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Probiotics in type 2 diabetes therapy - are they effective? A meta-analysis.

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Abstract

Introduction: Products containing probiotics may offer benefits to subjects with type 2 diabetes mellitus (T2DM).

Objectives: To assess the ability of probiotics to modify several cardiometabolic risk factors in subjects with T2DM.

Methods: PubMed, Embase, Cochrane Library and Scopus databases were thoroughly reviewed up to January 2015 to search for randomized controlled trials (RCTs) that examine the effect of probiotics on some modifiable cardiometabolic risk factors, including fasting plasma glucose (FPG), insulin concentration, insulin resistance (IR), glycated haemoglobin (HbA1c), level of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and C-reactive protein level (CRP) in subjects with T2DM. In total, 571 articles were identified and eight trials with 438 T2DM individuals were selected for meta-analysis. The effects of probiotics on each single modifiable cardiometabolic risk factor were calculated using STATISTICA ver. 10.

Results: In subjects with T2DM probiotics may significantly reduce the level of HbA1c (SMD = -0.81, 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). Probiotics supplementation was not found to alter the level of fasting glucose in plasma, insulin concentration, lipid profile or CRP level.

Conclusions: This meta-analysis of available RCTs suggests that probiotic supplementation might improve, at least to some extent, metabolic control in subjects with T2DM. However, regarding their future use in the supportive treatment of T2DM larger, well – designed long-term RCTs are needed to confirm any potentially beneficial relationship between the use of probiotics and modifiable cardiometabolic risk factors.

Key words: diabetes, gut microbiota, probiotics, metabolism, meta-analysis

Introduction

For centuries, one of the most effective methods of maintaining the balance of the intestinal microbiome was the use of probiotics –“live microorganisms which when administered in adequate amounts confer a health benefit on the host” [1]. Products which contain probiotic bacteria have been increasingly applied to prevent or treat various disorders such as irritable bowel syndrome, inflammatory bowel disease, chronic idiopathic constipation, obesity, allergic and pulmonary diseases, and various types of diarrhea [2]. It has also been suggested that probiotic supplementation alone or foods supplemented with probiotics may positively modify the metabolic disturbances associated, directly or indirectly, with chronic hyperglycemia.

While the human organism comprises approximately 60 trillion somatic cells, the gut microbiota consists of hundreds of trillions (over 100×10^{18}) of bacteria, mainly *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* [3]. It was found that lean and obese individuals have different ratios of *Bacteroidetes* and *Firmicutes* [4-6]. Interestingly enough, other analyses of intestinal microbiota have shown that patients with T2DM have a significantly lower number of bacteria, which produce butyrate (*Roseburia*, *Faecalibacterium prauznitzii*), when compared to healthy people [7,8]. Butyrate, a short-chain fatty acid (SCFA), is an important source of energy for intestinal cells [9]. Several studies have demonstrated that SCFAs serve as substrates for gluconeogenesis and lipogenesis, and affect the proliferation, differentiation and modulation of gene expression [10]. SCFAs bind to G-protein coupled receptors (GPCRs) and exert various biological effects, including the regulation of glucagon-like peptide-1 (GLP1), which is associated with the improvement of insulin secretion and thus, lower glucose level [11]. Additionally, SCFAs affect metabolism via interaction with histone deacetylases, which in turn influences the expression of genes, including those concerning the metabolism [12]. It has also been suggested that SCFAs may

directly prevent the low-grade inflammatory response, a condition closely associated with T2DM, through maintaining intestinal integrity. As the result probiotics may prevent the translocation of pro-inflammatory lipopolysaccharides into the bloodstream, associated with a decrease in inflammatory-related Toll-like 4 receptor (TLR4) signaling [11,13]. Interestingly, recent clinical trials have revealed increased number of butyrate-producing bacteria in insulin-resistant males with metabolic syndrome after infusion of feces from lean donors, accompanied by beneficial metabolic effects [14]. Thus, the appropriate balance of gut microbiota may be of great importance for glucose, lipid and protein metabolism. As a growing body of evidence suggests an association between probiotic consumption and metabolic profile in subjects with T2DM, the aim of the study was to assess the efficacy of probiotic supplementation on selected modifiable cardiometabolic risk factors in T2DM by a meta-analysis of existing research.

Patients and methods

Data extraction and selection criteria

The present study was ~~developed~~ made with ~~according to~~ PRISMA guidelines [15]. 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 of the 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 involved in the process of studies selection, referring from abstract to full text articles, quality assessment and data extraction. Any disagreements were dealt by compromise. Only randomized controlled trials were taken into consideration. Concerning population, inclusion criteria were being an adult with diagnosed T2DM assessed in the original study. Further, interventions of included studies covered specified probiotic, probiotic

mixes, synbiotics or dairy products containing probiotic bacteria, while comparison was placebo in the form of identically looking capsules, tablets or liquids.

