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The effect of probiotics/synbiotics supplementation on renal and liver biomarkers in patients with type 2 diabetes: a systematic review and meta-analysis of randomised controlled trials

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Abstract

Despite the apparent beneficial effects of probiotics/synbiotics on glucose haemostasis, lipid profile and inflammatory responses, it is not clear whether these beneficial effects also impact renal and hepatic function in diabetes. Therefore, we sought to assess the effect of probiotics/synbiotics supplementation on renal and liver biomarkers in adults with type 2 diabetes mellitus (T2DM) using a systematic review and meta-analysis of randomised controlled trials (RCT). PubMed, Scopus, Web of Science and Cochrane Library were systematically searched, up to February 2021. The pooled weighted mean difference (WMD) was estimated using a random-effects model. The methodological quality of studies, as well as certainty of evidence, was assessed using standard scales. Fifteen related trials were identified. Meta-analysis of six trials, involving 426 participants, indicated that probiotics/synbiotics supplementation reduced serum levels of creatinine (WMD = −0·10 mg/dl, 95 % CI −0·20, −0·00; $P = 0{\cdot}01$; $I^2 = 87{\cdot}7$ %; *P*-heterogeneity < 0·001), without any significant effect on blood urea nitrogen (BUN), glomerular filtration rate or microalbuminuria. No significant improvement was found on liver biomarkers following probiotics/synbiotics supplementation. The subgroup analysis showed a significant improvement in BUN when follow-up duration lasted for 12 weeks or more (WMD = −1·215 mg/dl, 95 % CI −1·933, −0·496; $P = 0{\cdot}001$) and in creatinine levels in patients with renal dysfunction (WMD = −0·209 mg/dl, 95 % CI −0·322, −0·096; $P < 0{\cdot}001$). Our results are insufficient to advocate the use of probiotics/synbiotics for improving renal or liver function in patients with T2DM. Indeed, due to the low certainty of evidence, these findings need to be affirmed in further high-quality RCT.

Key words: **Probiotic: Type 2 diabetes: Meta-analysis: Glomerular filtration rate: Kidney: Liver: Synbiotics**

With the increasing prevalence of obesity, sedentary lifestyles and urbanisation, type 2 diabetes mellitus (T2DM) has become a global health issue, affecting 463 million people in 2019, and is predicted to reach 700 million cases in 2045[1]. T2DM can lead to a series of additional complications, particularly micro- and macro-vascular damage, and negatively affecting multiple vital organs, including the kidneys, liver, eyes and cardiovascular system[2].

Studies have reported that 20–40 % of patients with diabetes suffer from renal dysfunction, characterised by urine albumin excretion or reduced glomerular filtration rate (GFR), and 40 % of them may progress to end-stage renal disease[3–6]. The exact cause of diabetic renal impairment is complex and is proposed to be contributed to hyperglycaemia, dyslipidaemia, atherosclerotic vascular, obesity, hyperuricaemia, and increased systemic and intra-glomerular pressure[7,8].

Accumulating evidence also indicates that the liver, as an insulin-sensitive tissue and the main regulator of metabolism, is prone to damage by hyperglycaemia, leading to further impaired metabolism and inflammatory reactions[9,10]. Steatosis, elevated liver enzymes, cirrhosis and carcinoma are among several important liver abnormalities in patients with T2DM[11,12].

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; GFR, glomerular filtration rate; RCT, randomised controlled trial; T2DM, type 2 diabetes mellitus; WMD, weighted mean difference.

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The most well-known strategy to prevent the progression of diabetes-related complications is maintaining glycaemic control[13]. In addition to weight control, lifestyle modifications and medical solutions, there is evidence supporting the effect of gut microbiome in regulating metabolism and energy haemostasis[14,15]. Recently, studies reported alternations of gut microbiota in patients with diabetes[16–18], and probiotics/synbiotics supplementation was able to exert beneficial effects on lipid profile, glycaemic control, blood pressure and inflammation in these patients[19–25].

The exact mechanism of beneficial effects manifest following probiotic supplementation is not well known. However, its anti-inflammatory properties are very likely contributory. A recent meta-analysis study showed that probiotic therapy significantly decreased C-reactive protein concentration and increased serum levels of glutathione, malondialdehyde and total antioxidant capacity in patients with chronic kidney diseases[22]. Moreover, probiotics may improve insulin resistance by increasing liver natural killer T cells and down-regulating TNF-α and NF-κB activity[26]. Probiotics have also shown angiotensin-converting enzyme inhibitor properties, and consequential antihypertensive effects[20,27].

Although there is evidence regarding the beneficial effects of probiotics/synbiotics on the improvement of metabolic control in patients with diabetes[24,25,28–30], so far, no study has systematically examined the effects of probiotics/synbiotics on renal and liver function in these patients. Therefore, we sought to investigate whether probiotic supplementation could improve renal and liver biomarkers, by conducting a systematic and meta-analysis of randomised controlled trials (RCT).

Methods

We performed the present meta-analysis in accordance with the Cochrane Handbook for Systematic Reviews of Interventions and[31] adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines[32]. This review was registered at the centre of Open Science Framework as https://doi.org/10.17605/OSF.IO/UKXBD.

Search strategy

We searched for references indexed in PubMed, Scopus, Web of Science and Cochrane Library, from database inception to 10 February 2021. The terms used in search strategy are provided in online Supplementary Table S1. We did not impose any keywords in terms of interested outcomes and did not apply any restriction for language or publication year. The reference lists of the meta-analyses that examined the effect of probiotic or synbiotic supplementation/fortified foods in T2DM were also searched manually. A specific question was also defined according to the Participants, Interventions, Control, Outcomes and Study design principle (Table 1).

