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Original Article

Effect of alive probiotic on insulin resistance in type 2 diabetes patients: Randomized clinical trial

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ABSTRACT

Background: Probiotics have beneficial effect on obesity related disorders in animal models. Despite a large number of animal data, randomized placebo-controlled trials (RCT) concluded that probiotics have a moderate effect on glycemic control-related parameters. However, effect of probiotics on insulin resistance are inconsistent.
Aim: In a double-blind single center RCT, effect of alive multistrain probiotic vs. placebo on insulin resistance in type 2 diabetes patient were assessed.
Methods: A total of 53 patients met the criteria for inclusion. They were randomly assigned to receive multiprobiotic "Symbiter" (concentrated biomass of 14 probiotic bacteria genera *Bifidobacterium, Lactobacillus, Lactococcus, Propionibacterium*) or placebo for 8-weeks administered as a sachet formulation. The primary main outcome was the change HOMA-IR (homeostasis model assessment-estimated insulin resistance) which calculated using Matthews et al.'s equation. Secondary outcomes were the changes in glycemic control-related parameters, anthropomorphic variables and cytokines.
Results: Supplementation with alive multiprobiotic for 8 weeks was associated with significant reduction of HOMA-IR from 6.85 ± 0.76 to 5.13 ± 0.49 ($p = 0.047$), but remained static in the placebo group. With respect to our secondary outcomes, HbA1c insignificant decreased by 0.09% ($p = 0.383$) and 0.24% ($p = 0.068$) respectively in placebo and probiotics groups. However, in probiotic responders ($n = 22$, patient with decrease in HOMA-IR) after supplementation a significant reduction in HbA1c by 0.39% ($p = 0.022$) as compared to non-responders was observed. In addition, from markers of chronic systemic inflammatory state only TNF-α and IL-1β changes significantly after treatment with probiotics.
Conclusion: Probiotic therapies modestly improved insulin resistance in patients with type 2 diabetes.

1. Introduction

Type 2 diabetes (T2D) is an epidemic, consider to be a challenge to public health and economy due to its complications that lead to disability. Annually the number of patients suffered from T2D are rapidly increases. WHO estimates that, globally, 422 million adults aged over 18 years were living with diabetes in 2014 [1]. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, and now about 1 in 11 adults worldwide have diabetes mellitus, 90% of whom have T2DM [2]. T2D results in the significant enhance in mortality. In 2012 there were 1.5 million deaths worldwide directly caused by diabetes. It was the eighth leading cause of death among both sexes [3]. Despite efforts to find radical treatments the growth and spread of the epidemic can not be stopped. Treatment strategies are heterogeneous and impact on different pathogenic links. But high risk of complications are remains even when patients achieving an optimal glycemic control. Given this all above, these problem needs the ensuing study, perhaps a new approach to understanding and finding new methods of treatments.

Family history of diabetes, overweight and obesity, unhealthy diet, physical inactivity and smoking are the strongest risk factors for T2D. Recent evidence suggests that the gut microbiota are involved in diabetes and metabolic disorders [4,5]. A clear relationship has been demonstrated between T2D and

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compositional changes in the gut microbiota, with a lower relative abundance of Firmicutes and a higher proportion of Bacteroidetes and Proteobacteria in T2D patients as compared to non-diabetic counterparts [6–8].

Data from animal studies revealed that altered microbiota may contribute to the pathogenesis of insulin resistance (IR) and thereby T2D by several mechanisms [9]. Gut microbiota increased production of short chain fatty acids (SCFAs) - acetate, propionate and butyrate. SCFAs are important source of energy for *de novo* lipogenesis and are ligands for receptors of free fatty acids (FFAR) - Gpr41 (FFAR3) (Gpr41 - G-protein- coupled receptors) and Gpr43 (FFAR2) in intestine enteroendocrine cells [10]. SCFAc by activating these receptors can decrease insulin resistance, promote pancreatic ß-cells proliferation and development [11,12]. Secondly gut microbiota by itself or via SCFAs can stimulate the secretion of GLP-1 and GLP-2, thus increasing insulin and adiponectin expression, might contribute to the enhanced insulin sensitivity, and decrease low-grade inflammation associated with T2D [13–15]. A compromised gut barrier function with an increased intestinal permeability lead to lipopolysaccharide (LPS) elevation and involved in the development of metabolic endotoxemia [16]. LPS binding to CD14 TLR-4 (toll-like receptor-4) receptor complex on the surface of macrophage and epithelial cells can acts as a trigger factor that induces the secretion of proinflammatory cytokines and aggravate the pre-existing low-grade inflammation which lead to IR development [16,17]. Finally, gut microbiota via modulation of bile salt hydrolase enzyme (BSH) activity, can directly increase the levels of primary bile acid which in turn binds and activates the farnesoid X receptor (FXR) and TGR5. Activation of 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 [18,19]. Activation of TGR5 lead to increased energy expenditure and have been linked with beneficial effects on glucose metabolism, such as improved insulin sensitivity and postprandial glycemic control [20,21].

