 0.814 | −0.3 ± 1.7 | −0.1 ± 1.5 | | |
| Placebo group | 53 | 7.9 ± 2.2 | 8.0 ± 2.3 | 8.2 ± 2.3 | 0.577 | 0.1 ± 2.2 | 0.3 ± 2.1 | | |
| HbA1c (%)[a] | | | | | | | | <0.05 | 0.582 |
| Probiotics group | 48 | 7.46 ± 1.2 | 7.33 ± 1.2 | 7.32 ± 1.4 | 0.061 | −0.14 ± 0.41 | −0.14 ± 0.62 | | |
| Placebo group | 53 | 7.29 ± 1.6 | 7.28 ± 1.6 | 7.31 ± 1.7 | 0.094 | −0.01 ± 0.41 | 0.02 ± 0.56 | | |
| Insulin (μU/mL)[a] | | | | | | | | <0.05 | <0.05 |
| Probiotics group | 48 | 13.1 ± 8.6 | 10.8 ± 6.8 | 10.2 ± 6.3 | 0.050 | −2.3 ± 6.8 | −2.9 ± 8.5 | | |
| Placebo group | 53 | 12.0 ± 6.7 | 12.5 ± 6.8 | 13.8 ± 6.3 | 0.231 | 0.5 ± 12.2 | 1.8 ± 9.0 | | |
| HOMA-IR (units)[a] | | | | | | | | 0.155 | 0.263 |
| Probiotics group | 48 | 3.1 ± 1.7 | 2.7 ± 1.3 | 2.7 ± 1.6 | 0.593 | −0.4 ± 1.7 | −0.4 ± 1.8 | | |
| Placebo group | 53 | 3.8 ± 2.1 | 4.2 ± 2.3 | 4.7 ± 2.8 | 0.057 | 0.4 ± 2.0 | 0.9 ± 2.0 | | |
| QUICKI (units) | | | | | | | | 0.833 | 0.277 |
| Probiotics group | 48 | 0.24 ± 0.04 | 0.24 ± 0.04 | 0.24 ± 0.04 | 0.989 | 0.00 ± 0.36 | 0.00 ± 0.04 | | |
| Placebo group | 53 | 0.23 ± 0.04 | 0.22 ± 0.04 | 0.22 ± 0.04 | 0.380 | −0.01 ± 0.04 | −0.01 ± 0.02 | | |
| hs-CRP (mg/L)[b] | | | | | | | | 0.401 | 0.570 |
| Probiotics group | 48 | 2.35 ± 2.81 | 2.65 ± 3.09 | 2.58 ± 2.80 | 0.845 | 0.30 ± 2.9 | 0.23 ± 2.7 | | |
| Placebo group | 53 | 2.76 ± 3.00 | 2.89 ± 3.14 | 2.40 ± 2.56 | 0.514 | 0.13 ± 3.1 | −0.36 ± 3.0 | | |
| Anthropometric parameters | | | | | | | | | |
| Weight (kg) | | | | | | | | 0.302 | 0.288 |
| Probiotics group | 48 | 75.3 ± 15.9 | 75.1 ± 15.2 | 75.0 ± 15.2 | 0.415 | −0.2 ± 1.4 | −0.3 ± 1.7 | | |
| Placebo group | 53 | 74.2 ± 14.3 | 74.4 ± 15.5 | 75.1 ± 14.7 | 0.526 | 0.2 ± 1.6 | 0.9 ± 1.6 | | |
| BMI (kg/m$^2$) | | | | | | | | 0.364 | 0.345 |
| Probiotics group | 48 | 29.5 ± 5.7 | 29.4 ± 5.6 | 29.4 ± 5.5 | 0.695 | −0.1 ± 0.7 | −0.1 ± 0.7 | | |
| Placebo group | 53 | 28.4 ± 5.1 | 28.5 ± 5.4 | 29.4 ± 5.7 | 0.387 | 0.1 ± 0.6 | 1.0 ± 0.6 | | |
| Waist circumference (cm) | | | | | | | | | |
| Males | | | | | | | | 0.294 | 0.876 |
| Probiotics group | 26 | 100 ± 13 | 102 ± 12 | 101 ± 12 | 0.535 | 2 ± 7 | 1 ± 6 | | |
| Placebo group | 28 | 98 ± 13 | 102 ± 12 | 100 ± 11 | 0.315 | 4 ± 22 | 2 ± 8 | | |
| Female | | | | | | | | 0.678 | 0.986 |
| Probiotics group | 21 | 100 ± 15 | 98 ± 15 | 98 ± 15 | 0.044 | −2 ± 5 | −2 ± 3 | | |