Selection criteria

The titles/abstracts and full text of retrieved references were screened according to the inclusion and exclusion criteria independently by two authors (SS and FM), and any discrepancies were resolved by discussion with a third author (SA). The inclusion criteria of this article were as follows: the RCT (parallel or crossover) that compared the effects of probiotic/synbiotic supplements or fortified foods (any strains and dosages) with placebo in pre-diabetic or T2DM patients. All included studies needed to report mean and standard deviation of baseline, post or change from baseline for at least one of the following liver enzymes or kidney function indicators, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase, alkaline phosphatase (ALP), bilirubin, creatinine, blood urea nitrogen (BUN), uric acid, microalbuminuria, proteinuria or GFR, or any other renal and liver biomarkers. The exclusion criteria were as follows: (1) trials with less than 1-week period, (2) trials without a placebo-controlled group, (3) duplicated publications from the same population, (4) trials with insufficient information for calculating the mean or standard deviation change in the outcome measure(s), (5) trials including pregnant or lactating women and (6) trials that used probiotic or synbiotic in combination with other treatments and/or the comparator group did not received the same treatment.

Table 1. Participants, Interventions, Control, Outcomes and Study design criteria for inclusion and exclusion of studies

Parameter	
Participants	Adults (≥ 18 years) of both sexes and all nationalities, with pre-diabetes or type 2 diabetes mellitus
Interventions	Probiotic/synbiotic supplements or fortified foods (any strains and dosages)
Control/comparator group	Placebo or non-fortified foods
Outcomes	Any biomarker of renal or liver function, including aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, alkaline phosphatase, bilirubin, creatinine, blood urea nitrogen, uric acid, microalbuminuria, proteinuria, or glomerular filtration rate, etc.
Study design	Randomised controlled trials (parallel or cross-over)

Data extraction

The relevant data were extracted by one author and then cross-checked by another (SS, FM), and any discrepancies resolved by discussion with a third author (SA). The following data were extracted: the first author's name, year of publication, study characteristic (study design, follow-up duration, study location, sample size in the intervention and control groups, the species and dosage of probiotic or synbiotic supplementation and interested outcomes) and participant characteristic (age, sex, health status). The means, along with the respective SD values, of before and after the intervention or change for AST (U/L), ALT (U/L), γ-glutamyl transferase (U/L), ALP (U/L), bilirubin (mg/dl), creatinine (mg/dl), BUN (mg/dl), microalbuminuria (albumin/creatinine ratio), GFR (ml/min per 1·73 m^2) and any other liver or renal-related biomarkers also were extracted.

Study quality and quality of evidence

The quality of the selected articles was evaluated using the Cochrane Collaboration's tool for assessing risk of bias[33]. The quality of evidence assessment was performed with the use of

the Grading of Recommendations Assessment, Development and Evaluation approach, which includes five domains: risk of bias, inconsistency of results, imprecision of results, indirectness of evidence and publication bias. The quality of evidence of RCT was initially considered as high and was downgraded by the following limitations: methodological errors[34], inconsistency[35], imprecision of estimates[36], indirectness[37] or evidence of publication bias[38]. All quality evaluation and evidence were performed independently by two reviewers (SS and FM), and disagreements were resolved through discussion with a third author (SA).

Statistical analyses

For each outcome, where at least ≥ 3 RCT reported sufficient data, the net change in mean and its 95 % CI between the intervention and control groups as the effect size is calculated in the meta-analysis. In term of trials that did not provide change values, the mean change was calculated by the minus mean final value from baseline mean value in each arm, and standard deviation of the mean change estimated formula suggested by the Cochrane Handbook of Systematic Review[39] where correlation coefficient was imputed (r 0·68 ALP[40], r 0·42 AST[41–43], r 0·48 ALT[41], r 0·73 bilirubin[41,42], r 0·82 creatinine[44–48], r 0·71 BUN[45–47,49], r 0·77 microalbuminuria[40,46], r 0·82 GFR[44,48]) from included studies reporting both baseline, final values and changes from baseline for each interested outcome. The random-effects model described by Dersimonian and Laird was used to calculate the overall pooled effect[50].

Regarding trials that multiple intervention (probiotic or synbiotic) compared with the single control group, the calculated effect size related to probiotic supplementation was included in main analysis to avoid counting the control group twice in the analysis.

Inconsistencies across trials were assessed with the use of the Cochrane's χ^2 test and the I^2 statistic, where significant heterogeneity was evident as $I^2 \geq 50\,\%$[51,52]. The subgroup analyses were conducted to detect source of heterogeneity if there are adequate trials for each outcome. Sensitivity analysis was conducted to evaluate the impacts of each trial on the meta-analysis results. The presence of publication bias was evaluated by the 'Begg's funnel plot' and Egger's test whenever if possible (at least ten trials included)[53,54]. Statistical analyses were conducted using STATA version 14 (STATA Corp.). Two-tailed P values of 0·05 were, *a priori*, considered as statistically significant.