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [22]. The data from animal studies suggested that probiotics beneficial effects can affect body weight, influence on glucose and fat metabolism, improve insulin sensitivity and reduce chronic systemic inflammation [4]. Probiotics have beneficial effect on obesity related disorders in animal models. Despite a large number of animal data, randomized placebo-controlled trials (RCT) concluded that probiotics have a moderate effect on glycemic control-related parameters and basically reported for Lactobacillus and/or Bifidobacterium strains [23,24]. However, effect of probiotics on insulin resistance are inconsistent.

Here we performed a double-blind single center RCT, effect of alive multistrain probiotic vs. placebo on insulin resistance in type 2 diabetes patient. We chose this probiotic based on our previous comparative experimental analysis of different probiotic strains in obesity prevention. In this animal study, we assess beneficial effects of lyophilized mono-probiotic (*B.animalis VKL*, *B.animalis VKB*, *L.casei IMVB-7280*), the combination of this three strains and multiprobiotic "Symbiter" containing biomass of 14 alive probiotic strains(*Lactobacillus* + *Lactococcus* (6×10^{10} CFU/g), *Bifidobacterium* (1×10^{10}/g), *Propionibacterium* (3×10^{10}/g), *Acetobacter* (1×10^{6}/g). We have shown that supplementation of probiotic composition, with preference to alive strains, led to a significantly lower prevalence of obesity, reduction of visceral adipose tissue weight and serum lipid levels as compared to single-strain probiotic [25,26].

Our primary aim was to investigate the effect of supplementation on IR. Secondary aims were to investigate effects on other glycemic control-related parameters, anthropomorphic variables and cytokines.

2. Methods

The study protocol was approved by local ethics committees of Kyiv City Clinical Endocrinology Center and was conducted according to the guidelines of the 1975 Declaration of Helsinki, and was registered at the US National Institutes of Health Web site (http://www.clinicaltrials.gov) #NCT03434860. Prior to the study, purpose and methodology of the study were fully explained to the participants by the researchers, and all patients gave written informed consent before any study procedures were initiated.

2.1. Study design

In this single-center double blind, placebo controlled, parallel group study, 53 T2D patients from the Kyiv City Clinical Endocrinology Center were selected. They were randomly assigned to receive multiprobiotic “Symbiter” or placebo for 8-weeks administered as a sachet formulation in double-blind treatment. Randomization was done by the study statistician based on a computer-generated list. The groups were homogeneous according to age, sex and diagnostic criteria. The assignment of groups was blind to participants, research staff and outcome assessors moreover, to maintain blind parallel study the statistician was not aware of the allocation of participants to intervention.

The multiprobiotic "Symbiter" was supplied by Scientific and Production Company “O.D. Prolisok”. It contains of 14 alive probiotic strains of *Lactobacillus* + *Lactococcus* (6×10^{10} CFU/g), *Bifidobacterium* (1×10^{10}/g), *Propionibacterium* (3×10^{10}/g), *Acetobacter* (1×10^{6}/g) genera. Over 8 weeks of interventional period, the patient received 1 sachet (10 g) of probiotic and placebo per day. All sachets were identical with similar organoleptic characteristics (e.g., taste and appearance).

The pre-randomization period was designed to minimize the effects of dietary changes on metabolic markers. For this purpose, 2 weeks before the study start, after inform consent signed, patients were instructed in one-on-one sessions with a dietitian to follow a therapeutic lifestyle-change diet as classified by the NCEP. In addition, participants were instructed to continue with stable antihyperglycemic treatment and received standardized mild physical training for 1 h per day.

Patients who underwent study were instructed to take the trial medication as prescribed. Throughout the study, weekly phone follow-up visits were provided for assessment of compliance, adherence to the protocol, as well as the recording of adverse events. The effectiveness of therapy was compared and evaluated separately in the two groups.

2.2. Inclusion criteria

Adult participants (ages 18–75, BMI ≥ 25 kg/m^2) diagnosed with T2D according WHO (1999) for at least 6 months prior to the study; treated with diet and exercise alone or metformin, SUs and insulin on a stabilized dose for at least 3 months before the study; with presence of insulin resistance established as HOMA-IR ≥ 2.0; had HbA1c between 6.5 and 11.0%; written informed consent.

2.3. Exclusion criteria

Main exclusion criteria included type 1 diabetes, treatment with other than mention in inclusion criteria antidiabetic drugs (pioglitazone, GLP-1 analogues, DPP IV inhibitors etc); regular use of a probiotic or prebiotic supplement within 3 months prior to enrollment; antibiotic use within 3 months prior to enrollment; uncontrolled cardiovascular or respiratory disease, decompensated liver disease including ascites, encephalopathy or variceal

bleeding, active malignancy, or chronic infections; participation in other clinical trials, and presence of pregnancy or lactation.

