ENDOCRINOLOGY RESEARCH AND PRACTICE

SOCIETY OF ENDOCRINOLOGY AND METABOLISM OF TURKEY

A Systematic Review of the Effect of Probiotics, Prebiotics, and Synbiotics on Gut Microbiota in Type 2 Diabetes Mellitus

REVIEW
Endocrinol Res Pract. 2025;29(3):267-276

ABSTRACT

Type 2 diabetes mellitus (T2DM) occurrence and progression are attributed to the gut microbiota environment. Probiotics have a key role in epithelium proliferation, gut barrier stabilization, metabolic endotoxemia, gut permeability, and systemic inflammation. The aim of this study was to assess the gut microbiota-modulatory properties of probiotic, prebiotic, and synbiotic on zonulin, lipopolysaccharide (LPS), short-chain fatty acids (SCFA), lipopolysaccharide binding protein (LBP), and endotoxin in T2DM. A comprehensive search up to November 2023 was conducted in Web of Science, PubMed, and Scopus databases. The Cochrane Risk of Bias tool was selected for assessing the methodological quality and risk of bias of selected studies. The findings of 16 studies showed that probiotic, prebiotic, and synbiotic administration in T2DM patients was efficient in decreasing endotoxin and LPS, while there were no significant changes regarding LBP, and controversial results for SCFA and zonulin. Probiotic, prebiotic, and synbiotic administration is efficient in improving some aspects of gut microbiota integrity, gut immunity markers, and subsequently improving glucose homeostasis in T2DM patients. Nevertheless, multiple randomized controlled trials are warranted.

Keywords: probiotics, prebiotics, synbiotics, gastrointestinal microbiome, diabetes mellitus type 2, systematic review

Introduction

Type 2 diabetes mellitus (T2DM) is recognized as a mutual endocrine and metabolic disorder manifested by impaired function of pancreatic beta cells and insulin resistance.[1] Besides genetic factors, lifestyle risk factors such as lack of physical activity and poor dietary quality are involved in the generation and progression of T2DM.[2] According to recent research, T2DM and the gut microbiota have a tight relationship.[3,4] Gut microbial dysbiosis leads to chronic mild inflammation and insulin resistance, augmenting the risk of developing T2DM.[5-7] This relationship is manifested by the alteration of regular gastrointestinal homeostasis, gut barrier integrity, hormone secretion, gut immunity, and inflammatory factors.[8] In T2DM, long-lasting chronic inflammation increases the level of inflammatory biomarkers, which are invariably correlated with insulin resistance by disrupting the insulin sensitivity and signaling cascade.[9] Thus, gut microbiota-modulatory agents may be considered as novel therapeutic methods for enhancing gut microbiome health and immunity.[10] The pancreas and the gastrointestinal tract are directly connected by the pancreatic duct system, leading to an unavoidable relationship with the gut microbiota. In fact, the connection between diabetes and the gut microbiota is due to the interaction between the immune system, pro-inflammatory status, and dysbiosis. Therefore, modifying the intestinal microbiome may have imperative benefits for diabetes complications.[11]

Consuming sufficient amounts of probiotics (live microorganisms with microflora-modifying properties in the gut),[12] and efficiently enhances immune system integrity.[13] Probiotics, alone or in combination with prebiotics, stimulate the production of beneficial bacteria. Prebiotics are non-digestible carbohydrates that, by exclusively stimulating the growth and/or action of specific bacteria, deliver beneficial health outcomes to the gut microbiota. Prebiotics mainly consist of inulin, fructo-oligosaccharide, inulin, galacto-oligosaccharides, starch and glucose-derived oligosaccharides, and non-carbohydrate oligosaccharides.[14] but synbiotics are products containing both pro- and prebiotics.[15] Probiotics efficiently decrease pathogenic bacterial colonization and stimulate non-digestive flora growth due to their immune system-modulating features.[16] Furthermore, probiotics efficiently augment

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Received: November 28, 2024
Revision Requested: January 23, 2025
Last Revision Received: February 6, 2025
Accepted: March 3, 2025
Publication Date: May 16, 2025

Cite this article as: Payahoo L, Ghalichi F, Fathi M, Ehsani A. A systematic review of the effect of probiotics, prebiotics, and synbiotics on gut microbiota in type 2 diabetes mellitus. *Endocrinol Res Pract.* 2025;29(3):267-276.