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

Quality and risk of bias

The quality of the methodology of the included RCTs was assessed using the Jadad criteria [16], as performed recently in other meta-analysis, which we considered as a model for our study [17]. While assessing the number of points the following were taken into consideration: quality of randomization, correctness of blinding and reason for subject withdrawal from the 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, and the risk of bias were evaluated.

Statistical analysis

Statistical analysis was carried out with STATISTICA version 10 (StatSoft, Inc.). All of the endpoints of interest constituted continuous data. Therefore, a t-test for mean difference between two independent groups with 95% confidence interval (95% CI) using 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 mean outcome between groups divided by a standard deviation of outcome among participants [18]. SMD is usually interpreted as a relative 'small' (0.2-0.3), 'medium' (~0.5) and 'large' (0.8 to ∞) effect [19]. Combining groups (if reasonable) and missing data were calculated using methods described in the Cochrane Handbook for Systematic Reviews of Interventions [19]. Heterogeneity across the included studies was assessed using I^2 statistics, representing the percentage of actual variation in relation to total variation [20]. Additionally, sensitivity analyses were performed.

Results

The detailed process of study identification and selection is presented in Figure 1. Eventually, 8 RCTs with 438 subjects met the inclusion criteria and were included in the meta-analysis (Table 1). All studies were small-scale, recruiting between 20 and 108 participants and had diversified quality. The quality of studies selected for meta-analysis is described in details in the Supplementary material online, Table S1. Sensitivity analyses corresponding to attached forest plots may be also found in supplementary material (Figure S1 – Figure S6).

Probiotics and fasting plasma glucose (FPG) level

Of 6 RCTs [22-25,27,28], five showed a statistically significant decrease of FPG after the consumption of probiotics, while only one did not [23]. A random-effect meta-analysis, showed no evidence of effect of probiotics supplementation on FPG level (SMD = -1.05, CI - 2.66 to 0.56, $P= 0.2017$) (Figure 2). The included studies showed high significant heterogeneity ($I^2=97.66\%$, $P< 0.001$).

Probiotics and HbA1c level

Glycosylated hemoglobin is a marker of average blood glucose level over prolonged time periods, which reflects the adequacy of metabolic control [29]. A pooled analysis of 3 RCTs

[22,24,25] showed a significant decrease in the level of HbA1c in diabetics consuming probiotics compared with those consuming placebo (SMD = -0.81, CI -1.33 to -0.29, $P=0.0021$) (Figure 3). The heterogeneity of included studies was moderate ($I^2=68.44\%$, $P=0.0421$).

Probiotics and insulin level

Three [22,23,27] out of five studies included in the analysis [22-25, 27] showed a significant decrease of insulin level after probiotic consumption. However, no significant difference in mean insulin level was observed between probiotics and placebo users, based on a pooled estimate (SMD = -1.27, CI - 2.56 to 0.02, $P= 0.0546$; $I^2 = 96.49\%$, $P<0.001$ for heterogeneity) (Figure 4).

Probiotics and insulin resistance

A pooled analysis of three RCTs [22,23,27] demonstrated a significant decrease of HOMA-IR after consumption of probiotics (SMD = -2.10, CI -3.00 to -1.2, $P<0.0001$, $I^2=82.91\%$, $P=0.0029$ for heterogeneity) (Figure 5).

Probiotics and total cholesterol

Only two [22,28] out of five RCTs included in this analysis [20,23,26-28] showed a significant decrease of total cholesterol level after administration of probiotic formula. Pooled effect was found to be insignificant (SMD = 0.12, CI -1.32 to 1.57, $P= 0.8664$, $I^2=96.48\%$; $P<0.001$ for heterogeneity).

Probiotics and triglyceride level

Five RCTs were included in this analysis [22,23,26-28]. Of these, three studies [22,27,28] showed a significant decrease of triglyceride level after administration of probiotic formulas. Nevertheless, a statistically insignificant association was found between supplementation of

probiotics vs. placebo in subjects with T2DM (SMD = -0.27, CI -2.04 to 1.50, $P= 0.7655$, $I^2=97.43\%$, $P<0.001$ for heterogeneity).

Probiotics and LDL level

Out of four RCTs included in this analysis [22,23,26,27] only one [22] showed a significant decrease of LDL level after administration of probiotics. The total effect was found to be statistically insignificant (SMD = 0.37, CI - 0.69 to 1.43, $P= 0.4947$, $I^2=93.68\%$, $P<0.001$ for heterogeneity).

Probiotics and HDL level

Only two [23,26] out of five [22,23,26-28] RCTs included in this analysis demonstrated a statistically significant increase of HDL level after probiotic consumption. However, no significant overall association was found between probiotic use and HDL level (SMD = 0.73, CI -0.50 to 1.96, $P=0.2472$) (Figure 6). The included studies were highly heterogeneous ($I^2=95.22\%$, $P= 0.0003$).

