Effectiveness of probiotics in type 2 diabetes: a meta-analysis

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INTRODUCTION
An increasing number of studies suggest that the use of probiotics may have a beneficial effect in patients with type 2 diabetes.

OBJECTIVES
The aim of the study was to assess the ability of probiotics to modify selected cardiometabolic risk factors in subjects with type 2 diabetes.

METHODS
PubMed, Embase, Cochrane Library, and Scopus databases were thoroughly reviewed up to January 2015 to search for randomized controlled trials (RCTs) that examined the effect of probiotics on selected modifiable cardiometabolic parameters in patients with type 2 diabetes. The following endpoints were considered: fasting plasma glucose (FPG), insulin concentration, insulin resistance, hemoglobin A₁c (HbA₁c), as well as the levels of total cholesterol, triglycerides, low-density and high-density lipoprotein cholesterol, and C-reactive protein (CRP). A total of 571 RCTs were initially identified, of which 8 trials with 438 individuals were selected for meta-analysis. The effects of probiotics were calculated for each parameter.

RESULTS
The meta-analysis showed a significant effect of probiotics on reducing HbA₁c levels (standardized mean difference [SMD], –0.81; confidence interval [CI], –1.33 to –0.29, \( P = 0.0023; \) \( I^2 = 68.44\% \); \( P = 0.0421 \) for heterogeneity) and HOMA-IR (SMD, –2.10; CI –3.00 to –1.20, \( P < 0.001; \) \( I^2 = 82.91\% \); \( P = 0.0029 \) for heterogeneity). Supplementation with probiotics did not have a significant effect on FPG, insulin, and CRP levels as well as the lipid profile.

CONCLUSIONS
Our meta-analysis suggests that probiotic supplementation might improve, at least to some extent, metabolic control in subjects with type 2 diabetes. 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.
SCFAs bind to G protein-coupled receptors and exert various biological effects, including the regulation of glucagon-like peptide 1, which is associated with the improvement of insulin secretion and thus, with lower glucose levels. Additionally, SCFAs affect metabolism via interaction with histone deacetylases, which in turn influences the expression of genes, including those related to metabolism. It has also been suggested that SCFAs may directly prevent the low-grade inflammatory response, a condition closely associated with type 2 diabetes, through maintaining intestinal integrity. As a result, probiotics may prevent the translocation of proinflammatory lipopolysaccharides into the bloodstream, associated with a decrease in inflammatory-related Toll-like 4 receptor signaling. Interestingly, recent clinical trials have revealed an increased number of butyrate-producing bacteria in insulin-resistant men with metabolic syndrome after infusion of feces from lean donors, accompanied by beneficial metabolic effects. Thus, the appropriate balance of gut microbiota may be of great importance for glucose, lipid, and protein metabolism. Since a growing body of evidence suggests an association between probiotic consumption and metabolic profile in subjects with type 2 diabetes, we aimed to assess the effect of probiotic supplementation on selected modifiable cardiovascular risk factors in type 2 diabetes using a meta-analysis of existing research.

PATIENTS AND METHODS Data extraction and selection criteria The present study was performed according to PRISMA guidelines. The PubMed, Embase, Cochrane Library, and Scopus databases were searched using the terms “probiotics” and “diabetes” connected via the logical (Boolean) operator “AND”, which restricted the search to trials focusing on both aspects at the same time. The search was last updated in January 2015 and involved only full-text articles published in English. Both authors were equally involved in the process of study selection, starting with the initial verification of abstracts followed by the assessment of full texts as well as quality assessment and data extraction. Any disagreements were resolved by compromise. Only randomized controlled trials (RCTs) were taken into consideration. Concerning the population, only adults with type 2 diabetes assessed in the original study were included in the meta-analysis. Furthermore, interventions of the included studies covered specified probiotic, probiotic mixes, synbiotics, or dairy products containing probiotic bacteria compared with placebo in the form of identically looking capsules, tablets, or liquids.

After establishing the most relevant endpoints, the obtained data were extracted from the studies and collated in a computer spreadsheet in the form of a table. The tables, individual for each single endpoint, included the number of subjects in the study and control groups, and values of tested parameters before and after the administration of probiotic or placebo. The outcomes of interest were fasting plasma glucose (FPG), insulin concentration, insulin resistance estimated using the homeostatic model assessment (HOMA-IR), hemoglobin A1c (HbA1c), and the levels of total cholesterol (TC), triglycerides, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and C-reactive protein (CRP). Subsequently, all collected data were transferred into a statistical software.