**Table 3** continued

| | *n* | Mean ± SD | | | Within-group *p* value | Week 6 − baseline | Week 12 − baseline | Interaction *p* values | |
|---|---|---|---|---|---|---|---|---|---|
| | | Baseline | Week 6 | Week 12 | | | | PP | ITT |
| Placebo group | 26 | 95 ± 10 | 94 ± 10 | 94 ± 11 | 0.707 | −1 ± 4 | −1 ± 5 | | |
| Lipid profile | | | | | | | | | |
| TG (mmol/L) | | | | | | | | 0.156 | 0.230 |
| Probiotics group | 48 | 1.37 ± 0.45 | 1.32 ± 40 | 1.25 ± 0.43 | 0.131 | −0.05 ± 0.40 | −0.12 ± 0.49 | | |
| Placebo group | 53 | 1.23 ± 0.46 | 1.27 ± 0.48 | 1.24 ± 0.41 | 0.973 | 0.04 ± 0.41 | 0.01 ± 0.35 | | |
| TC (mmol/L) | | | | | | | | 0.368 | 0.749 |
| Probiotics group | 48 | 4.76 ± 0.93 | 4.81 ± 0.98 | 4.70 ± 0.94 | 0.565 | 0.05 ± 0.73 | −0.06 ± 0.72 | | |
| Placebo group | 53 | 4.39 ± 0.95 | 4.60 ± 0.83 | 4.56 ± 0.81 | 0.208 | 0.21 ± 0.80 | 0.17 ± 0.81 | | |
| HDL (mmol/L) | | | | | | | | 0.944 | 0.974 |
| Probiotics group | 48 | 1.34 ± 0.33 | 1.31 ± 0.31 | 1.31 ± 0.30 | 0.489 | −0.03 ± 0.19 | −0.03 ± 0.19 | | |
| Placebo group | 53 | 1.31 ± 0.37 | 1.28 ± 0.35 | 1.27 ± 0.34 | 0.585 | −0.03 ± 0.18 | −0.04 ± 0.19 | | |
| LDL (mmol/L) | | | | | | | | 0.727 | 0.564 |
| Probiotics group | 48 | 2.65 ± 0.82 | 2.80 ± 0.89 | 2.66 ± 0.80 | 0.268 | 0.15 ± 0.68 | −0.05 ± 0.67 | | |
| Placebo group | 53 | 2.65 ± 0.73 | 2.69 ± 0.80 | 2.56 ± 0.72 | 0.467 | 0.04 ± 0.66 | −0.09 ± 0.55 | | |
| Blood pressure | | | | | | | | | |
| Systolic (mmHg) | | | | | | | | 0.631 | 0.549 |
| Probiotics group | 48 | 139.5 ± 27.0 | 133.6 ± 14.7 | 131.4 ± 15.2 | 0.023 | −5.9 ± 14.6 | −8.1 ± 19.4 | | |
| Placebo group | 53 | 133.1 ± 16.5 | 129.7 ± 16.6 | 128.5 ± 12.9 | 0.103 | −3.4 ± 14.1 | −4.6 ± 13.4 | | |
| Diastolic (mmHg) | | | | | | | | 0.931 | 0.835 |
| Probiotics group | 48 | 80.8 ± 10.4 | 79.4 ± 12.0 | 77.9 ± 10.8 | 0.218 | −1.4 ± 9.4 | −2.9 ± 10.1 | | |
| Placebo group | 53 | 77.9 ± 10.1 | 76.2 ± 9.0 | 77.5 ± 9.0 | 0.202 | −1.7 ± 9.2 | −0.4 ± 7.1 | | |
| Quantities of *Lactobacillus* spp. and *Bifidobacterium* spp. | | | | | | | | | |
| *Lactobacillus* spp. (CFU/g) | | | | | | | | <0.05 | 0.224 |
| Probiotics group | 18 | $6.4 \times 10^6 \pm 1.1 \times 10^6$ | – | $1.2 \times 10^7 \pm 6.4 \times 10^6$ | <0.05 | – | $5.6 \times 10^6 \pm 1.2 \times 10^2$ | | |
| Placebo group | 18 | $1.8 \times 10^7 \pm 0.7 \times 10^7$ | – | $2.1 \times 10^7 \pm 9.2 \times 10^6$ | 0.647 | – | $3 \times 10^6 \pm 1.2 \times 10^6$ | | |
| *Bifidobacterium* spp. (CFU/g) | | | | | | | | <0.05 | <0.05 |
| Probiotics group | 18 | $3.4 \times 10^6 \pm 0.2 \times 10^6$ | – | $1.4 \times 10^7 \pm 1.5 \times 10^7$ | <0.05 | – | $10.6 \times 10^6 \pm 1.6 \times 10^5$ | | |
| Placebo group | 18 | $2.7 \times 10^6 \pm 0.8 \times 10^6$ | – | $5.3 \times 10^6 \pm 1.9 \times 10^6$ | 0.749 | – | $2.6 \times 10^6 \pm 8.0 \times 10^5$ | | |