2.4. Outcomes assessment

The primary main outcome was change of the HOMA-IR. Insulin resistance was assessed by the validated homeostasis model assessment (HOMA) index [27] using the Matthews et al.'s equation: HOMA-IR = (FPG * FPI)/22.5, where FPG and FPI are fasting plasma glucose (mmol) and fasting plasma insulin (μU/ml), respectively. FPG was determined using the Trinder's glucose oxidase method. FPI was measured with the double radioimmunoassay (RIA) method (AIA-Pack IRI; Tosoh, Tokyo).

Secondary outcomes were the changes in glycemic control-related parameters, anthropomorphic variables and cytokines (TNF-α, IL-1β, IL-6, IL-8, INF-γ) levels. All values were determined following a 12-h fasting period, by the hospital clinical laboratory. HbA1c was measured by ion-exchange high-performance liquid chromatography using the Tosoh G8 HPLC HbA1c analyzer (AIA-Pack IRI; Tosoh, Tokyo).

The contents of serum interleukins (TNF-α, IL-1β, IL-6, IL-8, INF-γ) were measured by ELISA using specific mono- and polyclonal antibodies (Sigma) to these proteins. Studied molecules were immobilized in 96-well plates with sorption surface.

Anthropometric data including weight and height were measured to an accuracy of 0.1 kg and 0.5 cm, respectively. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of the participant's height in meters (weight/(height^2). Waist (narrowest diameter between xiphoid process and iliac crest) circumferences (WC) was measured, also.

2.5. Statistical analysis

The SPSS statistical package, version 20.0 (SPSS, Inc., Chicago, Illinois) and GraphPad Prism, version 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) were used for all statistical analyses and a P value less than 0.05 was considered statistically significant. All data in this study were expressed as mean ± standard error (M ± SEM) or %. Data distribution was analyzed using the Kolmogorov-Smirnov normality test. The baseline characteristics of participants in the 2 groups were compared using independent sample t-tests and chi-squared ($\chi 2$) test. The changes in outcomes of the participants after the initiation of therapy and end of the trial were compared by paired sample t-tests. Analysis of covariance (ANCOVA) was used to identify any differences between the 2 groups after intervention, adjusting for baseline measurements and confounders (BMI and sex).

3. Results

A total of 53 patients were randomly divided into two groups receiving either probiotic (n = 31) or placebo (n = 22), respectively. All subjects completed the study and received more than 85% of prescribed sachets, with comparable compliance rate (placebo = 86.4% vs probiotics = 90.3%, p = 0.654). Both probiotic and placebo were well tolerated and generally acceptable by the participants. Patients were satisfied with the organoleptic properties of it. During study period the participants reported only several minor adverse events. In both groups main adverse events were also gastrointestinal symptoms. In the probiotic group one patient complained with short-term diarrhea and nausea, and another two with mild abdominal pain. In the placebo group Two patients reported nausea and complained of one mild abdominal pain. However, there prevalence of adverse events was comparable between groups (placebo = 13.6% vs probiotics = 12.9%, p = 0.938) and didn't cause withdrawn patients from the study.

Table 1 presents the baseline clinical, anthropometric, and laboratory characteristics of the participants. Participants were treated with oral anti-diabetic (OAD) agents, insulin or their combination. The proportion of participants on insulinotherapy (p = 0.406) and the mean daily dosage of insulin were comparable between the groups (p = 0.811). In light of latest evidence, metformin is known to have pleiotropic effects beyond glucose reduction, including improvement of lipid profiles, GLP-1, bile acids and finally gut microbiota [28]. Moreover, in a double-blind RCT, treatment of naive T2D for 4 months showed that metformin had strong effects on the gut microbiome as compared to placebo [29]. To avoid impact of metformin, in our study we randomized equal portion of patient, treated with stable dose of drug at least 4 weeks prior to study start. Moreover, mean dosage of metformin were well balanced and comparable between the groups at baseline (p = 0.235).

There were no significant differences between the groups at baseline in terms of age, sex, diabetes duration and treatment or anthropometric measurements. Baseline characteristics of primary and secondary outcomes were evenly distributed across the two groups of enrolled patients (Table 1).

Table 1
Baseline anthropometric, clinical and laboratory parameters in examined patients (M ± SEM).