Quality and risk of bias The quality of the methodology of the included RCTs was assessed using the Jadad criteria, as performed recently in another meta-analysis, which we considered as a model for our study. While assessing the number of points, we considered the quality of randomization, correctness of blinding, and reason for subject withdrawal from a specific study. Each RCT was granted a score from 0 to 5 with a higher score indicating higher credibility. Moreover, the allocation concealment and intention-to-treat analysis as well as the risk of bias were evaluated.

Statistical analysis A statistical analysis was conducted with STATISTICA version 10 (StatSoft Inc., Tulsa, Oklahoma, United States; Statsoft Polska, Kraków, Poland). All endpoints of interest constituted continuous data. Therefore, the t test for mean difference between 2 independent groups with 95% confidence interval (95% CI) using the random-effects model, implying variation between single effects resulting from normal distribution, was used for the calculation. Effect size (standardized mean difference [SMD]) defined in the software as Cohen’s d was calculated as the difference in the mean outcome between the groups divided by a standard deviation of outcome among participants. SMD is usually interpreted as a relative “small” (0.2–0.3), “medium” (0.5), and “large” (0.8 to ∞) effect. Combining groups (if reasonable) and missing data were calculated using methods described in the Cochrane Handbook for Systematic Reviews of Interventions. Heterogeneity across the included studies was assessed using the I^2 statistics, representing the percentage of actual variation in relation to total variation. Additionally, sensitivity analyses were performed.

RESULTS A total of 8 RCTs with 438 subjects met the inclusion criteria and were included in the meta-analysis (Table 1). The detailed process of study identification and selection is presented in Figure 1. All studies were small-scale, recruiting between 20 and 108 participants, and had diversified quality. The quality of studies selected for the meta-analysis is described in detail in Supplementary material online, Table S1. Sensitivity analyses corresponding to attached forest plots may be also found in Supplementary material online (Figures S1–S6).
studies showed highly significant heterogeneity ($I^2 = 97.66\%$; $P < 0.001$).

### Probiotics and hemoglobin A\textsubscript{1c} levels

HbA\textsubscript{1c} is a marker of average blood glucose levels over prolonged time periods, which reflects the adequacy of metabolic control. A pooled analysis of 3

### Table 1: Characteristics of randomized controlled trials assessing the metabolic effects of probiotics in subjects with type 2 diabetes included in the meta-analysis