Log-transformed

*FBG* fasting blood glucose, *HbA1c* glycated hemoglobin, *CFU* colony-forming unit, *HDL* high-density lipoprotein, *HOMA-IR* homeostasis model assessment-estimated insulin resistance, *hs-CRP* high-sensitivity C-reactive protein, *ITT* intention to treat, *LDL* low-density lipoprotein, *PP* per protocol, *SD* standard deviation, *spp* subspecies plural, *TC* total cholesterol, *TG* triglycerides, *QUICKI* quantitative insulin sensitivity check index

of these studies were conducted among OW/OB participants [33, 34]. This could partly be explained by our observation among OW/OB participants that have less impact following probiotics supplementation [33, 34]. Lack of improvement in the study conducted by Mazlom et al. [11] could be due to the small sample size. The discrepancy of the results from different studies may also be partly attributed to the medications used by the participants. Different studies imposed different inclusion criteria with regard to the medications. Pharmacodynamics may have been affected by probiotics, especially gliclazide [35].

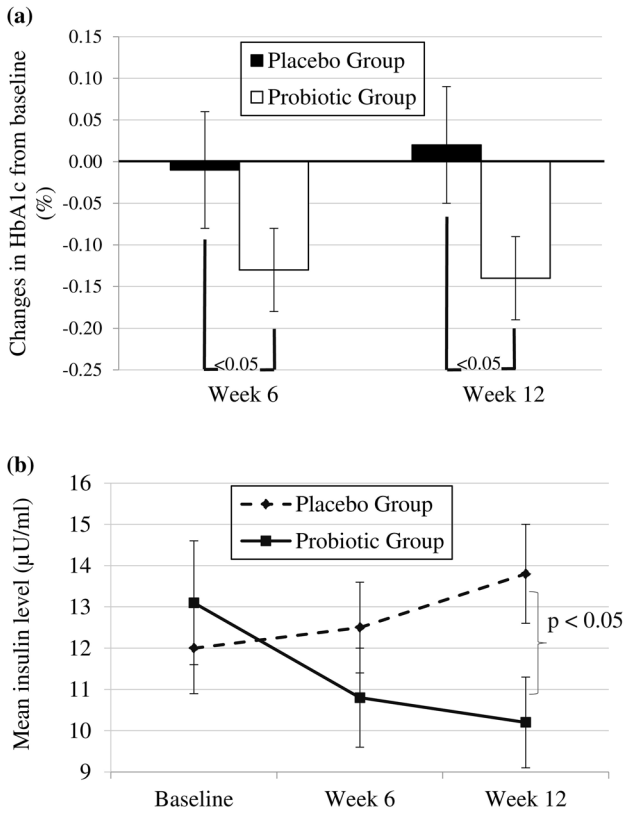


**Fig. 2** Mean ± SE of changes in HbA1c (**a**) and insulin (**b**) from baseline to week 6 and week 12 in probiotics and placebo groups

In this study, probiotics supplementation significantly improved HbA1c, while it has no effect on FBG. A notable fact is that in fairly controlled diabetes, FBG contributes approximately 30 % and postprandial blood glucose contributes approximately 70 % to HbA1c [36]. Thus, it can be assumed that improvements in postprandial blood glucose led to decreasing HbA1c in the probiotics group. Indeed, FBG is also influenced by several factors including physical activity [37], duration since the last meal [38] and a range of other neuroendocrine factors [39]. Hence, any change in the FBG levels due to intervention is difficult to be assessed.

Probiotics supplementation improved fasting insulin in this study which contradicts the results of the previous studies [10–12]. One of the possible reasons for this discrepancy could be the short duration of intervention in these studies which was 6 [10, 11] and 8 weeks [12] on the basis of PP analyses. We observed no significant changes within the first 6 week, yet the improvement in fasting insulin appeared significant after 12 week of intervention. Insulin resistance showed some improvements in this study, while insulin sensitivity did not change in this and previous studies [10–12].