Results

Study selection and characteristics

The study selection process is detailed in Fig. 1. Our initial systematic search identified 4905 potentially relevant studies, after removing duplicates (n 1348). Following title/abstract review, ninety-eight articles were retained for full-text screening, and then, eighty-three further articles were excluded due to the wrong population (n 4), wrong intervention (n 16), wrong outcome (n 51), wrong comparison (n 2), insufficient data (n 1), repeated reports (n 6) and without full text (n 3). The excluded studies as well as the reasons are shown in online Supplementary Table S2. Finally, fifteen trials were eligible for inclusion in the systematic review and reported following outcomes: ALP (n 4), ALT (n 6), AST (n 6), bilirubin (n 3), BUN (n 5), creatinine (n 6), GFR (n 3), microalbuminuria (n 3), uric acid (n 2), cystatin-C (n 1), albumin (n 1), γ-glutamyl transferase (n 1) and neutrophil gelatinase-associated lipocalin (n 1).

The study characteristics are described in Table 2. Except for two studies[41,42], all the included studies were parallel in design. Most of the included studies were carried out in Iran[40–48,55–57], and the rest of the studies were performed in Ukraine[58], Sweden[59] and Malaysia[60]. Participants were composed of both male and female in all the included studies and were with T2DM, although patients with both type 1 and type 2 diabetes were eligible for inclusion in two studies[47,48], and one study did not provide information about the type of diabetes[45]. Participants in seven studies suffered from nephropathy[40,44,45,47,57,59], dialysis[48] and non-alcoholic fatty liver[58]. The mean baseline BMI presented an obesity (> 30 kg/m^2) condition in six studies[42,43,45,56,58,59], and participants in other studies were in overweight category. Participants in five studies were treated with exogenous insulin[47,48,57–59], and oral anti-hyperglycaemic drugs were given in rest of the studies.

The duration of intervention ranged from 6 to 12 weeks. All the included studies administered synbiotics[46] or probiotics[47,48,55,56,58–60] in solid pharmaceutical formulations (powder or table form), and six studies used soya milk[40,57], bread[43], honey[45] and an unknown food containing synbiotic[41,42] as carrier. One study included two doses of probiotic, where the higher dose was considered for analysis[59]. There was also one study that presented data on synbiotic, probiotic and placebo supplementation, separately, where the probiotic in comparison with placebo was included in the analysis[43]. Common adverse effects were reported, such as gastric disturbance[59,60], headache, hypoglycaemia and musculoskeletal symptoms[59].

Risk of bias and quality of evidence

The Cochrane Collaboration's tool was used to assess the methodological quality of studies. Participants, personnel and outcomes assessor were blind in all the included studies. Of the fifteen included randomised studies, two did not describe the randomisation and allocation concealment process[40,59]. Furthermore, one study was funded partly by a non-academic source; however, the authors declared no conflict of interest, and the company did not interfere with the decision to exploit research results; therefore, we did not downgrade for funding domain[60]. No concern was also found about incomplete data or selective reporting. Altogether, most of the included studies were rated as good quality, and two studies were fair in methodological quality[40,59] (online Supplementary Table S3). The quality of evidence showed very low certainty for ALT, ALP, bilirubin, creatinine, GFR and microalbuminuria, and low certainty for AST and BUN (online Supplementary Table S4).

Meta-analysis

Effect of probiotics/synbiotics supplementation on liver biomarkers. Pooling data from RCT revealed probiotics/synbiotics supplementation had no significant effect on ALP[41,43,56,60]

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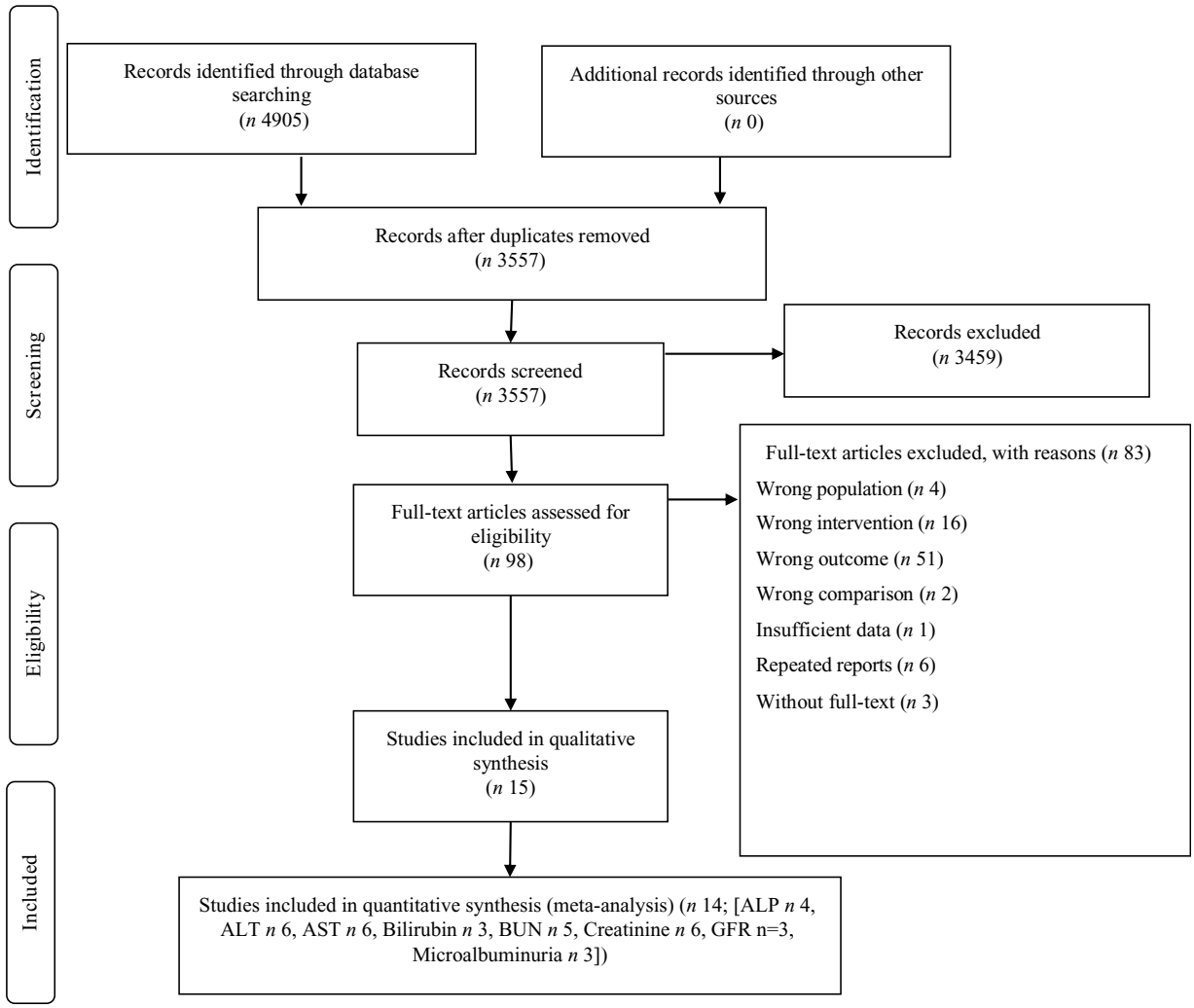