Parameters	Placebo group (n = 22)	Probiotic group (n = 31)	P
Age, years	**57.18 ± 2.06**	**52.23 ± 1.74**	**0.073**
Duration of T2D, years	**5.91 ± 0.87**	**6.16 ± 0.92**	**0.844**
Metformin, % (n)	**81.8 (18)**	**62.1 (18)**	**0.110**
Metformin daily dosage, mg	**1563.88 ± 144.66**	**1768.42 ± 94.21**	**0.235**
Sulfonilureas, % (n)	**45.5 (10)**	**51.7 (15)**	**0.436**
Insulinotherapy, % (n)	**27.3 (6)**	**34.5 (10)**	**0.406**
Insulin daily dosage, IU	**32.16 ± 2.27**	**31.5 ± 1.62**	**0.811**
BMI, kg/m^2	**35.65 ± 1.57**	**34.70 ± 1.29**	**0.642**
Weight, kg	**96.95 ± 4.35**	**99.32 ± 3.23**	**0.665**
Waist circumference, cm	**96.36 ± 1.48**	**96.58 ± 1.10**	**0.907**
Insulin, μU/ml	**18.43 ± 1.89**	**17.76 ± 1.75**	**0.797**
Glucose, mmol/	**9.2 ± 0.75**	**8.68 ± 0.47**	**0.562**
HOMA-IR	**7.24 ± 0.74**	**6.85 ± 0.76**	**0.720**
HbA1c, %	**8.31 ± 0.29**	**8.4 ± 0.22**	**0.830**
TNF-α, pg/ml	**50.16 ± 4.46**	**50.27 ± 3.07**	**0.985**
IL-1β, pg/ml	**39.43 ± 4.69**	**38.21 ± 2.78**	**0.726**
IL-6, pg/ml	**15.75 ± 2.11**	**15.37 ± 2.43**	**0.963**
IL-8, pg/ml	**27.72 ± 2.77**	**27.58 ± 1.24**	**0.906**
γ-INF	**176.53 ± 18.67**	**177.58 ± 11.23**	**0.962**

Supplementation with alive multiprobiotic for 8 weeks was associated with significant reduction of our primary endpoint - HOMA-IR from 6.85 ± 0.76 to 5.13 ± 0.49 (p = 0.047), but remained static in the placebo group (7.24 ± 0.74 to 7.95 ± 1.01; p = 0.573) (Fig. 1A,B). But when we compare, in between group analysis using ANCOVA, mean changes in absolute values (Fig. 1C) and percentages from baseline (Fig. 1D), HOMA-IR values throughout the study changes insignificantly. However, as compared to placebo group were HOMA-IR increased after intervention, in probiotic group reduction of this parameter was observed (Fig. 1C,D).

With respect to our secondary outcomes, HbA1c insignificant decreased by 0.09% (p = 0.383) and 0.24% (p = 0.068) respectively in placebo and probiotics groups (Table 2, Fig. 2C). However, in probiotic responders (n = 22, patient with decrease in HOMA-IR) after supplementation a significant reduction in HbA1c by 0.39% (p = 0.022) as compared to non-responders was observed (Fig. 2D). In placebo group in responders as compared to non-responders the reduction of HbA1c was insignificant (p = 0.094) (Fig. 2F).

In terms of other glycemic-related parameters, we didn't observed significant changes for fasting glycemia (Fig. 2B) and insulin (Fig. 2A) in both within and between group analysis (Table 2).

Slight significant reduction of weight, BMI and waist circumference only within probiotic group was also noted (Fig. 3A–C). However, in between group analysis using ANCOVA, from all anthropometric parameters, significant reduction in absolute values (p = 0.032) and percentages (p = 0.039) from baseline remains only for waist circumference (Table 2).

From markers of chronic systemic inflammatory state only TNF-α (7.95 ± 1.27 pg/ml, $p < 0.001$, paired *t* test between baseline values and week 8), IL-1β (5.44 ± 1.51 pg/ml, $p < 0.001$, paired *t* test between baseline values and week 8) and IL-6 (3.45 ± 1.48 pg/ml, p = 0.027, paired *t* test between baseline values and week 8) changes significantly after 8-week of treatment with probiotics. However, ANCOVA analysis stated statistically significant differences between 2 groups for mean changes only in terms of TNF-α and IL-1β (Table 2). Other cytokines levels did not change significantly in both interventional groups (Fig. 4A–D).

4. Discussion

In this study, we performed a randomized placebo-controlled trial and showed that the alive multi-strain probiotic mixture administered once daily for 8 weeks to patients with T2D associated with significant reduction of HOMA-IR (our primary endpoint), weight, BMI, waist circumference and cytokines (TNF-α, IL-1β, IL-6) when compared with placebo. These changes, however, remain significant in the between-group analyses only for waist circumference, TNF-α and IL-1β. Glycemic-related parameters (our main secondary endpoint) also did not affected by therapy throughout the study in both within and between group analysis. However, in sub-group analysis, we noted significant reduction of HbA1c only in probiotic responders (patient with decrease in HOMA-IR) as compared to non-responders.