DOI: 10.5152/erp.2025.24587

epithelium proliferation, intestinal barrier functioning, diminish gut endotoxin production and permeability, as well as systemic inflammation.[17] Hence, due to the actions mentioned for probiotics, they can be commonly used for preventing and improving diabetes.[3,18] Short-chain fatty acids (SCFA), such as acetate, propionate, and butyrate, are the main metabolic outputs of the gut microbiome and are considered as nutrient sources for enterocytes.[19] SCFAs promote the gut barrier function, exert anti-inflammatory effects, boost the gut epithelium integrity, gut immunity, glycemic homeostasis, and lipid panel, and alleviate insulin resistance.[20-22] Individuals with T2DM exhibit a significant decline in SCFA-producing bacteria. Subsequently, prebiotics produce SCFA in the gut and enhance glucose hemostasis.[19] Inflammation and gut microbiota diversities may lead to lipopolysaccharide (LPS) and endotoxin modulation.[23] LPS is the main constituent of gram-negative bacteria. In fact, in T2DM patients, the levels of circulating LPS are higher and have a tight relationship with glycemic homeostasis and the insulin signaling pathway.[24] In T2DM patients, endotoxin enters the circulation and induces systemic inflammation. Considering the fact that endotoxin is a major component of the gut microbiome, supplementing with probiotics may efficiently change endotoxin levels and modify the composition and strength of the gut epithelial barrier.[25] LPS-binding protein (LBP) is a metabolic syndrome marker and has a direct relationship with interleukin-6 (IL-6) plasma levels and inflammatory cytokines in patients with T2DM.[26] Zonulin is recognized as a regulator of endogenous tight junctions and a notable biomarker for assessing gut permeability.[19]

The objective of the present systematic review was to assess the gut microbiota-modulatory effects of probiotics, prebiotics, and synbiotic in T2DM, in order to highlight evidence-based findings and take a further step in T2DM prevention and management.

Material and Methods

Search Strategy

The current systematic review was accomplished using the Preferred Reporting Items for Systematic Review and Meta-Analyses guidelines (PRISMA) checklist, and a thorough search was conducted up to November 2023. Web of Sciences, PubMed, and Scopus databases were used to perform a thorough search for available papers. This study was approved by the Ethics Committee of Maragheh University (approval no: IR.MARAGHEHPHC.REC.1403.014, date: June 12, 2024).

MAIN POINTS

- Probiotic, prebiotic, and synbiotic administration is efficient in improving some aspects of gut microbiota integrity, gut immunity markers.
- Probiotic, prebiotic, and synbiotic administration was efficient in decreasing endotoxin and lipopolysaccharide levels in type 2 diabetes mellitus (T2DM) patients.
- Probiotic, prebiotic, and synbiotic administration was not efficient in reducing lipopolysaccharide binding protein level in T2DM patients.
- Controversial results regarding short-chain fatty acids and zonulin levels were observed after probiotic, prebiotic, and synbiotic administration.

The strategy of search was based on the following keywords: ("Probiotics" OR "Prebiotics" OR "Synbiotics" OR "Lactobacillus" OR "Bifidobacterium" OR "Streptococcus" OR "Saccharomyces cerevisiae" OR "VSL#3") AND (treatment) OR (supplementation) OR (intake) AND ("Microbiome") OR ("Gut") OR ("Microbiota") OR ("microflora") OR ("Gut bacteria") OR (Gastrointestinal Microbiome) OR ("Dysbiosis") AND ("Diabetes Mellitus, Type 2") OR ("Type 2 diabetes") AND ("randomized controlled trial") OR ("controlled clinical trial") OR ("supplementation") OR ("clinical trial") OR ("random*") OR ("trial").

Eligibility Criteria

The inclusion criteria in data extraction are defined as randomized controlled trial design studies, ages between 20 and 70 years, supplemented by oral probiotics, prebiotics, and synbiotics in both genders. According to the criteria considered for inclusion, 2 authors (FGH and MF) assessed the obtained papers by limiting them to "title and abstract" and removing the duplicate papers. In case of disagreement about extracted data, the third author (LP) resolved and helped to make the right decision.

By confirming each paper according to inclusion criteria, the data, including study design, country, sample size, randomization, intervention, dose of supplementation, duration, age, gender, body mass index, participants, and outcomes (zonulin, LPS, SCFA, LBP and endotoxin), were collected in an Excel file.