<table>
<thead>
<tr>
<th>Trial</th>
<th>Type</th>
<th>Participants</th>
<th>Intervention</th>
<th>Duration</th>
<th>Effects</th>
<th>Follow-up</th>
<th>Jadad score, AC, ITT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andreasen\textsuperscript{21}</td>
<td>DB-RCT</td>
<td>45 adult patients (18 with type 2 diabetes, 5 with impaired glucose tolerance, 22 with normal glucose tolerance)</td>
<td>$L$. acidophilus NCFM</td>
<td>4 weeks</td>
<td>insulin sensitivity, inflammatory markers</td>
<td>no</td>
<td>4</td>
</tr>
<tr>
<td>Asemi\textsuperscript{22}</td>
<td>DB-RCT</td>
<td>54 adult diabetic patients</td>
<td>7 viable and freeze-dried strains: $L$. acidophilus (2 x 10^8 CFU), $L$. casei (7 x 10^9 CFU), $L$. rhamnosus (1.5 x 10^9 CFU), $L$. bulgaricus (2 x 10^9 CFU), $B$. breve (2 x 10^9 CFU), $B$. longum (7 x 10^8 CFU), $S$. thermophilus (1.5 x 10^8 CFU), and 100 mg fructo-oligosaccharide</td>
<td>8 weeks</td>
<td>metabolic profiles, hs-CRP, biomarkers of oxidative stress</td>
<td>no</td>
<td>4</td>
</tr>
<tr>
<td>Asemi\textsuperscript{23}</td>
<td>DB-RCT</td>
<td>62 adult diabetic patients</td>
<td>probiotic viable and heat-resistant $L$. sporogenes (1 x 10^8 CFU), 0.04 g insulin (HPX) as prebiotic with 0.38 g isomalt, 0.36 g sorbitol and 0.05 g stevia as sweetener per 1 g</td>
<td>6 weeks</td>
<td>metabolic profiles, hs-CRP, biomarkers of oxidative stress</td>
<td>no</td>
<td>4</td>
</tr>
<tr>
<td>Ejtahed\textsuperscript{24}</td>
<td>DB-RCT</td>
<td>60 adult diabetic patients</td>
<td>300 g/d of probiotic yogurt containing $L$. acidophilus La5 and $B$. lactis Bb12</td>
<td>6 weeks</td>
<td>fasting blood samples, 24-hour dietary recalls, and anthropometric measurements</td>
<td>no</td>
<td>5</td>
</tr>
<tr>
<td>Judiono\textsuperscript{25}</td>
<td>RCT</td>
<td>108 adult diabetic patients</td>
<td>clear kefir</td>
<td>30 days</td>
<td>HbA\textsubscript{1c}, FBG, PBG, insulin, C-peptide</td>
<td>no</td>
<td>1</td>
</tr>
<tr>
<td>Mahboobi\textsuperscript{26}</td>
<td>DB-RCT</td>
<td>55 adult prediabetic patients</td>
<td>7 x 10^8 CFU $L$. casei, 2 x 10^6 CFU $L$. acidophilus, 1.5 x 10^8 CFU $L$. rhamnosus, 2 x 10^8 CFU $L$. bulgaricus, 2 x 10^9 CFU $B$. breve, 7 x 10^8 CFU $B$. longum, 1.5 x 10^8 CFU $S$. thermophilus, fructooligosaccharide (as prebiotic), B-group vitamins, maltodextrin, lactose, and magnesium stearate</td>
<td>8 weeks</td>
<td>lipid profile, blood pressure</td>
<td>no</td>
<td>5</td>
</tr>
<tr>
<td>Mazloom\textsuperscript{27}</td>
<td>SB-CT</td>
<td>34 adult diabetic patients</td>
<td>$L$. acidophilus, $L$. bulgaricus, $L$. bifidum, and $L$. casei</td>
<td>6 weeks</td>
<td>glucose, insulin, TG, TC, LDL-C, HDL-C, malondialdehyde, hs-CRP, and IL-6</td>
<td>no</td>
<td>3</td>
</tr>
<tr>
<td>Moroti\textsuperscript{28}</td>
<td>DB-RCT</td>
<td>20 adult diabetic patients</td>
<td>synbiotic shake containing 10^8 CFU/ml $L$. acidophilus, 10^9 CFU/ml $B$. bifidum and 2 g oligofructose</td>
<td>30 days</td>
<td>standard lipid profile (TG, TC, HDL-C) and glycemia, or blood sugar levels</td>
<td>no</td>
<td>5</td>
</tr>
</tbody>
</table>

Abbreviations: AC, allocation concealment; CFU, colony-forming unit; DB-RCT, double-blind randomized controlled trial; FBG, fasting blood glucose; HbA\textsubscript{1c}, hemoglobin A\textsubscript{1c}; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; ITT, intention-to-treat; LDL-C, low-density lipoprotein cholesterol; PBG, postprandial blood glucose; SB-CT, single-blind controlled trial; TC, total cholesterol; TG, triglycerides

**Probiotics and fasting plasma glucose levels** Of 6 RCTs,\textsuperscript{22,25,27,28} 5 showed a significant decrease of FPG after the consumption of probiotics, while only 1 did not:\textsuperscript{27} A random-effects meta-analysis did not show the effect of supplementation with probiotics on FPG levels (SMD, -1.05; CI, -2.66 to 0.56; $P = 0.2017$; \textbf{FIGURE 2}). The included
FIGURE 1 Flowchart demonstrating the selection of trials assessing the metabolic effect of probiotics in subjects with type 2 diabetes

FIGURE 2 Forest plot of the association between probiotic use and fasting plasma glucose levels. The shaded squares indicate the effect of probiotics in a particular study. The horizontal lines represent 95% confidence intervals (CIs). The diamond data marker indicates the pooled effect.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Cohen’s d</th>
<th>95% CI</th>
<th>P</th>
<th>Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asemi22</td>
<td>-3.70</td>
<td>(-4.58 to -2.82)</td>
<td>0.0000</td>
<td>16.46%</td>
</tr>
<tr>
<td>Mazloom27</td>
<td>-1.21</td>
<td>(-1.94 to -0.48)</td>
<td>0.0012</td>
<td>16.71%</td>
</tr>
<tr>
<td>Moroti28</td>
<td>-2.94</td>
<td>(-4.20 to -1.67)</td>
<td>0.0000</td>
<td>15.64%</td>
</tr>
<tr>
<td>Ejtahed24</td>
<td>-0.66</td>
<td>(-1.18 to -0.14)</td>
<td>0.0125</td>
<td>17.00%</td>
</tr>
<tr>
<td>Asemi23</td>
<td>2.43</td>
<td>(1.96–2.89)</td>
<td>0.0000</td>
<td>17.06%</td>
</tr>
<tr>
<td>Judiono25</td>
<td>-0.47</td>
<td>(-0.87 to -0.06)</td>
<td>0.0242</td>
<td>17.12%</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td>-1.05</td>
<td>(-2.66 to 0.56)</td>
<td>0.2017</td>
<td>100.00%</td>
</tr>
</tbody>
</table>
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Probiotics and insulin resistance

A pooled analysis of 3 RCTs showed a significant decrease of HOMA-IR after the use of probiotics.