Fig. 1. Study selection process.

(*n* 4 studies, 310 participants; weighted mean difference (WMD) = 7·26 U/L, 95 % CI −3·39, 17·91; $P = 0·18$; $I^2 = 63·3$ %; *P*-heterogeneity = 0·04), ALT[41,43,56,58–60] (*n* 6 studies, 397 participants; WMD = −0·76 U/L, 95 % CI −4·12, 2·58; $P = 0·65$; $I^2 = 57·7$ %; *P*-heterogeneity = 0·03), AST[41,43,56,58–60] (*n* 6 studies, 397 participants; WMD = −0·91 U/L, 95 % CI −3·05, 1·22; $P = 0·4$; $I^2 = 28·1$; *P*-heterogeneity = 0·22) and bilirubin levels (*n* 3 studies, 256 participants; WMD = −0·04 mg/dl, 95 % CI −0·16, 0·08; $P = 0·52$; $I^2 = 86·2$ %; *P*-heterogeneity = 0·001) (Fig. 2, online Supplementary Table S5). Between-study heterogeneity was moderate to high, although the small number of studies precluded a comprehensive subgroup analysis, the duration of intervention and liver complications could justify the observed heterogeneity to some extent (online Supplementary Tables S6 and S7).

Effect of probiotics/synbiotics supplementation on renal biomarkers. Our analysis found probiotics/synbiotics supplementation reduced creatinine levels[44–48,60] (*n* 6 studies, 426 participants; WMD = −0·10 mg/dl, 95 % CI −0·20, −0·00; $P = 0·01$; $I^2 = 87·7$ %; *P*-heterogeneity < 0·001), without any significant effect on GFR[44,48,60] (*n* 3 studies, 236 participants; WMD = 4·55 ml/min per 1·73 m^2, 95 % CI −0·94, 10·05; $P = 0·1$; $I^2 = 90·7$ %; *P*-heterogeneity < 0·001), microalbuminuria[40,46,59] (*n* 3 studies, 139 participants; WMD = −10·36 Alb/Cr (mg/gr), 95 % CI −22·87, 2·16; $P = 0·1$; $I^2 = 80·9$ %; *P*-heterogeneity = 0·005) or BUN[45–48,60] (*n* 5 studies, 386 participants; WMD = −0·87 mg/dl, 95 % CI −1·91, 0·18; $P = 0·1$; $I^2 = 36·1$ %; *P*-heterogeneity = 0·18) (Fig. 3, online Supplementary Table S5). Subgroup analysis was performed when the number of studies was sufficient for each outcome, and the results showed a significant reduction in BUN levels when intervention lasted for 12 weeks or more (*n* 4 studies, 316 participants; WMD = −1·215 mg/dl, 95 % CI −1·933, −0·496; $P = 0·001$; $I^2 = 0·0$ %; *P*-heterogeneity = 0·41) and also showed a significant reduction in creatinine levels in patients with renal complications (*n* 4 studies, 220 participants; WMD = −0·209 mg/dl, 95 % CI −0·322, −0·096; $P < 0·001$; $I^2 = 46·7$ %; *P*-heterogeneity = 0·13). Subgroup analysis also identified duration of intervention and renal complication as the potential source of heterogeneity.