Probiotics and CRP level

C-reactive protein level (CRP) is an indicator of an inflammatory state considered as an integral element of T2DM [30]. Two [22,23] out of four RCTs [21-23,27] showed a significant decrease of CRP level after probiotic intake. However, the overall effect was demonstrated as statistically insignificant (SMD = -1.73, CI -3.54 to 0.08, $P= 0.0617$) (Figure 7). The included studies were of high heterogeneity ($I^2=96.85\%$, $P<0.001$).

Risk of bias

All of included studies were randomized controlled trials. Allocation concealment was provided in original evidence. Random and blinded assignment to study and control groups, blinded performance of trials and outcome assessment authentically limit the probability of cumulative risk of bias. However, incorporated studies analyzed only outcome data of patients

who completed the study. Data on patients who were withdrawn during the study – mostly from the reasons not related to intervention, e.g. need for therapy change - is missing. Details of quality assessment may be found in supplementary material.

As included studies reported both beneficial effects of intervention, as well as lack of beneficial effect of intervention, the risk of publication bias may be evaluated as low.

Furthermore, incorporated studies reported both statistically significant as well as statistically non-significant results of intervention, therefore the risk of selective outcome reporting bias is also reduced.

Only four papers referred to the issue were written in other than English language. However, on the basis of abstract written in English, we assessed these studies as not-fulfilling adopted eligibility criteria. Therefore, we considered the risk of language bias as low. Additionally, risk of multiple publication bias as well as citation bias may be also provided as non-significant. Unfortunately, due to low number of included studies it was impossible to assess the risk of bias on the funnel plot.

Discussion

To our knowledge this is the first meta-analysis assessing the effect of probiotics on modifiable cardiometabolic risk factors in subjects with T2DM conducted to date. Although individual studies report that probiotic use has varied effects on these parameters, the present meta-analysis indicates that probiotics only have a significant impact on HbA1c and HOMA-IR levels in subjects with T2DM when compared with placebo, indicating a potential of probiotics in glycaemia-related parameters. However, it is important to note that Ejtahed et al. [24] report that probiotics have an insignificant effect on HbA1c level (Figure 3).

Furthermore, all of the studies included in our analysis but one showed a substantial decrease in blood glucose concentration in subjects with diabetes after probiotic use. Only Asemi, et al. [23] reported a significant rise of glycaemia in the intervention group in comparison with

subjects administered with placebo. Interestingly enough, studies performed by Asemi [22,23] clearly stand out from the rest in several analyses. Also, sensitivity analyses (supplementary material) highlight their great impact on the total effect. It is difficult to assess what is the reason of such distinction. Probably, the cause for this situation may be the longest duration of the trials (8 and 6 weeks, respectively). Additionally, it seems that using high-dose multispecies probiotic supplements or synbiotic may be more effective than single-strain supplement. The analysis of the association between probiotic intake and insulin level also demonstrated inconsistent findings of original research. It cannot be excluded that the differences observed between the results of these studies are based on the protocols of the particular studies. Probiotic supplementation has been reported to be associated with reduced adipose tissue mass and Body Mass Index (BMI), and that these changes may play a role in T2DM prevention [31,32]. Hulston et al. reported probiotics consumption to have a positive influence on blood glucose concentration and insulin sensitivity in healthy subjects fed with an obesogenic diet [33]. Furthermore, studies have demonstrated probiotics may have potential benefits in the prevention of other diabetes-related changes [34-37], which has been confirmed, at least partially, by the results of our meta-analysis.

The nature of the beneficial effects of probiotics on glycaemia-related parameters is not fully understood. It has been suggested that probiotics may increase GLP-1 secretion from enteroendocrine L-cells to improve carbohydrate metabolism, decrease glucotoxicity and increase insulin sensitivity of target cells [11]. Probiotic intake influences 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 pro-inflammatory signaling and enhances insulin sensitivity [11,38].

Our findings did not reveal probiotics to have any significant effects on other cardiometabolic risk factors, including lipid profile components and CRP level; it is possible that this may be

due to the various probiotic strains used in the studies and their short duration. An elevated HDL level is generally regarded as a factor reducing the likelihood of cardiovascular disease (CVD). Interestingly, it is also considered as a protective factor in metabolism-related disorders, including diabetes [39]. Our study did not show statistically significant effect of probiotics use on HDL level. However, several previous RCTs have shown a significant rise of HDL cholesterol after the administration of probiotic-containing products to non-diabetic subjects [40,41]. It is worth underlining that the association between other lipid parameters and probiotic consumption is also inconsistent [31,40,42].

Present meta-analysis failed to confirm that probiotics have any effect in lowering the level of triglycerides, total cholesterol or LDL. In contrast with our findings, previous meta-analyses report that probiotics effectively reduced the levels of total cholesterol and LDL-cholesterol in subjects with originally high or normal lipid levels[43,44]. This discrepancy may result from the characteristics of the study group (healthy or obese subjects in previous analyses vs. subjects with diabetes in our meta-analysis), the length of intervention or choice of probiotic strain.