The Cochrane Risk of Bias (RoB) tool was used for assessing the quality and risk of bias of studies. Performance bias, selection bias, reporting bias, detection bias, attrition bias, and others (10 items) were the components of RoB (Figures 1 and 2).

Results

The flow diagram of selecting studies is presented in Figure 3. Studies that did not provide required information, those that didn't have an appropriate design, and irrelevant studies were excluded. Finally, 16 eligible articles were selected for inclusion in the current study.

Characteristics of Studies Included

Among 16 articles, 4 studies were carried out in Iran, 3 in Japan, 2 in the United States, and 1 in Australia, Norway, Chile, Indonesia, Thailand, Saudi Arabia, and Brazil. The sample size of the studies was in the range of 24 to 110 patients. Six studies used probiotics, 8 studies used prebiotics, and 2 studies used synbiotics as intervention. The BMI of participants was between 25 and 45 kg/m^2. Table 1 shows the detailed characteristics of the studies included. In 11 studies, both genders were included, but in the remaining studies, only females were assessed.

The Effects of Probiotics, Prebiotics, and Synbiotic on LPS and LBP

The findings of 4 RCTs concerning the supplementation of probiotics, prebiotics, and synbiotics on LPS concentration were consistent. In the study by Angela Horvath *et al*,[19] 26 diabetic patients (12 in synbiotic, 14 in control group) with BMI between 30 and 40 kg/m^2 were recruited. The intervention group received synbiotics (probiotics including *B. bifidum* W23, *B. lactis* W51, *B. lactis* W52, *L. acidophilus* W37, *L. casei* W56, *L. brevis* W63, *L. salivarius* W24, *Lc. lactis* W58, and *Lc. lactis* W19; prebiotic components included galacto-oligosaccharides P11 (GOS) and fructo-oligosaccharides P6 (FOS), konjac glucomannan P13 (E425), and a matrix containing maltodextrin) for 6 months. Results indicated a significant decline in LPS levels. In a randomized-controlled clinical trial by Dehghan *et al*,[24] 49 (n=24