Table 2. The characteristics of trials that investigated the effect of probiotics/synbiotics supplementation on liver and renal biomarkers in adults with type 2 diabetes and were eligible for inclusion in the meta-analysis

Author, year	Participants, sex	Mean age	Mean BMI	Country, study design	Condition	Type of diabetes	Type of supplement	Probiotic agent	Duration (weeks)	Outcomes	Results
Studies investigated renal biomarkers											
Abbasi, 2017[40]	40, M & F	56·9 (int), 53·6 (cont)	26·68 (int), 26·58(cont)	Iran, P	Nephropathy	2	Probiotic soya milk	Lactobacillus plantarum A7	8	Microalbuminuria	Significant decrease in microalbuminuria
Abbasi, 2018[40]	40, M & F	56·9 (int), 53·6 (cont)	26·68 (int), 26·58(cont)	Iran, P	Nephropathy	2	Probiotic soya milk	Lactobacillus plantarum A7	8	Creatinine, GFR	Significant decrease in serum creatinine and significant increase in GFR in probiotic group
Arani, 2018[45]	60, M & F	62·7 (int), 60·3 (cont)	30·3 (int), 31·1(cont)	Iran, P	Nephropathy	–	Probiotic honey	Bacillus coagulans	12	Creatinine, BUN	No significant change
Asemi, 2013[56]	54, M & F	50·51 (int), 52·59(cont)	31·61 (int), 30·17 (cont)	Iran, P	–	2	Multispecies probiotic	Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Bifidobacterium breve, Bifidobacterium longum, Streptococcus thermophilus and fructooligosaccharide	8	Uric acid	No significant change
Asemi, 2014[42]	62, M & F	53·1 (int), 53·1 (cont)	29·60 (int), 29·90 (cont)	Iran, C	–	2	Synbiotic food	Lactobacillus sporogenes, inulin, isomalt, sorbitol and stevia	6 (three times a day)	Uric acid	Significant increase in serum uric acid in synbiotic group
Ebrahimi, 2017[46]	70, M & F	58·71 (int), 58·63(cont)	28·13 (int), 27·30 (cont)	Iran, P	–	2	Synbiotic	Lactobacillus, Bifidobacterium, Streptococcus thermophilus, Prebiotics (fructooligosaccharide)	9	Creatinine, urea, microalbuminuria, BUN	Significant decrease in microalbuminuria in synbiotic group
Firouzi, 2015[60]	136, M & F	52·9 (int), 54·2 (cont)	29·2 (int), 29·3 (cont)	Malaysia, P	–	2	Multistrain probiotic	Lactobacillus, acidophilus, Lactobacillus casei, Lactobacillus lactis, Bifidobacterium bifidum, Bifidobacterium longum and Bifidobacterium infantis	6 and12	Creatinine, urea, GFR, BUN	Significant decrease in serum urea in probiotic group
Mafi, 2018[47]	60, M & F	58·9 (int), 60·9 (cont)	25·3 (int), 26·3 (cont)	Iran, P	Nephropathy	1 and2	Multistrain probiotic	Lactobacillus acidophilus strain ZT-L1, Bifidobacterium bifidum strain ZT-B1, Lactobacillus reuteri strain ZT-Lre, and Lactobacillus fermentum strain ZT-L3	12	Creatinine, BUN, proteinuria	Significant decrease in serum creatinine and BUN in probiotic group
Miraghajani, 2017[57]	40, M & F	56·9 (int), 53·6 (cont)	26·68 (int), 26·58 (cont)	Iran, P	Nephropathy	2	Probiotic soya milk	Lactobacillus plantarum A7	8	Cystatin-C, NGAL	Significant decrease in

Table 2. (*Continued*)

Author, year	Participants, sex	Mean age	Mean BMI	Country, study design	Condition	Type of diabetes	Type of supplement	Probiotic agent	Duration (weeks)	Outcomes	Results
											cystatin-C in probiotic group
Mobini, 2017[59]	29, M & F	64 (int), 66 (int), 65 (cont)	32·3 (int), 30·6 (int), 30·7(cont)	Sweden, P	–	2	Probiotic (low and high dose)	Lactobacillus reuteri DSM 17938	12	Microalbuminuria	No significant change
Soleimani, 2017[48]	60, M & F	54 (int), 59·4 (cont)	25·5 (int), 27·0 (cont)	Iran, P	Dialysis	1 and 2	Multistrain probiotic	Lactobacillus acidophilus, Lactobacillus casei and Bifidobacterium bifidum	12	Creatinine, BUN, GFR	No significant change
Studies investigated liver biomarkers											
Asemi, 2017[41]	62, M & F	–	29·7 (int), 30·1 (cont)	Iran, C	–	2	Synbiotic food	Lactobacillus sporogenes, inulin, isomalt, sorbitol and stevia	6 (three times a day)	ALP, AST, ALT, bilirubin	Significant decrease in total bilirubin in synbiotic group
Asemi, 2015[55]	58, M & F	49·6 (int), 52·1(cont)	31·9 (int), 30·7 (cont)	Iran, P	–	2	Multispecies Probiotic	Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Bifidobacterium breve, Bifidobacterium longum, Streptococcus thermophilus and fructooligosaccharide	8	ALP, AST, ALT, bilirubin	Significant decrease in serum ALT in synbiotic group
Bahmani, 2015[43]	5, M & F	51·3 (int), 52·0 (int), 53·4(cont)	30·8 (int), 29·8 (int), 30·5 (cont)	Iran, P	–	2	Synbiotic bread/probiotic	Lactobacillus sporogenes and inulin/Lactobacillus sporogenes	8 (three times a day)	ALP, AST, ALT	No significant change
Firouzi, 2015[60]	136, M & F	52·9 (int), 54·2 (cont)	29·2 (int), 29·3 (cont)	Malaysia, P	–	2	Multistrain probiotic	Lactobacillus, acidophilus, Lactobacillus casei, Lactobacillus lactis, Bifidobacterium bifidum, Bifidobacterium longum and Bifidobacterium infantis	6 and 12	ALP, AST, ALT, bilirubin, albumin	No significant change
Kobyliak, 2018[58]	58, M & F	53·4 (int), 57·2 (cont)	34·82 (int), 34·26 (cont)	Ukraine, P	NAFLD	2	Multistrain probiotic	Lactobacillus, Lactococcus, Bifidobacterium, Propionibacterium, Acetobacter	8	AST, ALT, GGT	Significant decrease in serum AST and GGT levels in probiotic group
Mobini, 2017[59]	29, M & F	64 (int), 66 (int), 65 (cont)	32·3 (int), 30·6 (int), 30·7(cont)	Sweden, P	–	2	Probiotic (low and high dose)	Lactobacillus reuteri DSM 17938	12	AST, ALT	No significant change

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; cont, control group; C, cross-over; F, female; GFR, glomerular filtration rate; int, intervention group; M, male; NAFLD, non-alcoholic fatty liver disease; NGAL, neutrophil gelatinase-associated lipocalin; P, parallel.