Several clinical trials, with similar to our study outcomes, reported previously [30–36]. They were conducted among Iranian

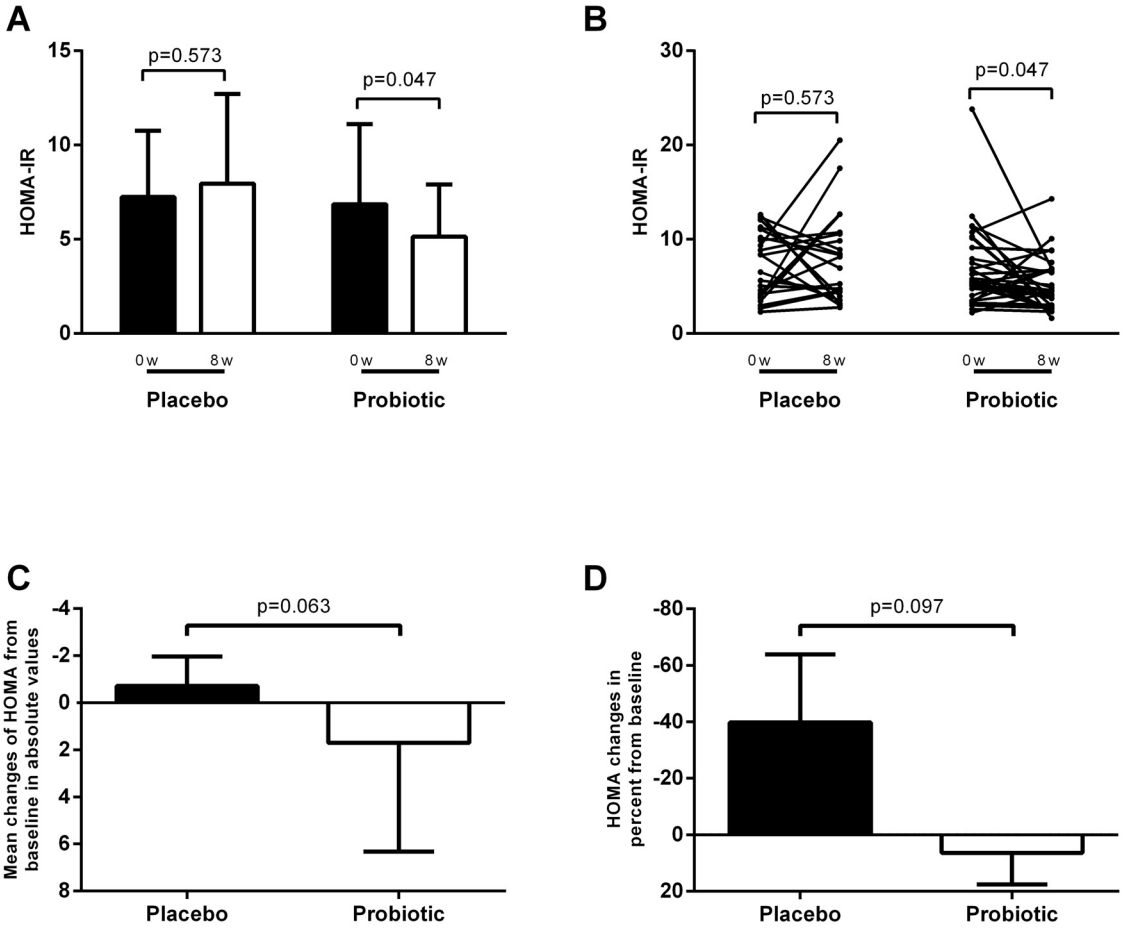

Fig. 1. Primary outcomes analysis with accent on HOMA-IR changes. A, B – intra-group analysis of changes at baseline and after interventon. Data expressed in mean ± SEM (A) and individual values at baseline and after 8 weeks of treatment. C, D – analysis of inter-group mean changes of absolute values (C) or percentages (D) from baseline to end of treatment throughout the study. Data expressed as mean ± SEM. ANCOVA was used to identify any differences between the 2 groups after intervention.

Table 2
Changes in secondary outcomes parameters between baseline and week 8.