The anti-inflammatory properties of bacteria are known to be strain-specific, which in turn depends on the nature of the superficial antigens on the bacterium [45,46-48]. Therefore, the variable effectiveness of probiotic bacteria in reducing an inflammatory state, assessed in the analysis of the influence of probiotics on CRP level, may be connected with the variation in their probiotic preparations and their strain-specific efficacy. However, as the efficiency of probiotics is suggested to be beneficial in other inflammation-related disorders, they may also appear an effective tool in diabetes treatment [49,50].

Limitations

Our meta-analysis has several limitations. Regarding search, several other search strategies on

probiotics and diabetes are used. There are many synonyms for probiotics, comprising designations of different probiotic species. Likewise, a variety of synonyms for diabetes exists. However, we believe that adding subsequent probiotic strains into the searching query might in fact lead to the exclusion of other probiotic types, especially those which are used less frequently. In our opinion using only two expressions – probiotics and diabetes – expands the number of results, in practice, as these terms are commonly used as the keywords. The number of identified RCTs (eight) that met the inclusion criteria is relatively low. This could be associated with the fact that the topic has not been previously considered: the first reports investigating the effect of probiotics on modifiable cardiometabolic risk factors in diabetics have been published only recently. This low number of involved RCTs implies a relatively low number of enrolled subjects (438 subjects), which reduces the credibility of the meta-analysis. Furthermore, the low number of included RCTs and their diversified setting makes it impossible to assess the effect of a particular probiotic strain on all analyzed metabolic parameters. The majority of analyzed studies exploited probiotic mixes or dairy products containing several probiotic strains; only one study [21] assessed the effect of a single probiotic strain. Therefore, no subgroup analysis to estimate which probiotic preparations could be more effective in improving metabolic parameters in diabetics was possible. Another important point is that the analyzed RCTs only had a maximum length of 8 weeks and had no follow-up. It 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 (supplementary material) highlight how the effect of potential exclusion of a study would affect the total effect. Further, they show that studies affect the standard error differentially and it is mostly associated with the number of subjects enrolled. 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 studies were included in the analysis, while factors possibly affecting its homogeneity were noted. Most probably, the reason for this heterogeneity is the diversified setting of included RCTs. Firstly, interventions in considered trials involved different probiotic formulas, be they a specified single probiotic strain, a multispecies probiotic preparation, synbiotic 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 analyses. All of these issues greatly reduces the clarity and explicit nature of the conclusions. Nevertheless, they may indicate a trend that requires further scientific evidence.

In conclusion: this meta-analysis of available RCTs suggest that probiotic supplementation has a beneficial effect on selected cardiometabolic parameters in T2DM patients. However, before they can be recommended for use in supportive treatment of T2DM, 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 (MK and JD) contributed to the design of the research. Both authors were independently involved in data collection, selection and assessment of its quality. Both authors analyzed the data. Both authors edited and approved the final version of the manuscript.

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Table 1.Characteristics of RCTs assessing the metabolic effects of probiotics in subjects with T2DM included in the meta-analysis