Probiotics and insulin levels

Three of five studies included in the analysis showed a significant decrease in insulin levels after probiotic consumption. However, no significant difference in mean insulin levels was observed between probiotic and placebo users, based on a pooled estimate (SMD, –1.27; CI, –2.56 to 0.02; P = 0.0546; I² = 96.49%; P < 0.001 for heterogeneity; FIGURE 4).

Probiotics and insulin resistance

A pooled analysis of 3 RCTs demonstrated a significant decrease of HOMA-IR after the use of probiotics.
studies were highly heterogeneous ($I^2 = 95.22\%$; $P = 0.0003$).

**Probiotics and C-reactive protein levels**

CRP levels indicate an inflammatory state considered as an integral element of type 2 diabetes.

Two of four RCTs showed a significant decrease in CRP levels after probiotic intake. However, the overall effect was nonsignificant (SMD, –1.73; CI, –3.54 to 0.08; $P = 0.0617$; **FIGURE 7**). The included studies were highly heterogeneous ($I^2 = 96.85\%$; $P < 0.001$).

**Risk of bias**

All of the included studies were RCTs. Allocation concealment was provided in original evidence. Random and blinded assignment to study and control groups as well as blinded performance of trials and outcome assessment limit the probability of cumulative risk of bias. However, the studies analyzed only outcome data of patients who completed the study. There were no data on patients who were withdrawn during the study (mostly for reasons not related to the intervention, for example, the need for therapy change). Details on quality assessment may be found in Supplementary material online.

As the included studies reported both beneficial effects of intervention as well as the lack of beneficial effects of intervention, the risk of publication bias may be assessed as low. Furthermore, the studies reported both statistically significant and nonsignificant results of intervention; therefore, the risk of selective outcome-reporting bias is also reduced.

Only 4 papers were written in a language other than English. However, on the basis of an abstract in English, we assessed these studies as not

(SMD, –2.10; CI, –3.00 to –1.2; $P < 0.001$; $I^2 = 82.91\%$; $P = 0.0029$ for heterogeneity; **FIGURE 5**).

**Probiotics and total cholesterol levels**

Only 2 of 5 RCTs included in this analysis showed a significant decrease in TC levels after the administration of probiotic formulas. A pooled effect was found to be nonsignificant (SMD, 0.12; CI, –1.32 to 1.57; $P = 0.8664$; $I^2 = 96.48\%$; $P < 0.001$ for heterogeneity).

**Probiotics and triglyceride levels**

Five RCTs were included in this analysis. Of these, 3 studies showed a significant decrease in triglyceride levels after the administration of probiotic formulas. Nevertheless, a nonsignificant association was found between the supplementation of probiotics and placebo in subjects with type 2 diabetes (SMD, –0.27; CI, –2.04 to 1.50; $P = 0.7655$; $I^2 = 97.43\%$; $P < 0.001$ for heterogeneity).

**Probiotics and low-density lipoprotein cholesterol levels**

Of 4 RCTs included in this analysis, only 1 study showed a significant decrease in LDL cholesterol levels after the administration of probiotics. The total effect was found to be nonsignificant (SMD, 0.37; CI, –0.69 to 1.43; $P = 0.4947$; $I^2 = 93.68\%$; $P < 0.001$ for heterogeneity).

**Probiotics and high-density lipoprotein cholesterol levels**

Only 2 of 5 RCTs included in this analysis demonstrated a significant increase in HDL cholesterol levels after the administration of probiotics. However, no significant overall association was found between the use of probiotics and HDL cholesterol levels (SMD, 0.73; CI, –0.50 to 1.96; $P = 0.2472$; **FIGURE 6**). The included studies were highly heterogeneous ($I^2 = 95.22\%$; $P = 0.0003$).
fulfilling the eligibility criteria. Therefore, we considered the risk of language bias as low. Additionally, the risk of multiple-publication bias as well as citation bias may be also considered as nonsignificant. Unfortunately, owing to a low number of the included studies, it was impossible to assess the risk of bias on the funnel plot.