Parameters	Placebo group (n = 22)	p1	Probiotic group (n = 31)	p2	p3
BMI, kg/m^2 Absolute value Percentage from baseline	**0.05 ± 0.09 0.11 ± 0.29**	**0.549**	**0.26 ± 0.11 0.78 ± 0.31**	**0.027**	**0.176 0.129**
Weight, kg Absolute value Percentage from baseline	**0.15 ± 0.28 0.10 ± 0.30**	**0.608**	**0.94 ± 0.27 0.90 ± 0.26**	**0,002**	**0.052 0.052**
Waist circumference, cm Absolute value Percentage from baseline	**0.13 ± 0.2 0.13 ± 0.2**	**0.504**	**0.75 ± 0.12 0.74 ± 0.19**	**0.001**	**0.032 0.039**
HbA1c, % Absolute value Percentage from baseline	**0.09 ± 0.10 0.46 ± 1.26**	**0.383**	**0.23 ± 0.12 2.21 ± 1.31**	**0.068**	**0.367 0.345**
Insulin, μU/ml Absolute value Percentage from baseline	**−1.82 ± 3.02 −29.89 ± 17.0**	**0.514**	**3.30 ± 1.88 3.72 ± 9.96**	**0.090**	**0.158 0.097**
Glucose, mmol/l Absolute value Percentage from baseline	**0.27 ± 0.52 −4.4 ± 6.2**	**0.614**	**0.37 ± 0.42 0.98 ± 4.28**	**0.384**	**0.878 0.480**
TNF-α, pg/ml Absolute value Percentage from baseline	**1.03 ± 2.07 −2.11 ± 6.78**	**0.706**	**7.95 ± 1.27 14.8 ± 2.46**	**<0.001**	**0.014 0.011**
IL-1β, pg/ml Absolute value Percentage from baseline	**0.45 ± 1.97 −8.04 ± 7.29**	**0.982**	**5.44 ± 1.51 9.39 ± 3.97**	**0.001**	**0.035 0.043**
IL-6, pg/ml Absolute value Percentage from baseline	**1.89 ± 1.28 0.17 ± 7.98**	**0.155**	**3.45 ± 1.48 3.02 ± 9.17**	**0.027**	**0.432 0.815**
IL-8, pg/ml Absolute value Percentage from baseline	**3.85 ± 1.66 10.71 ± 5.05**	**0.030**	**3.80 ± 1.05 9.96 ± 4.32**	**0,001**	**0.978 0.972**
γ-INF, pg/ml Absolute value Percentage from baseline	**6.16 ± 8.88 −0.07 ± 4.67**	**0.495**	**13.80 ± 7.04 2.02 ± 4.13**	**0.060**	**0.504 0.738**

p1-2 – difference between placebo and probiotic groups after intervention.
p3 – difference within groups throughout the study.

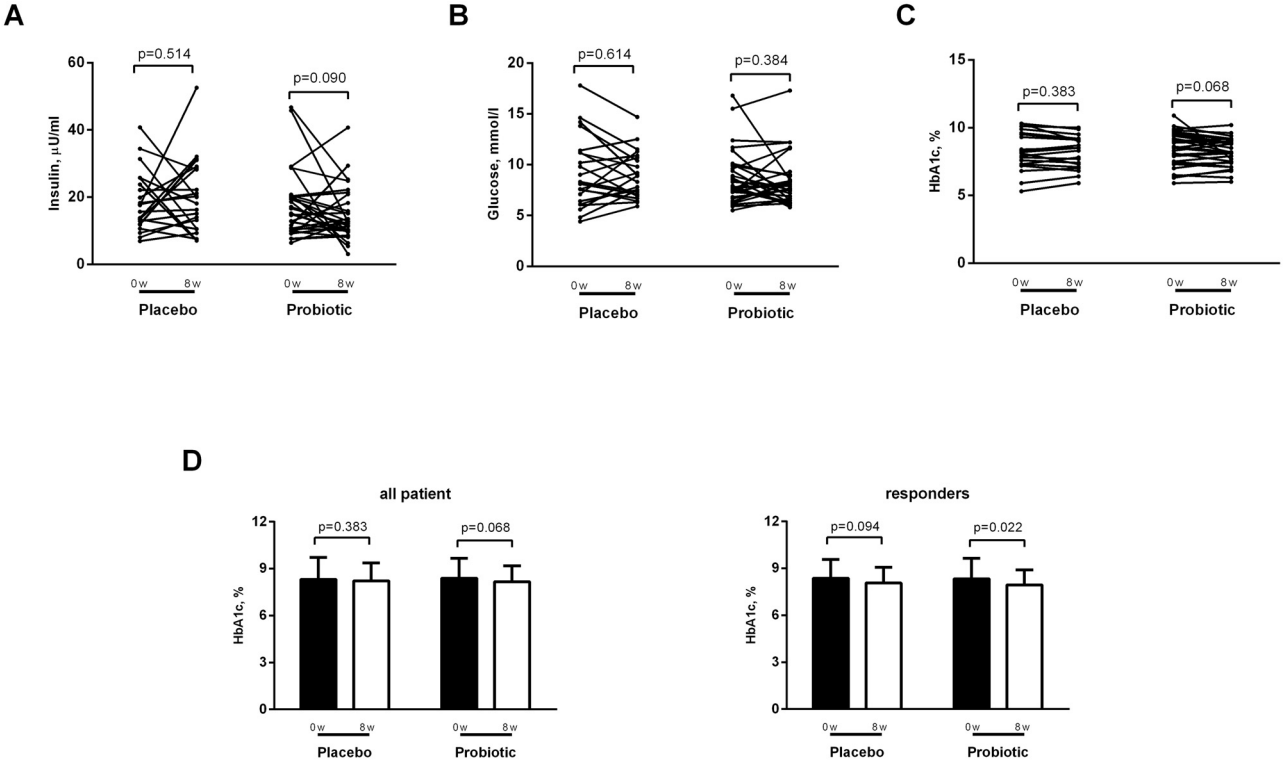

Fig. 2. Secondary outcomes analysis with accent on glycemic-related parameter changes (A – insulin; B – fasting glycemia; C – HbA1c). A–C – intra group analysis of changes at baseline and after interventon. Data expreesed as individual values at baseline and 8-week. D, F - changes of HbA1c in all patients (D) and in therapy responders(patient with decrease in HOMA-IR) (F). Data expressed as mean ± SEM.