Author	Ref	Year	Title	Type	Participants	Intervention	Duration	Effects	Follow up	Jadad's score, AC, ITT
Andreasen	21	2010	Effect of Lactobacillus acidophilus NCFM on insulin sensitivity and the systemic inflammatory response in human subjects	DB-RCT	45 adult patients (18 with T2DM, 5 with impaired glucose tolerance GT, 22 with normal GT)	L. acidophilus NCFM	4 weeks	Insulin sensitivity, inflammatory markers	No	4, ✓ -
Asemi 1	22	2013	Effect of Multispecies Probiotic Supplements on Metabolic Profiles, hs-CRP and oxidative stress in patients with Type 2 Diabetes	DB-RCT	54 adult diabetic patients	7 viable and freeze-dried strains: Lactobacillus acidophilus (2×10^9 CFU), L. casei (7×10^9 CFU), L. rhamnosus (1.5×10^9 CFU), L. bulgaricus (2×10^8 CFU), Bifidobacterium breve (2×10^{10} CFU), B. longum (7×10^9 CFU), Streptococcus thermophilus (1.5×10^9 CFU), and 100 mg fructo-oligosaccharide.	8 weeks	Metabolic profiles, hs-CRP, biomarkers of oxidative stress	No	4, ✓ -
Asemi 2	23	2014	Effect of synbiotic food consumption on metabolic status of diabetic patients: a double-blind randomized cross-over controlled trial	DB-RCT	62 adult diabetic patients	probiotic viable and heat-resistant Lactobacillus sporogenes (1×10^7 CFU), 0.04 g inulin (HPX) as prebiotic with 0.38 g isomalt, 0.36 g sorbitol and 0.05 g stevia as sweetener per 1 g	6 weeks	Metabolic profiles, hs-CRP, biomarkers of oxidative stress	No	4, ✓ -
Ejtahed	24	2012	Probiotic yogurt improves antioxidant status in type 2 diabetic patients	DB-RCT	60 adult diabetic patients	300 g/d of probiotic yogurt containing Lactobacillus acidophilus La5 and Bifidobacterium lactis Bb12	6 weeks	Fasting blood samples, 24-h dietary recalls, and anthropometric measurements	No	5, ✓ -
Judiono	25	2014	Effects of clear kefir on biomolecular aspects of glycemic status of T2DM patients in Bandung, West Java	RCT	108 adult diabetic patients	clear kefir	30 days	HbA1c, FBG, PBG, insulin, c-peptide	No	1, ✓ ?
Mahboobi	26	2014	The effects of probiotic supplementation on markers of blood lipids and blood pressure in patients with type 2 diabetes	DB-RCT	55 adult prediabetic patients	7×10^9 colony forming unit (CFU) Lactobacillus Casei, 2×10^9 CFU Lactobacillus Acidophilus, 1.5×10^9 CFU Lactobacillus Rhamnosus, 2×10^8 CFU lactobacillus Bulgaricus, 2×10^{10} CFU Bifidobacterium Breve, 7×10^9 CFU Bifidobacterium Longum, 1.5×10^{10} CFU Streptococcus Thermophilus, fructooligosaccharide (as prebiotic), B group vitamins, maltodextrin, lactose and magnesium stearate	8 weeks	Lipid profiles, blood pressure	No	5, ✓ -
Mazloom	27	2012	Effect of probiotics on lipid profile, glycemic control, insulin action, oxidative stress and inflammatory markers in patients with Type 2 Diabetes: a Clinical trial	SB-CT	34 adult diabetic patients	L. acidophilus, L. bulgaricus, L. bifidum, and L. casei	6 weeks	Glucose, insulin, TG, total cholesterol, LDL-C, HDL-C, malondialdehyde, high sensitive CRP (hs-CRP) and IL-6.	No	3, ✓ -
Moroti	28	2012	Effect of the consumption of a new synbiotic shake on glycemia and cholesterol levels in elderly people with T2DM	DB-RCT	20 adult diabetic patients	synbiotic shake containing 10^8 UFC/mL Lactobacillus acidophilus, 10^8 UFC/mL Bifidobacterium bifidum and 2 g oligofructose	30 days	Standard lipid profile (total cholesterol, triglycerides and HDL cholesterol)and glycemia, or blood sugar levels	No	5, ✓ -

Abbreviations: CFU – colony forming unit, AC – allocation concealment, ITT – intention-to-treat

Figure 1. **Flowchart demonstrating the selection of trials** assessing the metabolic effect of probiotics in subjects with T2DM