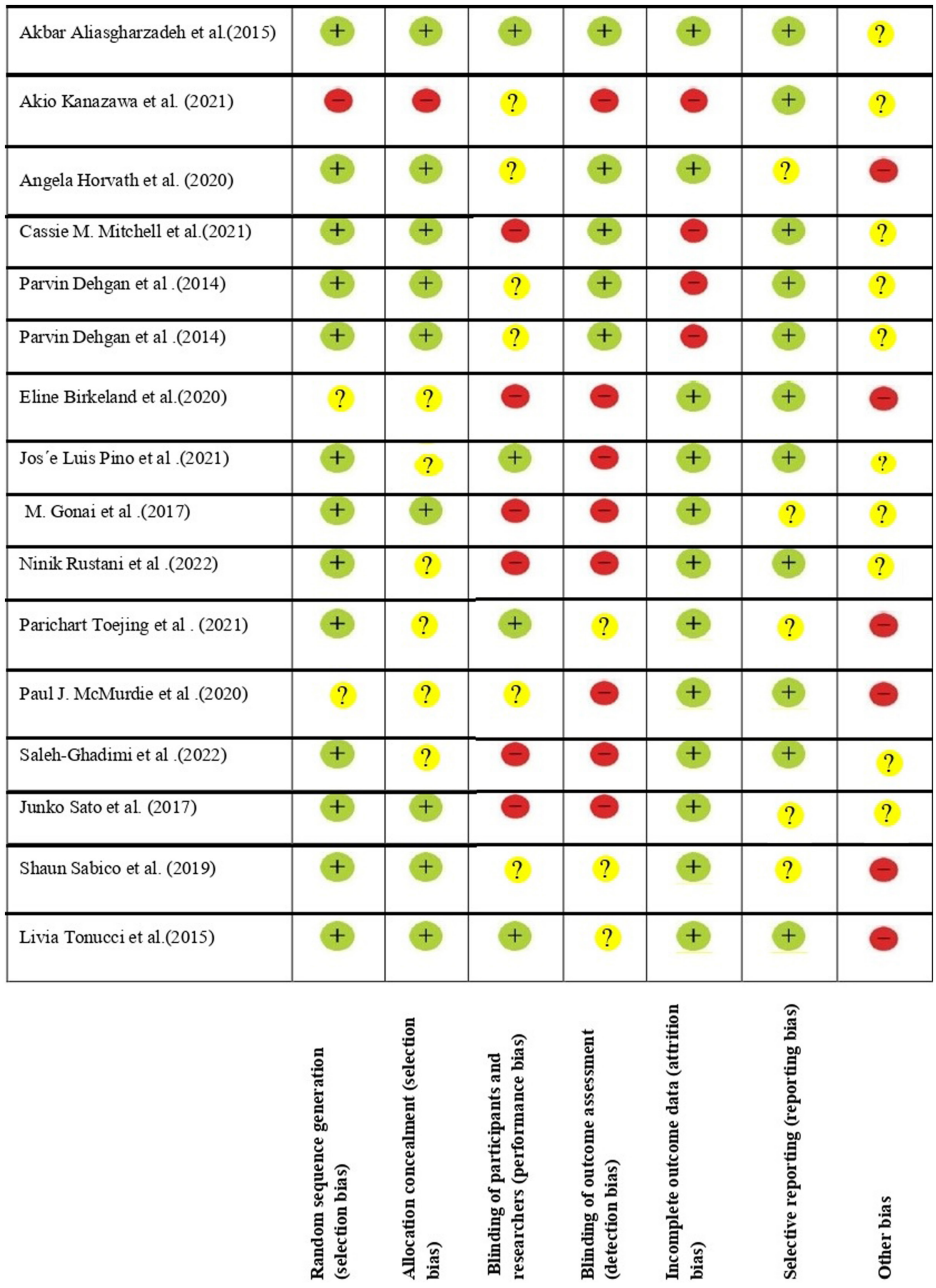

Figure 1. Risk of bias indicating studies' quality assessment at an individual level. (+) Low risk of bias. (−) High risk of bias. (?) Unclear risk of bias.

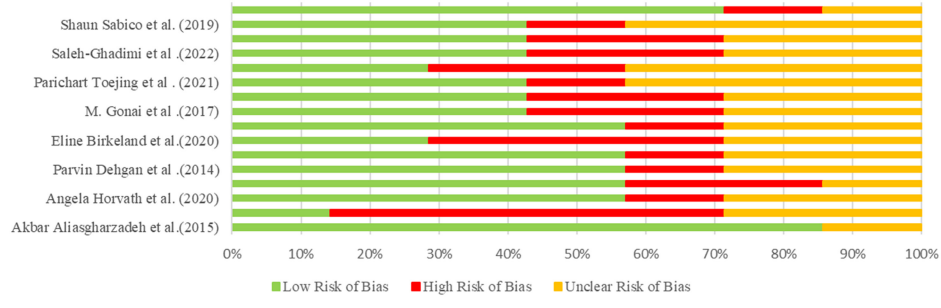

Figure 2. Risk of bias of included studies as percentages.