Recent evidences showed that intestinal microbiota may play an important role in the early development of metabolic disease [40]. In this context, the effect of probiotics supplementation on HbA1c and fasting insulin was important as it triggered the underlying cause of impaired metabolism rather than treating the consequences.

### Effect of probiotics on other diabetes-related parameters as secondary outcomes

We did not observe significant changes in body weight. In general, losing weight is more challenging in people with diabetes than healthy individuals, especially when the reduction was accompanied with the improvement in glycemic control [41]. In people with poorly controlled diabetes, the improvements in glycemic control will also lead to a reduction in energy expenditure, which prevents further weight loss [41].

The current study noted non-significant improvements in TG levels, while other lipid parameters did not show

**Table 4** Effect sizes of the interaction between supplementation and glycemic control variables over the course of study in normal weight and OW/OB participants

| Glycemic control parameters | Effect size | |
|---|---|---|
| | Normal weight group ($n = 28$) | OW/OB group ($n = 73$) |
| FBG | 0.057 (small) | 0.014 (small) |
| HbA1c | 0.204 (large)* | 0.015 (small) |
| Insulin | 0.104 (medium) | 0.049 (small) |
| HOMA-IR | 0.068 (medium) | 0.044 (small) |
| QUICKI | 0.010 (small) | 0.009 (small) |

* $p = 0.008$

any improvements. Numerous studies have shown controversial results, of which some reported that probiotics supplementation significantly reduces blood lipids [42, 43], while other studies did not report any improvements after probiotics supplementation [44, 45]. A study showed that insulin acts as anti-lipolysis agent in adipose tissue and stimulates lipoprotein lipase [45]. Thus, with the abnormal level of insulin in people with type 2 diabetes, improving lipid profile after probiotics supplementation might be more difficult compared to people without diabetes. Suboptimal lipid profiles may attribute to the poor glycemic management of Malaysians with type 2 diabetes [46].

Not achieving the significant outcomes in lipid profiles and blood pressure may be caused by the type of the supplements. Studies have proven that the cholesterol-lowering effects of probiotics were only seen when they were combined with dairy products [47]. Similarly, in terms of blood pressure, the angiotensin-converting enzyme inhibitors exist in the form of proteins in some fermentable foods and can be converted into active form through fermentation process by probiotics bacteria [48]. Therefore, consuming probiotics in the current form without any substrate might explain the non-significant improvement in blood pressure in this study.