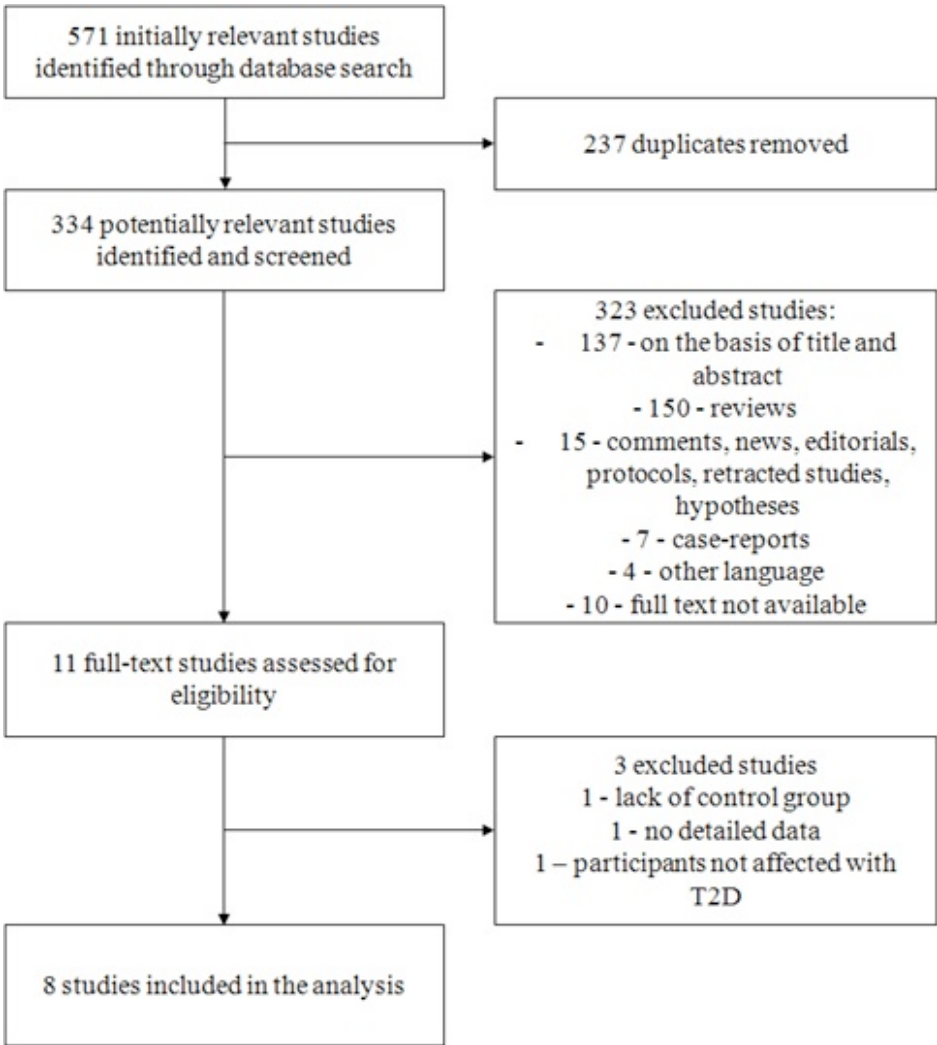


Figure 2. **Forest plot of the association between probiotic use and FPG level.** The shaded squares indicate the effect of probiotics in the particular study. The horizontal lines represent 95% CIs. The diamond data marker indicates the pooled effect.

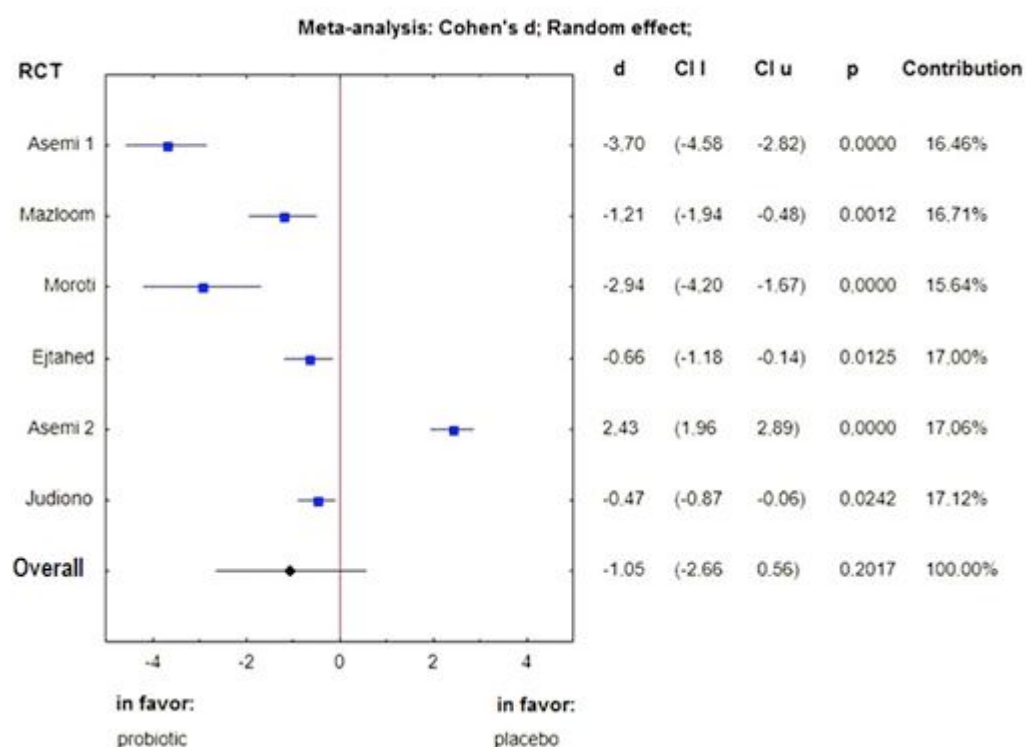


Figure 3. **Forest plot of the association between probiotic use and HbA1c level.**