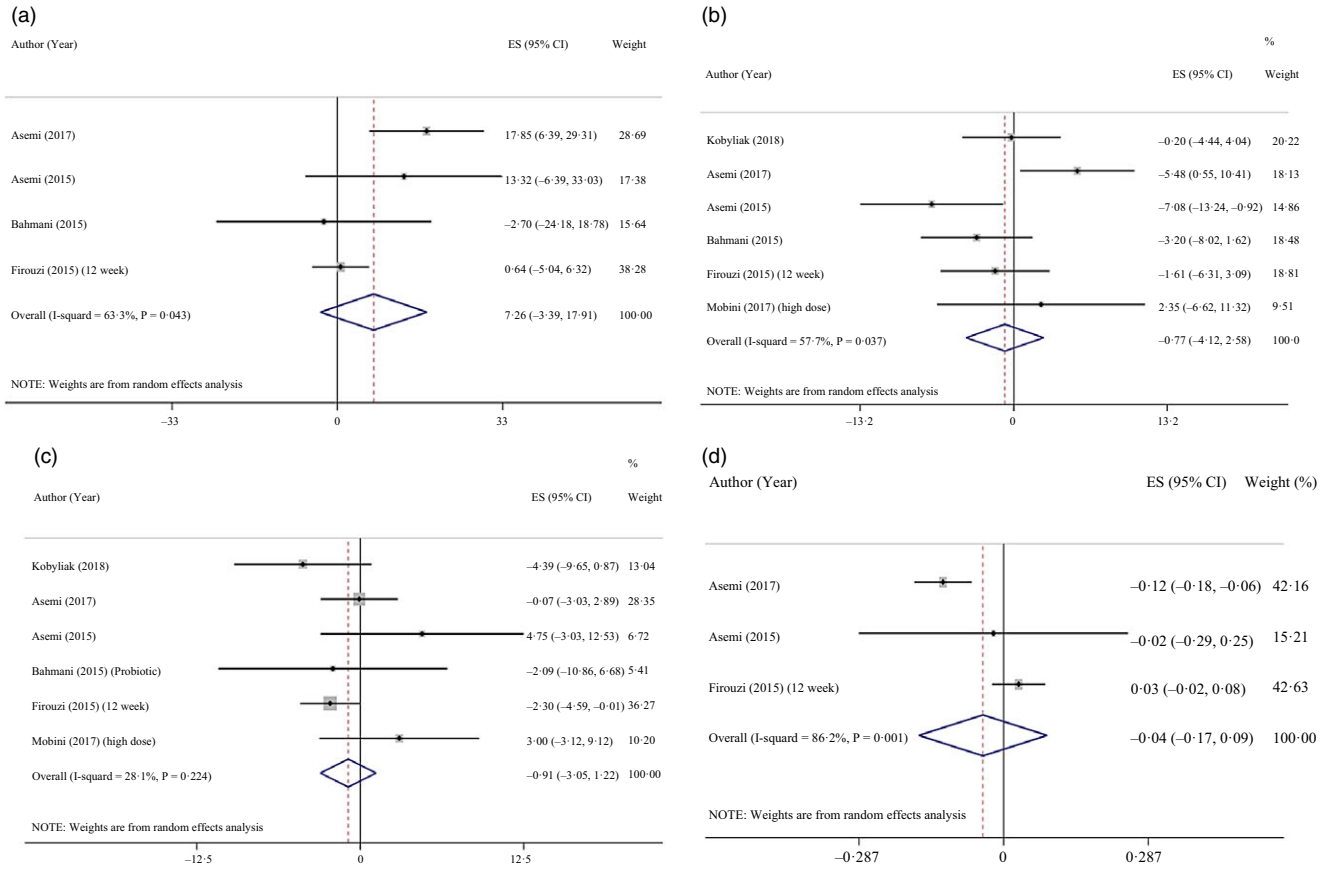

Fig. 2. Forest plot of randomised controlled clinical trials illustrating weighted mean difference (WMD) in (a) ALP change (U/L), (b) ALT change (U/L), (c) AST change (U/L) and (d): bilirubin change (mg/dl) between the probiotics/synbiotics supplementation and control groups for all eligible studies. Analysis was conducted using random-effects model.

Outcomes did not analyse

Uric acid. Two studies evaluated the effect of probiotic supplement and synbiotic food consumption on serum uric acid and reached to contradictory results. One study found synbiotic food supplementation significantly increased serum uric acid[42], while other study revealed no significant effect following probiotic supplementation[56].

γ-Glutamyl transferase. One study suggested a significant 12 % decrease in serum γ-glutamyl transferase following a multistrain probiotic supplementation in type 2 diabetes patients with non-alcoholic fatty liver disease[58].