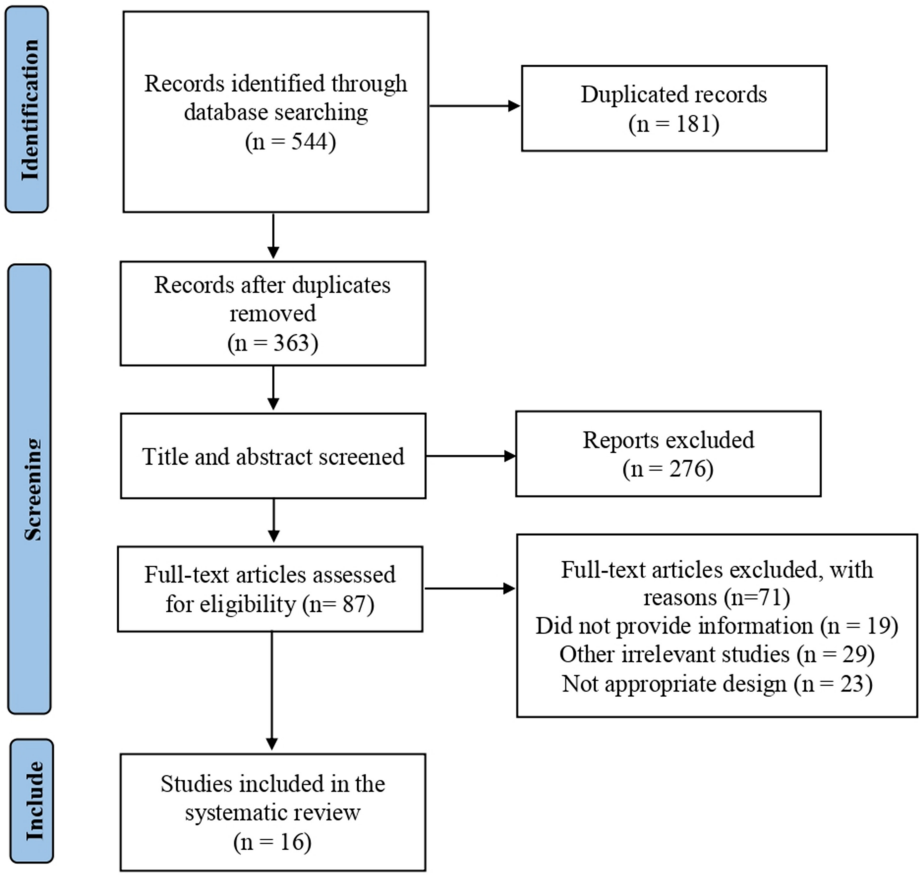

Figure 3. Flowchart of study selection.

intervention; n=25 placebo groups) obese T2DM patients were recruited. The first group received 10 g/d of high-performance inulin supplement and the placebo group received the same amounts maltodextrin for a duration of 8 weeks. The results revealed a significant decline in LPS levels and oxidative stress biomarkers, as well as inflammatory cytokines including tumor necrosis factor-α (TNF-α), IL-10, and highly sensitive C-reactive protein (hs-CRP), compared to the control group (approximately 28%, p<0.05). Another study was carried out on 36 type 2 diabetic patients with normal weights of both genders. The intervention group took L. *Paracasei* HII01 (50×10^9 CFU/day) and the placebo group received 10 mg/day of corn starch for 12 weeks. The results of the study were revealed a significant decrease in LPS levels as well as other inflammatory biomarkers including hs-CRP, IL-6, and TNF-α. In the study by Dehghan P *et* al.,[23] 54 women with T2DM were randomly allocated into 2 groups. The intervention group consumed 10 g per day oligofructose enriched inulin for 8 weeks. The findings indicated that the concentration of LPS, along with IL-6, INF-γ, TNF-α, and hs-CRP, significantly declined post-intervention (p<0.05). Based on these findings, LPS and inflammatory factors have a direct relationship. In studies where LPS levels declined after probiotics or prebiotics supplementation, inflammatory factors also decreased. Augmented circulating LPS results in metabolic endotoxemia, leading to inflammatory reactions and insulin resistance. Probiotics decrease insulin resistance by modulating LPS levels and suppressing pro-inflammatory biomarkers.[27] In fact, as can be seen in Figure 4, insulin resistance and inflammation have a 2-way relationship

In the study by Cassie M. Mitchell *et al*.,[28] supplementation of 24 obese type 2 diabetic patients with 10 g/day of inulin for 6 weeks increased LBP levels non-significantly at the end of the study. Nevertheless, another study mentioned an insignificant decline in LBP levels.[26] In one study, it was detected that the concentration of LBP in diabetic patients was significantly higher than in the healthy control group. At the end of intervention period, galacto-oligosaccharides (GOS) supplementation had no significant effect on LBP levels; however, it showed that long-term GOS intake may improve LBP status.[29] In other studies, the serum levels of LBP, TNF-α, IL-6 did not change significantly.[30]

The Effects of Probiotics, Prebiotics, and Synbiotic on Endotoxin

A study on 55 overweight females with type 2 diabetes in females who received 10 g/day of resistant dextrin for 8 weeks showed that the concentration of endotoxin declined significantly post-intervention. In this study, other oxidative stress biomarkers, including hs-CRP, TNF-α, MDA, and IL-6, also decreased significantly at the end of the intervention period ($P < .05$).[31] In the study by Saleh-Ghadimi *et al*,[32] 76 women with type 2 diabetes were recruited. The intervention group (n=38) consumed 10 g/day of resistant dextrin, and the placebo group received the same amounts maltodextrin for 8 weeks. The results revealed a significant reduction in endotoxin levels at the end of the study. Also, the level of other inflammatory biomarkers including IL-6, TNF-α, and IL-8, decreased post-intervention. In the study by Sabico *et al*,[25] administration of 61 participants with type 2 diabetes with 2.5×10^9 CFU Ecologic®Barrier (a multi-strain probiotic containing *Bifidobacterium*