Overall, the impact of probiotics supplementation on hs-CRP remains controversial. This is in line with other human studies [11] which found no significant improvements in hs-CRP after probiotics supplementation. The levels of hs-CRP were in the normal range at baseline in both the groups. It is possible that probiotics were unable to modulate the hs-CRP because the levels were already substantially low. Another possible explanation is that majority of the participants were on statins. Studies have shown that statins affect hs-CRP levels [49, 50].

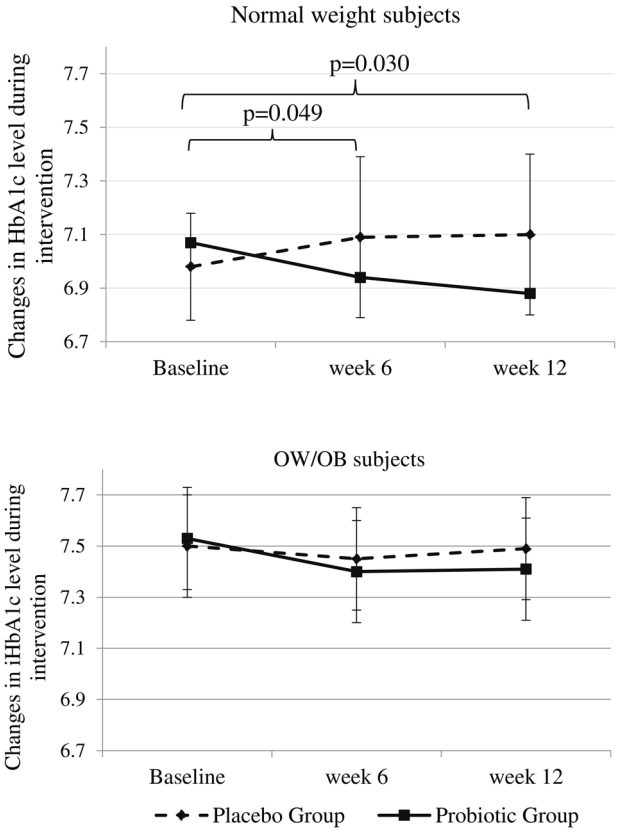


**Fig. 3** Mean ± SE of changes in HbA1c levels between study groups in week 6 and week 12 among normal weight and OW/OB participants in PP analysis