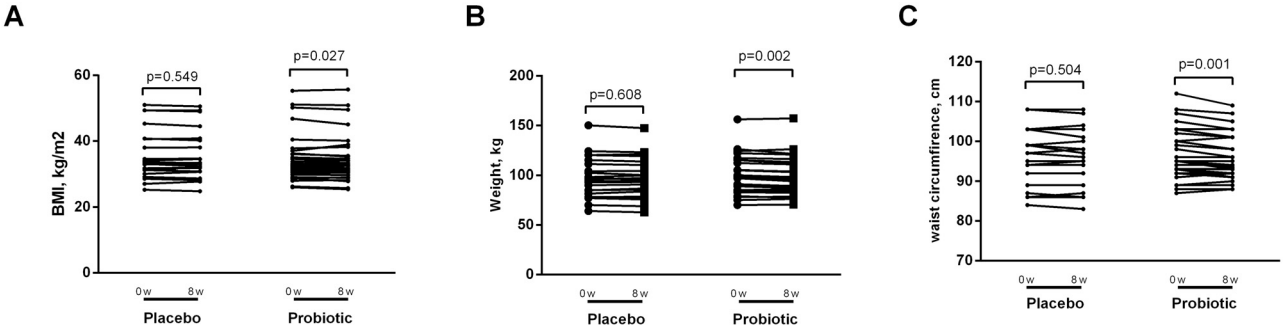

Fig. 3. Secondary outcomes analysis with accent on antropometric parameters (A – body mass index; B – weight; C – waist circumference). A–C – intra group analysis of changes at baseline and after interventon. Data expreesed as individual values at baseline and 8-week.