Cystatin-C, neutrophil gelatinase-associated lipocalin. One study showed significant reduction in cystatin-c and marginally significant reduction in neutrophil gelatinase-associated lipocalin levels in patients with type 2 diabetic nephropathy after the consumption of probiotic soya milk compared with control[57].

Sensitivity analysis and publication bias. The leave-one out sensitivity analysis did not identify any study with a significant influence on the pooled effects sizes. An additional sensitivity analysis was conducted excluding the studies that examined synbiotic supplementation, and the results showed significant decreases in creatinine and BUN levels, with a significant reduction in between-study heterogeneity (online Supplementary Table S7). Publication bias was not examined due to the insufficient study for each outcome.

Discussion

This meta-analysis pooled data from RCT investigating the effect of probiotics/synbiotics supplementation on kidney and liver parameters in patients with diabetes. Our results revealed probiotics/synbiotics supplementation has no significant effect on ALT, AST, ALP, BUN, bilirubin, GFR or microalbuminuria. However, it was shown that probiotics/synbiotics may elicit beneficial effects on creatinine levels.

Emerging data indicating gut microbiota modulation by probiotic, prebiotic or synbiotic supplementation can induce favourable effects on lipid profile, glycaemic control[61] and antioxidant capacity in patients with diabetes[21]. It has been suggested that inflammation is the major mechanism related to diabetes complications[62,63]. Indeed, patients with diabetes tend to suffer from chronic inflammation, exacerbated by impaired intestinal function[64]. The gut is known as a potential immune regulation gate[65], and several immune, endocrine and metabolic pathways accrue between intestinal and other organs[66]. SCFA, the main product of gut fermentation, reduce intestinal permeability, bacteria translocation[67] and down-regulate the expression of

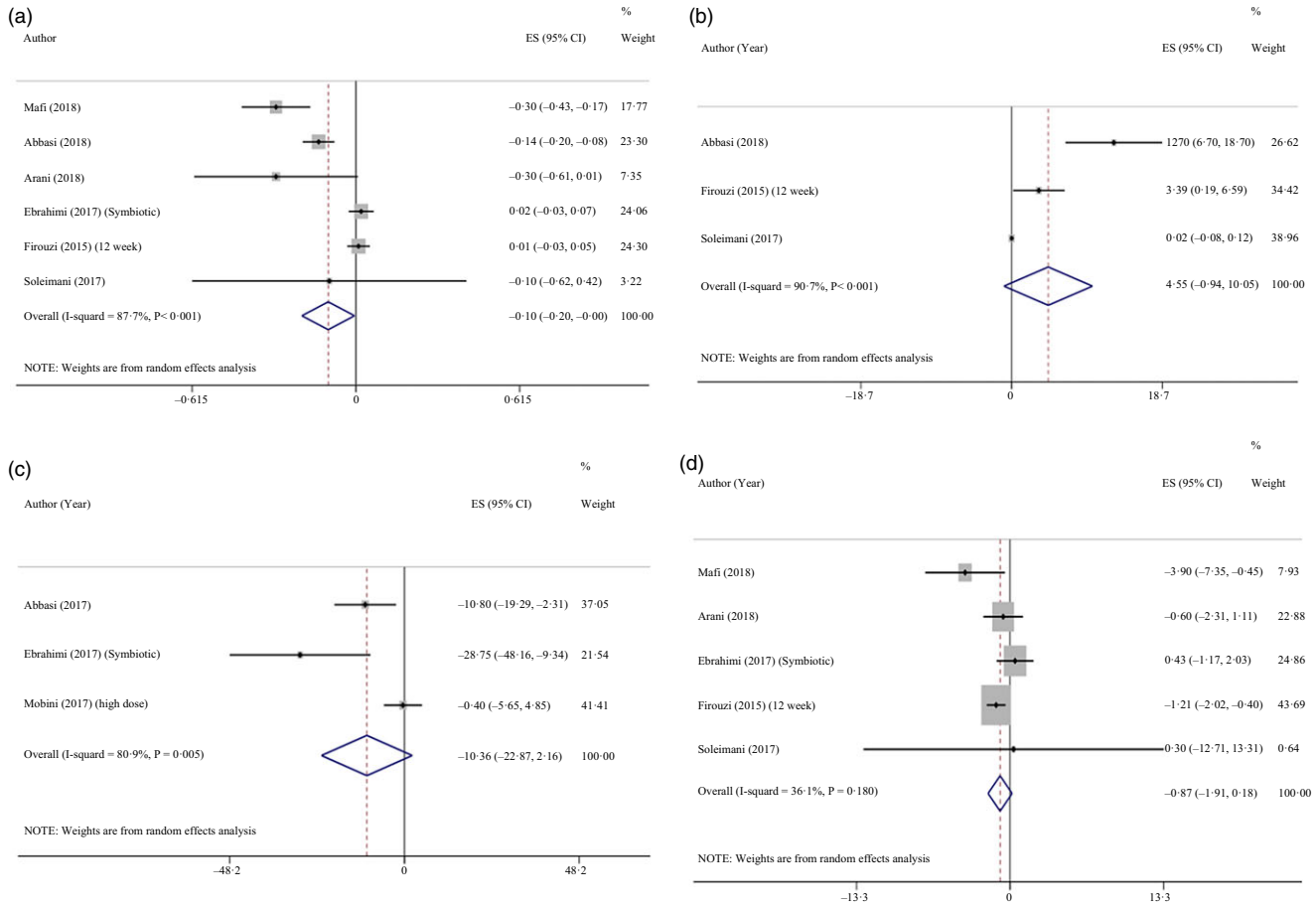

Fig. 3. Forest plot of randomised controlled clinical trials illustrating weighted mean difference (WMD) in (a) creatinine change (mg/dl), (b) GFR change (ml/min per 1·73 m^2), (c) microalbuminuria change (Alb/Cr (mg/gr)) and (d): BUN change (mg/dl) between the probiotics/synbiotics supplementation and control groups for all eligible studies. Analysis was conducted using random-effects model.