**DISCUSSION** To the best of our knowledge, this is the first meta-analysis assessing the effect of probiotics on modifiable cardiometabolic risk factors in subjects with type 2 diabetes. Although individual studies have reported that probiotic use has varied effects on these parameters, the present meta-analysis indicates that they have a significant impact only on HbA1c and HOMA-IR in subjects with type 2 diabetes when compared with placebo, indicating a potential effect of probiotics on glycemia-related parameters. However, importantly, Ejtahed et al\textsuperscript{24} reported a nonsignificant
Our findings did not show probiotics to have any significant effects on other cardiometabolic risk factors, including lipid profile components and CRP levels; this may have been caused by the use of various probiotic strains and short duration of studies. An elevated HDL cholesterol level is generally regarded as a factor reducing the risk of cardiovascular disease. Interestingly, it is also considered as a protective factor in metabolic disorders, including diabetes. Probiotic intake affects the structure of the gut flora, which might improve the integrity of the intestinal epithelium, weaken the immune responses, and diminish the Toll-like receptor 4 pathway, which in turn reduces proinflammatory signaling and enhances insulin sensitivity.

Our study did not show probiotics to have any significant effects on other cardiometabolic risk factors, including lipid profile components and CRP levels; this may have been caused by the use of various probiotic strains and short duration of studies. An elevated HDL cholesterol level is generally regarded as a factor reducing the risk of cardiovascular disease. Interestingly, it is also considered as a protective factor in metabolic disorders, including diabetes. Probiotic intake affects the structure of the gut flora, which might improve the integrity of the intestinal epithelium, weaken the immune responses, and diminish the Toll-like receptor 4 pathway, which in turn reduces proinflammatory signaling and enhances insulin sensitivity.
is widely believed that a substantially longer period of probiotic consumption is needed for its true effect to be demonstrated on various glucose and lipid metabolism markers. Furthermore, significant heterogeneity was observed between trials within the meta-analysis. Sensitivity analyses highlighted how the effect of potential exclusion of a study would affect the total effect. Furthermore, they show that studies affect the standard error differently and it is mostly associated with the number of subjects enrolled to a study. However, to avoid reducing reliability and giving rise to bias, despite the fact that the experimental exclusion of extreme results considerably increases experimental homogeneity, all of the studies were included in the analysis, while factors possibly affecting their homogeneity were noted. Most probably, the reason for this heterogeneity is the diversified setting of the included RCTs. Firstly, interventions in considered trials involved different probiotic formulas including a specified single probiotic strain, a multispecies probiotic preparation, symbiotic, or dairy product containing probiotic bacteria. Secondly, the duration of intervention across the studies varied considerably. Finally, the low number of studies also increases the heterogeneity of the analyses. All of these issues greatly reduce the clarity and explicit nature of the conclusions. Nevertheless, the results of our meta-analysis may indicate a trend that requires further scientific research.

In conclusion, this meta-analysis of available RCTs suggests that probiotic supplementation has a beneficial effect on selected cardiometabolic parameters in patients with type 2 diabetes. However, before they can be recommended for use in supportive treatment of type 2 diabetes, larger well-designed studies are needed to determine the true relationship between probiotic supplementation and modifiable cardiometabolic risk factors.

**Contribution statement** MK conceived the idea for the study. Both authors contributed to the design of the research. Both authors were independently involved in data collection, selection, and quality assessment. Both authors analyzed the data. Both authors edited and approved the final version of the manuscript.

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**Supplementary material online** Supplementary material is available with the online version of the article at www.pamw.pl.

**REFERENCES**


Skuteczność probiotyków w terapii cukrzycy typu 2 – metaanaliza

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STRESZCZENIE

WProwadzenie Rosnąca liczba badań sugeruje, że stosowanie probiotyków może mieć korzystny wpływ na stan zdrowia osób z cukrzycą typu 2.

CELE Celem badania była ocena zdolności probiotyków do modyfikowania wybranych czynników ryzyka sercowo-metabolicznego u osób z cukrzycą typu 2.


WYNIKI Metaanaliza wykazała istotny wpływ probiotyków na spadek poziomu HbA1c (standaryzowana średnia różnic [standardized mean difference – SMD] –0,81; CI od –1,33 do –0,29; p = 0,0023; niejednorodność: I² = 68,44%, p = 0,0421) i HOMA-IR (SMD –2,10; CI od –3,00 do –1,20; p <0,001; niejednorodność: I² = 82,91%, p = 0,0029). Suplementacja probiotyków nie miała istotnego wpływu na stężenie FPG, insuliny, CRP oraz profil lipidowy.