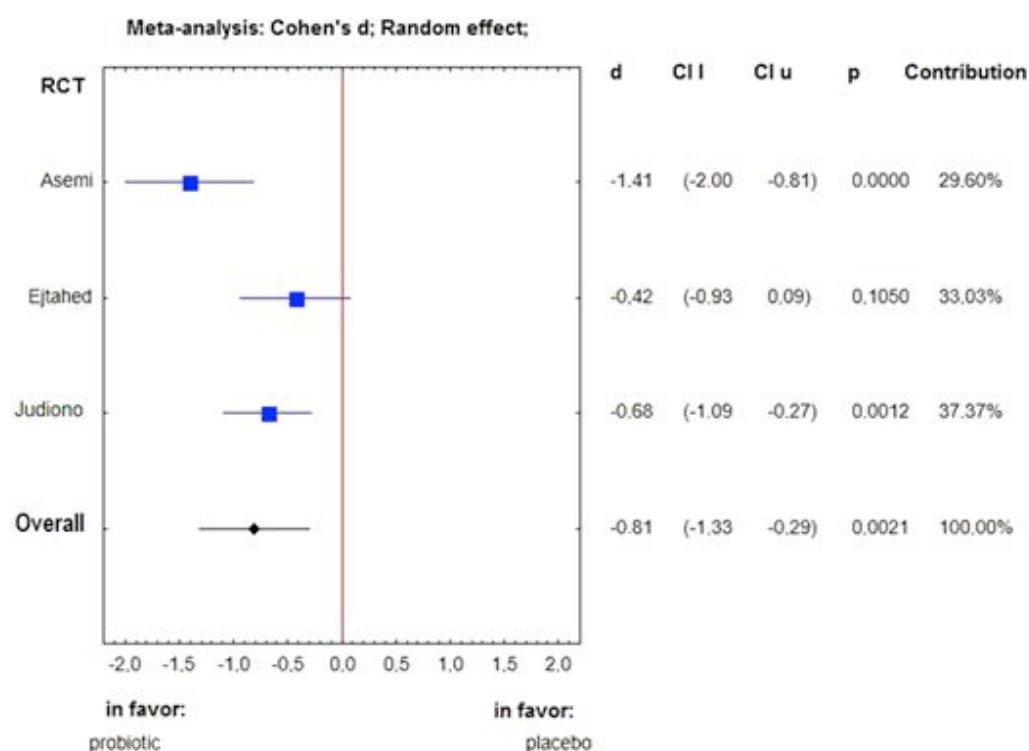


Figure 4. Forest plot of the association between probiotic use and insulin level.

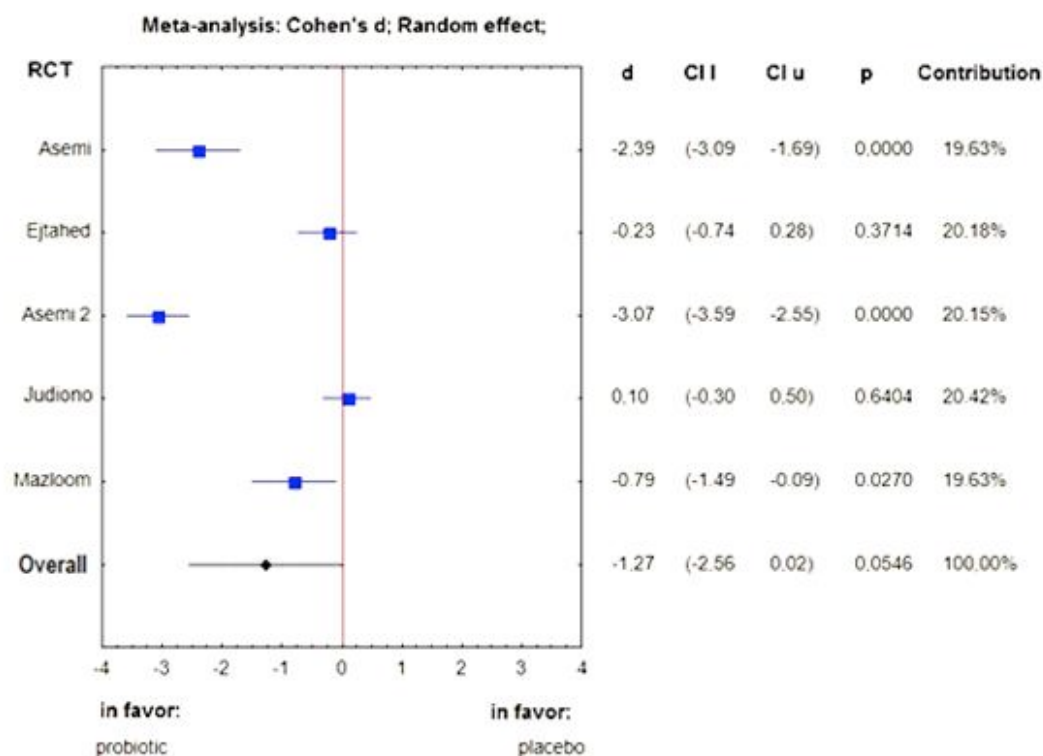


Figure 5. Forest plot of the association between probiotics use and HOMA-IR.