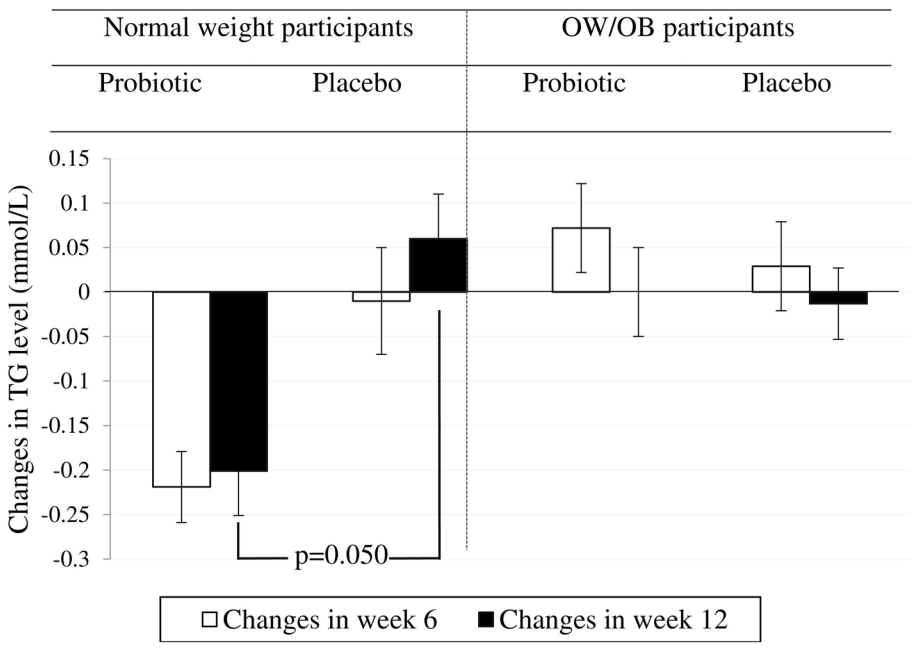


**Fig. 4** Mean ± SE of changes in TG levels between study groups in week 6 and week 12 among normal weight and OW/OB participants in ITT analysis

**Effect of probiotics on the quantities of *Lactobacillus* and *Bifidobacterium* spp.**

Probiotics supplementation significantly increased *Lactobacillus* and *Bifidobacterium* load in this study, thus proving successful passage of the supplement through the gastrointestinal tract. In the current study, the amount of *Lactobacillus* spp. and *Bifidobacterium* spp. increased in placebo group at week 12 though they were not statistically significant. Retrospective assessment of dietary intake to locate any increase in prebiotic and probiotics sources did not discover any foods that contributed to it.

*Effect of probiotics in subgroup analyses among normal weight and OW/OB participants*

After classification of BMI [51], we found probiotics supplementation has different outcomes in normal weight participants compared with OW/OB counterparts. While this subgroup was unplanned analysis in a small sample of size, the larger improvement observed in normal weight participants in terms of glycemic control and TG compared to OW/OB participants deserved further discussion. Even though it can be argued that we have more participants ($n = 78$) falling within OW/OB than the normal weight category ($n = 28$), the larger improvement documented within normal weight participants was unlikely related to sample size. The reason may be the fact that the *Bacteroidetes*-to-*Firmicutes* (B/F) ratio is lower in obese than in normal weight counterparts [52, 53], with *Actinobacteria* comprising a bigger proportion of the microbiota structure [52]. Since the quantity of *Firmicutes* is stable in both groups, the proportion is likely modulated by changes in *Bacteroidetes* [52]. The three phyla constitute more than 90 % of gut microbiota population [52, 53]. Considering the gut ecosystem as a competent ecosystem [50], supplementation with *Lactobacillus* and *Bifidobacterium*, which belong to the *Firmicutes* and *Actinobacteria* phyla, respectively [54], will change the ecology of gut microbiota. The B/F ratio, which has been found to be correlated with insulin resistance [55], appears to become much lowered in OW/OB compared with normal weight participants after supplementation with these phyla. The difference in this ratio between normal weight and OW/OB participants might explain differences in their response to probiotics. Indeed, there is a possibility that the dosage of probiotics may not be sufficient enough to allow any significant improvements in OW/OB participants, hence requiring the use of weight-based dosage regimens for significant changes.

Changes in TG levels after probiotics supplementation can be explained by the role of insulin in anti-lipolysis in adipose tissue and as a stimulant for lipoprotein lipase [56]. It can be hypothesized that a decrease in insulin levels in the current study leads to a reduction in the level or activity of lipoprotein lipase in adipose tissue. Therefore, less free fatty acids and TG are produced by the body. This hypothesis gains ground in the analysis of subgroups whereby the decrease in TG levels was more profound and significant among normal weight participants within probiotics group who had also better reduction in insulin levels compared with OW/OB participants in the same group.