Table 1. Study Characteristics of Included Studies

Citation (First Author et al., Year)	Study Design	Location	Sample Size	Randomized	Intervention	Dose	Duration	Mean Age	Gender	BMI	Condition	Zonulin	LPS	SCFA	LBP	Endotoxin
Akbar Aliasgharzadeh et al.(2015)	Randomized controlled clinical trial controlled clinical trial	Iran	55	Randomized	Prebiotic (Resistant dextrin)	10 g/day	8 week	47.5 (30-65)	female	>25	Type 2 diabetes, overweight					Sig decrease
Akio Kanazawa et al. (2021)	Randomized controlled study	Japan	86	Randomized	Synbiotic	3 g/day	24 week	55 (30-80)	Male and female	>25	Type 2 diabetes, overweight			Sig increase	Sig decrease	
Angela Horvath et al. (2020)	Randomized controlled pilot study	Austria	26	Randomized	Synbiotic	10 g/day	6 months	60 (56-65)	Male and female	30-40	Type 2 diabetes, obese	No change	Sig decrease		Insig increase	
Cassie M. Mitchell et al.(2021)	Pilot Randomized Controlled Trial	The Uited States	24	randomized	Prebiotic (Inulin)	10 g/day	6 weeks	57.5 (40-75)	Male and female	25-39.9	Type 2 diabetes, overweight and obese				No change	Insig decrease
Parvin Dehgan et al .(2014)	Randomized controlled clinical trial	Iran	54	Randomized	Prebiotic (oligofructose-enriched inulin)	10 g/day	8 week	42.5 (20-65)	Female	>25	Type 2 diabetes, overweight		Sig decrease			
Parvin Dehgan et al .(2014)	Randomized controlled clinical trial	Iran	54	Randomized	Prebiotic (high--performance inulin)	10 g/day	8 weeks	42.5 (20-65)	Female	>25	Type 2 diabetes, overweight		Sig decrease			
Eline Birkeland et al.(2020)	Randomized controlled trial	Norway	25	Randomized	Prebiotic (inulintype fructans)	16 g/day	6 weeks	56 (41-71)	Male and female	40<	Type 2 diabetes, obese			Sig increase		
Jos´e Luis Pino et al .(2021)	Randomized, double-blind, placebo-controlled clinical trial	Chile	37	Randomized	Prebiotic (oat β-glucan)	5 g/day	3-8 weeks	45 (30-60)	Male and female	30-35	Type 2 diabetes, obese	Insig decrease				
M. Gonai et al . (2017)	Randomized, double-blind, placebo-controlled trial	Japan	55	Randomized	Prebiotics (galacto-oligosaccharides)	10 g/day	4 week	45 (20-70)	Male and female	24.8	Type 2 diabetes, overweight			No change	No change	
Ninik Rustani et al .(2022)	Randomized Double-Blind Controlled Trial	Indonesia	40	Randomized	Probiotic (skim milk powder containing 10^{10} CFU/g *Lactobacillus plantarum*)	1 g/day	12 weeks	35 (20-50)	Female	>35	Type 2 diabetes, obese			Insig increase		
Parichart Toejing et al . (2021)	Randomized Clinical Trial	Thailand	50	Randomized	Probiotic ($5\text{-}10^{9}$ CFU/day *Lactobacillus paracasei* HII01)	10 mg / day	12 weeks	45 (20-70)	Male and female	23.24	Type 2 diabetes, obese	Insig decrease	Sig decrease	Sig increase		
Paul J. McMurdie et al .(2020)	Double-blind, randomized, placebo-controlled trial	The Unied States	104	Randomized	Probiotic (5-strains containing inulin, *Akkermansia muciniphila*, *Clostridium beijerinckii*, *Clostridium butyricum*, *Bifidobacterium infantis*, and *Anaerobutyricum hallii*)	N/A	12 weeks	53.7	Male and female	25-45	Type 2 diabetes, overweight and obese			No change		

(Continued)

Table 1. Study Characteristics of Included Studies (*Continued*)