pro-inflammatory cytokines[68]. However, findings from previous meta-analysis are inconsistent[69,70]. It seems that the anti-inflammatory effects of probiotics are increased when combined with the prebiotics. Moreover, as shown in a meta-analysis, the use of synbiotics may have more beneficial effects in reducing inflammatory factors than probiotics[70], because of the additional substrate for fermentation, and consequential growth stimulation of gut microbiota[71]. However, our results showed a significant reduction in creatinine and BUN levels when analysis restricted to probiotic supplementation. It may be due to the higher dose of probiotic in the studies administered probiotic, exclusively. Moreover, BUN levels improved in studies administered probiotic/synbiotic for 12 weeks or more. This association disappeared when a sensitivity analysis was conducted for studies with ≥ 8 weeks follow-up duration (data not shown). It seems, more than 12 weeks intervention may exert greater beneficial effects of probiotics. However, the number of included studies in our analysis was not enough to draw a definitive conclusion.

In line with a previous systematic review[72], we found probiotic/synbiotic supplementation may improve creatinine levels in patients with renal dysfunction, although a meta-analysis by AbdelQadir *et al* showed despite a significant improvement in antioxidant indices, there is no association between probiotic supplementation and creatinine, GFR or BUN levels in patients with diabetic nephropathy[73]. It may be contributed to misclassification of the study by Firouzi *et al*,[60] which the nephropathy was an exclusion criterion of this study, but it has been included in the analysis.

It is suggested probiotic may improve renal function through increasing anaerobic bacteria such as Lactobacillus and Bifidobacterium leading to decrease in PH and urea levels. Moreover, some probiotic species such as Bacteroides can reduce urea by their urease activity[74]. However, our analysis found no significant association for other renal biomarkers. There is accumulating evidence suggesting some new biomarkers for kidney function, such as cystatin-C or neutrophil gelatinase-associated lipocalin, are more affected in early stages of kidney injuries than BUN or GFR[71,72]. The Northern Manhattan study also indicated that cystatin-C-based GFR may be a better predictor of all-cause mortality in the elderly, in comparison with serum creatinine[73]. However, in our study, data were not enough to perform a meta-analysis on these predictor biomarkers.

Concordant with our findings, several previous studies showed contradictory effects of probiotic supplementation on liver enzymes in patients with diabetes[43,60] or fatty liver diseases[75–77]. As a possible explanation, metformin, which was used by most of our

included studied population, is known to improve lipid profile[78], liver function[79] and ovarian function[80], beyond glycaemic control. It is also evident that metformin reduces micro- and macro-vascular complications and also alters gut microbiota[81], which may affect our results. Moreover, different probiotic strains were supplemented in included studies, and it is shown that strain variation may produce different effects on the host[82,83]. However, because of the small number of studies, it was not possible to assess strain-specific effects on interested outcomes. On the other hand, we assessed liver function using liver enzymes, the factors that change in the later stages of liver damage. It is suggested that standard biomarkers such as ultrasound be used in future studies.

Strengths and limitations

As far as we are aware, this is the first meta-analysis comprehensively investigating the effect of probiotics/synbiotics supplementation on kidney and liver function in patients with type 2 diabetes. However, one previous meta-analysis study investigated the effect of probiotic supplementation on kidney function in patients with diabetic nephropathy, with non-significant results[73]. Pooling data from good quality RCT permits causal associations to be drawn; however, there are some considerable limitations. First, the number of included studies was small for each outcome, which affects the validity of the results. Second, there was varied setting among studies, which made it difficult to assess the isolate effect of probiotic supplementation on the outcomes, including probiotic species, probiotic carrier, the medication used and body weight. Third, although macronutrients intake was controlled in most of the included studies, fibre intake or antioxidant nutrients (such as vitamin E, C, D or *n*-3) were not considered in analyses. Fourth, renal and liver biomarkers in most of the included studies were secondary outcomes; therefore, the studies may not have an adequate sample size to detect a significant association. Fifth, none of the included studies used gold standard biomarkers, resulting reduced validity of the results. Sixth, the absence of any information on the composition of colon microbiota after the intervention with probiotics/synbiotics makes it difficult to draw conclusions about the effect of the supplement on changing the gut microbiota, which is suggested to be studied in future researches. Seventh, the certainty of evidence was low or very low; as, most of the included participants were from same location (Iran), and the point estimate was smaller than 5 % baseline value of interested outcomes, leading to downgrading for inconsistency and imprecision, respectively.

Conclusion

In the present systematic review and meta-analysis, we assessed the effects of probiotics/synbiotic treatment on the liver and kidney biomarkers in patients with T2DM. The results of our meta-analysis indicated that probiotics/synbiotic treatment may reduce creatinine levels. However, due to the very low certainty of evidence, more clinical data using gold standard biomarkers are needed, globally, to clarify the role of probiotics, the most beneficial bacteria and the optimal dosage in T2DM patients.

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Supplementary material

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