This study was unable to explain the possible mechanism behind the effect of probiotics on glycemic and lipid control as presented in the result. We have hypothesized several mechanisms of action according to the previous literature. The effect of multi-strain probiotics in improving glycemic control can be firstly explained through the action of primary bile acids on the farnesoid X receptor (FXR). Certain strains of *Lactobacillus* and *Bifidobacterium* are known to possess the bile salt hydrolase enzyme (BSH). This enzyme can directly increase the levels of primary bile acid which in turn binds and activates the FXR, leading to increased storage of glucose, decreased production of glucose from non-glucose nutrients, increases synthesis of insulin and increases the secretion of insulin [57, 58]. Secondly, probiotics are also known to increase glucagon-like peptide (GLP) 1 and GLP 2 which are able to decrease low-grade inflammation associated with diabetes, decrease insulin resistance which in turn decreases ß-cell toxicity and improves glycemic control. Furthermore, GLP 1 and GLP 2 also decrease hunger and increase satiety, thus decreasing energy intake which collectively improved glycemic control [59]. Another possible mechanism is the increased production of short-chain fatty acids (SCFAs) by mainly *Bifidobacterium* in the colon via its action through insoluble dietary fibers. These SCFAs especially butyrate can decrease insulin resistance by promoting pancreatic ß-cells differentiation, proliferation and development, increase the secretion of GLP-1, thus increasing secretion of insulin, and decrease the release of proinflammatory cytokines by adipose tissue [60, 61]. A compromised gut barrier function can aggravate the pre-existing low-grade inflammatory state associated with T2D. Probiotics are known for their activity in restoring the gut barrier function. When the gut barrier function is not intact, there is an onset of metabolic endotoxemia. This condition increases the expression of selected proteins that suppresses the insulin signaling pathways. As a result, the body becomes less responsive to insulin and results in insulin resistance [7].

This study has several strengths that contributed to the reliability and validity of the findings. Possible confounding factors (dietary intake, particularly fiber intake and physical activity level) were well controlled. The participants were also asked to refrain from any prebiotics supplement or probiotics consumption 2 weeks before commencement of study and throughout the study. The

significant increases in the quantities of *Lactobacillus* and *Bifidobacterium* species from stool samples enabled determination that the results could be attributed to probiotics supplementation.

The limitations of this study should be considered. Main researcher was in charge of randomization, while it would have had an added advantage if disclosing randomization sequence had been performed by a third person. Indeed, stool analysis was performed on a subsample of the last recruited participants which by right they should be randomly selected. Although this subsample was enough to draw a significant difference in the *Lactobacillus* and *Bifidobacterium* load before and after the intervention, the results would be more precise if a stool sample had been taken from all participants. Measuring the detailed microbial profile of the stool before and after intervention would have provided assurance in demonstrating that the observed improvements were probiotics mediated. Another area of concern is the length of the study. Diabetes-related complications usually occur over a long period of time and 12-week intervention might not be enough to reverse the condition.

## Conclusion

Probiotics supplementation was associated with improvements in HbA1c and fasting insulin. Normal weight participants experience significant improvement in HbA1c and TG with probiotics supplementation compared to OW/OB participants. The multi-strain probiotics in the study also proved viable through gut transit. This study conducted among type 2 diabetics who were moderately well controlled, but caution should be taken for those who are uncontrolled and those on insulin, as current research only specially investigated in this group. Future research should focus on identifying the role of and the complex interaction among probiotics and the proportion of the various phyla in the gut in normal weight and OW/OB individuals. Besides, the effect of this multi-strain probiotics supplement should be investigated in large cohorts and the possible pathways involved in its beneficial glycemic- or lipid-lowering effects should be uncovered.

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**Author's contribution** The authors' responsibilities were as follows: SF (PhD student) was involved in study conception, study coordination, study conduct, laboratory testing and analysis, statistical analysis and manuscript writing; HAM critically reviewed the manuscript; AI reviewed the manuscript; NAK was involved in administrative coordination; MY B-N served as principle investigator, was involved in study conception and study coordination and reviewed the manuscript. All authors were responsible for the study design.

**Compliance with ethical standards**

**Conflict of interest** The authors have nothing to disclose.

**Ethical approval** The protocol was approved by the Clinical Research and Ethics Committee of the Universiti Kebangsaan Malaysia Medical Center, conformed to the contents of the 1975 Declaration of Helsinki as revised in 1983, and was registered at the US National Institutes of Health Web site (http://www.clinicaltrials.gov) #NCT01752803.

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