Citation (First Author et al., Year)	Study Design	Location	Sample Size	Randomized	Intervention	Dose	Duration	Mean Age	Gender	BMI	Condition	Zonulin	LPS	SCFA	LBP	Endotoxin
Saleh-Ghadimi et al.(2022)	Randomized controlled trial	Iran	76	Randomized	Prebiotic (resistant dextrin)	10 g/day	3 months	47.5 (30-65)	Female	≥30	Type 2 diabetes, obese					Sig decrease
Junko Sato et al. (2017)	Randomised controlled study	Japan	70	Randomized	Probiotic (*Lactobacillus casei* strain Shirota-fermented milk)n	N/A	16 weeks			20-35.5	Type 2 diabetes, normal, overweweight and obese			Sig decrease	No change	
Shaun Sabico et al. (2019)	Randomized, double-blind, placebo-controlled trial	Saudi Arabia	30	Randomized	Probiotic (2.5 × 10^9 cfu/g Ecologic®Barrier)	2 g/day	6 months			29.75	Type 2 diabetes, overweight					Sig decrease
Livia Bordalo Tonucci et al.(2015)	Randomized, Double-Blind, Placebo-Controlled trial	Brazil	50	Randomized	Probiotic (fermented milk containing *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. lactis BB-12)	120 g/day	6 weeks			<35	Type 2 diabetes, normal, overweweight and obese			No change		

bifidum W23, Bifidobacterium lactis W52, Lactobacillus acidophilus W37, Lactobacillus brevis W63, Lactobacillus casei W56, Lactobacillus salivarius W24, Lactococcus lactis W19, and L. lactis W58) twice daily for 6 months resulted in a significant decrease in endotoxin levels up to 70% in the intervention group ($P < .05$). Moreover, the concentrations of inflammatory biomarkers such as TNF-α, IL-6, and CRP decreased 67%, 77%, and 53%, respectively ($P < .05$). Nevertheless, a non-significant decrease in endotoxin concentration was observed in other studies.[28]

Probiotics, Prebiotics, and Synbiotic on SCFA

In a randomized control trial, 80 overweight type 2 diabetic patients were recruited. The intervention group received the synbiotic supplement containing *Lacticaseibacillus paracasei* strain Shirota, *Bifidobacterium breve* strain Yakult, and galactooligosaccharides for 24 weeks. The results of the study showed that the concentration of acetic acid and butyric acid augmented significantly in the intervention group post-intervention. However, changes in other short-chain fatty acid levels were not significant.[26] In addition, Angela Horvath *et al*,[19] showed that administering synbiotic supplements to 26 obese type 2 diabetic patients (12 in synbiotic, 14 in control group) for 6 months increased the concentration of SCFAs non-significantly in the intervention group. In the study by Eline Birkeland et al.,[33] participants received 16 g of inulin-type fructans (a combination of oligofructose and inulin) for 6 weeks. The results manifested that the total concentration of SCFA ($P = .04$), acetic acid ($P = .02$), and propionic acid ($P = .04$) abundance increased significantly in the intervention group. Differences in other SCFAs such as isobutyric acid, butyric acid, valeric acid, isovaleric acid, caproic acid, and isocaproic acid were not statistically significant. Gonai M *et al*[29] study on 52 type 2 diabetic patients of normal weight (n = 25 placebo) and overweight (n = 27 intervention) of both genders. The intervention group received 10 g/day GOS for 4 weeks. The findings revealed that the concentration of SCFAs mostly increased in the intervention group compared with the placebo group. In the study by Rustanti N *et al*[34] 36 women with T2DM patients and BMI under 30 kg/m^2 (n = 18 intervention, n=18 placebo) consumed 1 g skim milk powder containing 10^{10} CFU *Lactobacillus plantarum Dad-13* for 11 weeks. The results displayed a non-significant increase in total SCFAs, acetic acid, propionic acid, and butyric acid abundance in the intervention group. Additionally, a significant increase in *L. plantarum* was noted in the feces of participants in the intervention group ($P < .001$). The counts of bacterial species did not differ significantly at the end of the intervention period. In the study by Parichart Toejing *et al*,[27] supplementation of 36 type 2 diabetic patients with *L. paracasei HII01* (50×10^9 CFU/day) for 12 weeks caused a significant increase in the prevalence of lactic acid, propionic acid, acetic acid, and butyric acid in the intervention group in comparison to baseline ($P < .05$); however, there were no significant differences between the groups. Paul J. McMurdie *et al*[35] showed that supplementation of 76 obese T2DM patients with synbiotic, including inulin, Clostridium beijerinckii, Clostridium butyricum, Bifidobacterium infantis, Akkermansia muciniphila, and Anaerobutyricum hallii for 12 weeks, exhibited no meaningful changes in terms of SCFA abundance between the 2 groups. However, the concentration of butyrate during 12 weeks increased in the intervention group. In the study by Junko Sato *et al*[30] 70 type 2 diabetic patients were enrolled. The first group consumed Shirota fermented milk containing Lactobacillus casei strain for 16 weeks. The analysis was done on 68 subjects and the results showed a significant decrease in the count of SCFAs, acetic, and butyric acids compared to the placebo. Changes in other