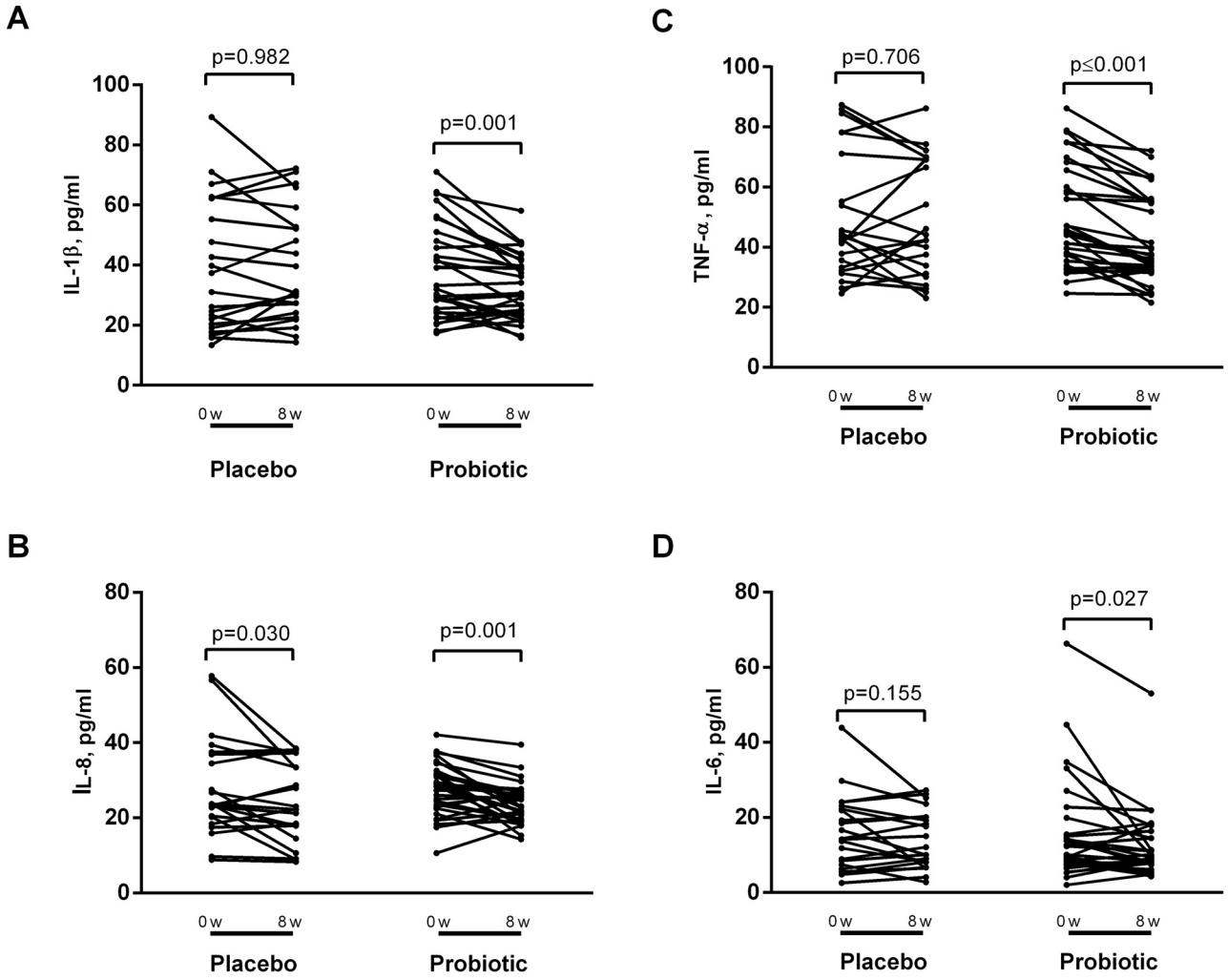

Fig. 4. Secondary outcomes analysis with accent on cytokines changes (A - IL-1β; B – IL-8; C - TNF-α; D – IL-6). A–D – intra group analysis of changes at baseline and after interventon. Data expreesed as individual values at baseline and 8-week.

[30–32], Malaysian [33], Sweden [34], Danish [35] or Brazilian [36] participants and their results may not be extrapolated to Ukrainian population due to the genetic differences, food consumption pattern, as well as environmental differences. Moreover, all of them used single- or multi-strain lyophilized probiotics; milk or yogurt fermented with two or more probiotic strains which belongs mostly to Lactobacillus and Bifidobacterium genera. In our study, we used alive probiotic mixture containing biomass of 14 strains (*Lactobacillus, Lactococcus, Bifidobacterium*, *Propionibacterium*, *Acetobacter*). In our previous study [25,26] we have showed higher efficacy of alive probiotic strains produced as bacterial cell suspensions in a special protective medium. Bacteria in such suspensions are biologically active and able to act in the human organism immediately following intake of the preparation. Also, multi-strain or multispecies formed mutualistic interactions in mixtures and therefore were able to share with different metabolites, affect different receptors and produced various biologically active compounds. So, their synergistic overall effect is greater than the sum of their individual effects. Finally, mixture in our study contained genus *Propionibacterium* which produce SCFA, mainly propionate and acetate, which at low amounts exert multiple advantageous effects on the host, including the prevention of intestinal inflammation and oxidative stress, improvement of intestinal barrier function [37], modulation insulin sensitivity and metabolic disorders [38]. So, we believe, that this probiotic composition will have benefits on IR and other glycemic-related and inflammation parameters in subjects with T2D.

Previous clinical trials [30–36] also shows controversial data related to effects of probiotics on glycemic related parameters in subjects with T2D. Several studies [30,32,33,36] reported the improvement of glycemic control after probiotics supplementation, milk or yogurt fermented with probiotic strains. By contrast, two trials [31,35] in T2D and another two [39,40] in obese/overweight subjects did not find any improvement in glycemic control after probiotics supplementation. In agreement with our data, study reported by Mobini et al [34]. In this RCT a significant reduction in HbA1c was observed only in patients who responded with increased ISI following *L. reuteri* DSM 17,938 (responders) but not non-responders after 12-week supplementation.

In our study, supplementation with alive multiprobiotic as compared to placebo for 8 weeks was associated with significant reduction of our primary endpoint - HOMA-IR. But in between group analysis using ANCOVA, HOMA-IR values throughout the study changes insignificantly. Interesting that recently published 2 meta-analysis to assess the effect of probiotics on glycemic control in subjects with type 2 diabetes have shown conflicting results in terms of HOMA-IR [41,42]. In meta-analysis, reported by Kasinska et al., which included 8 trials with 438 individuals a significant effect of probiotics on HOMA-IR (SMD, −2.10; CI −3.00 to −1.20, $P < 0.001$; $I2 = 82.91\%$; $P = 0.0029$ for heterogeneity) was founded

[41]. Li et al., analyzed data from 9 trials (n = 368) and did not found significant differences in HOMA-IR between the treatment group and the control group [42]. In contrast, Ruan et al, included 17 RCT (n = 635) in meta-analysis among obese, T2D and pregnant participants [43]. Probiotic consumption, compared with placebo, significantly reduced HOMA-IR (MD = 0.48; 95% CI −0.83, −0.13; p = 0.007) [43].

5. Conclusion

Probiotic therapies modestly improved insulin resistance in patients with type 2 diabetes. Application of probiotic agents and modulation with gut microbiota might become a new method for glucose management in T2DM. However, larger well-designed, long-term RCTs are needed to confirm any potentially beneficial relationship between the use of probiotics and modifiable cardiometabolic risk factors in patients with type 2 diabetes.

Conflicts of interest

None to declare.

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Author contributions

N.K. and IK conceived and designed the study. N.K., G.M., D.K and T.F. wrote the manuscript. All authors enrolled patients and approved the final version of the manuscript.

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