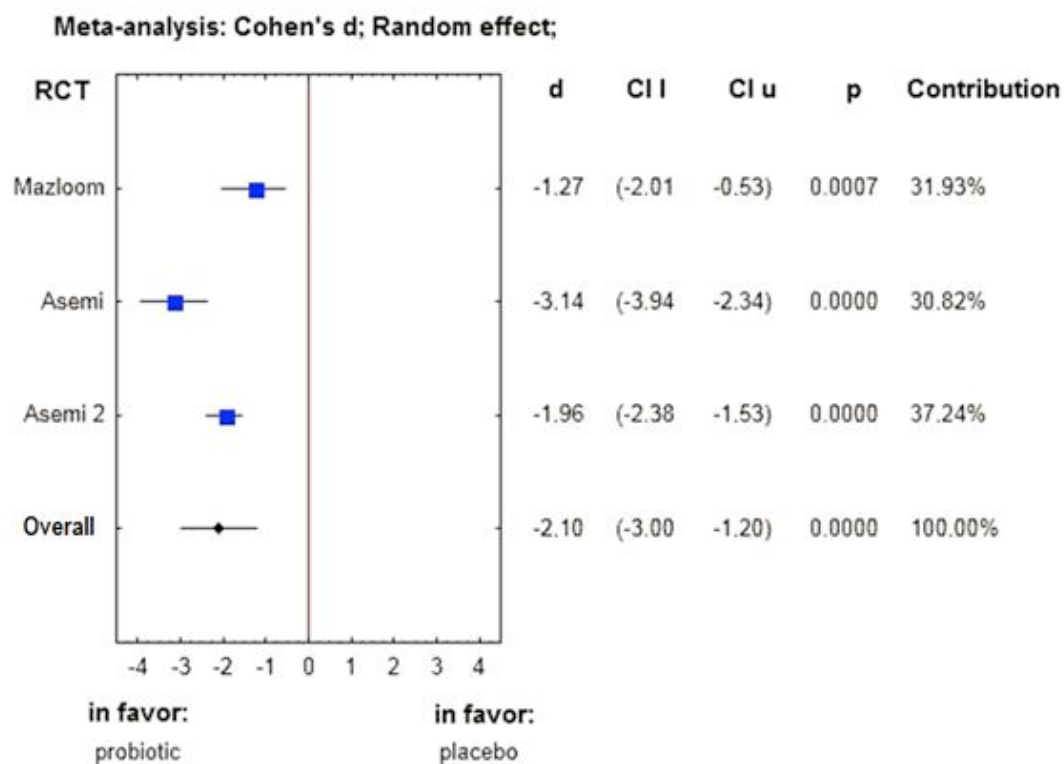


Figure 6. Forest plot of the association between probiotic use and HDL cholesterol level.

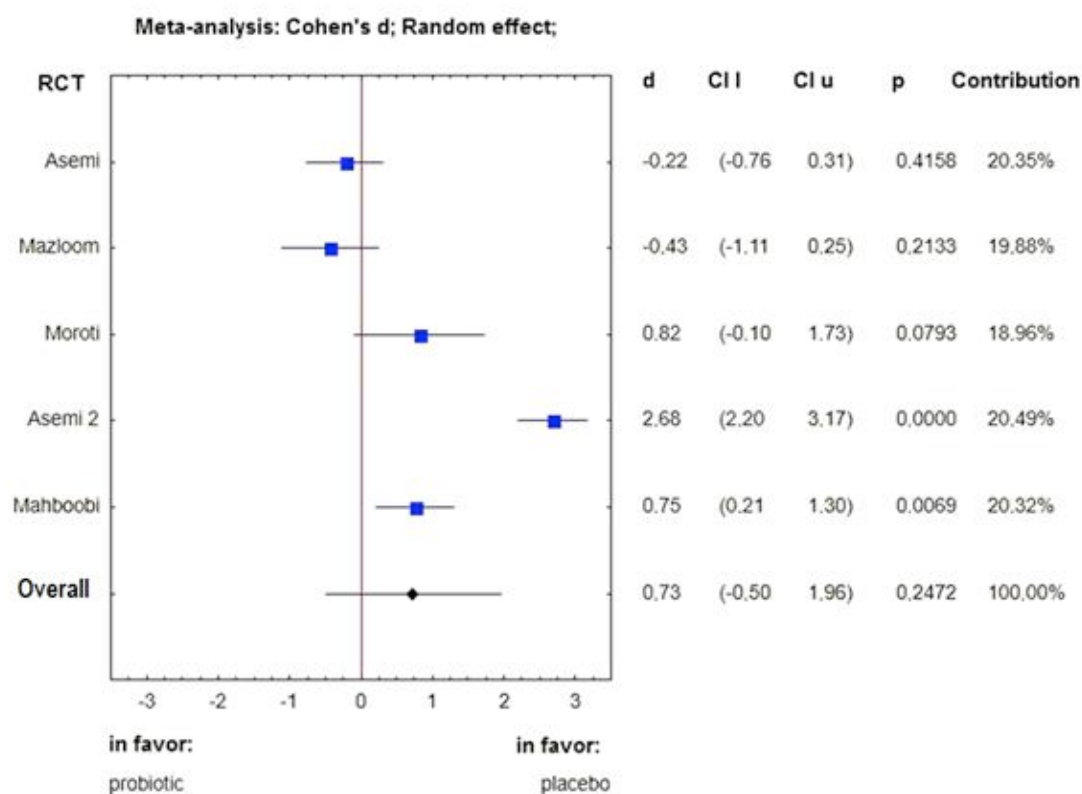


Figure 7. Forest plot of the association between probiotic use and CRP level.