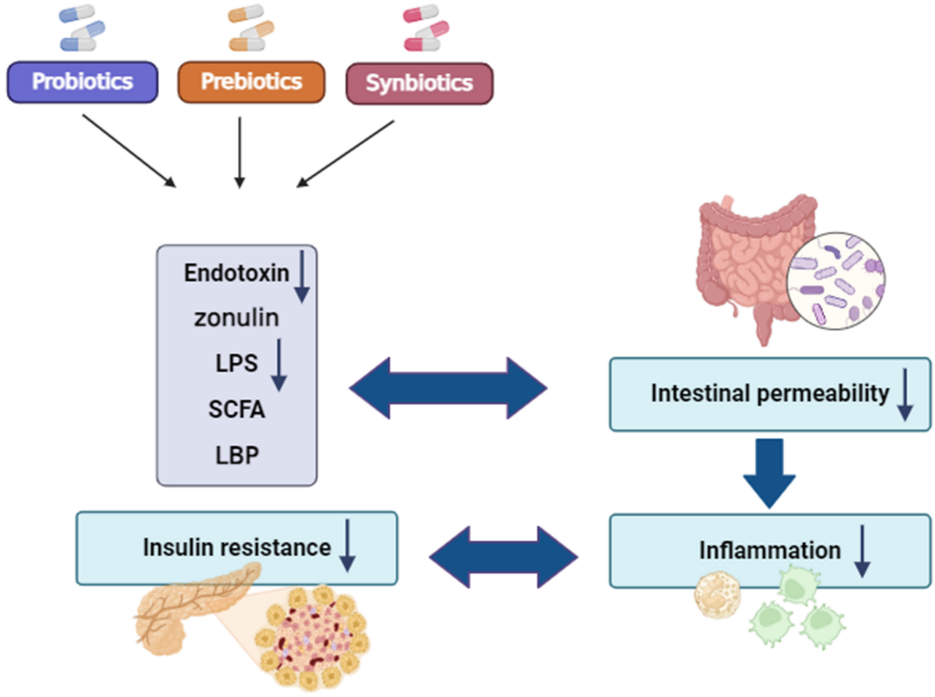

Figure 4. Mechanism of action of probiotics, prebiotics, and synbiotics on the gut microbiota and association with insulin resistance. Prebiotics, probiotics, and Synbiotics, decline the level of gut immunity markers, which subsequently affect intestinal permeability and inflammation. Inflammation and insulin resistance have a bilateral and reciprocal effect. Lipopolysaccharide (LPS), short-chain fatty acids (SCFA), and lipopolysaccharide binding protein (LBP).

SCFAs including propionic, isovaleric, valeric acid, succinic acid, formic acid, and lactic acid were statistically decreased as well. In the study by Livia Bordalo Tonucci *et al.*,[36] 50 overweight type 2 diabetic patients in both genders were enrolled. Volunteers consumed 120 g per day of fermented milk containing 10^9 units of *Lactobacillus acidophilus La-5* and *Bifidobacterium animalis lactis* BB-12 for 6 weeks. The analysis was applied to 45 participants. and the findings exhibited no significant differences among groups in terms of butyric acid, acetic acid, and propionic acid concentration. However, a significant increase was seen in acetic acid levels in both groups.

Probiotics, Prebiotics, and Synbiotic on Zonulin

In the study by Jose Luis Pino *et al*[37] the impact of oat β-glucan (5 g/day) for 12 weeks was assessed on zonulin concentration in 37 obese T2DM patients in both genders. In this study, the concentration of zonulin decreased non-significantly in the intervention group. Other studies showed no significant changes in serum concentration of zonulin.[19,27]

Discussion

The present systematic review of 16 studies showed that probiotic, prebiotic, and synbiotic administration in T2DM patients was efficient in decreasing endotoxin and LPS, while there were no significant changes regarding LBP, and controversial results for SCFA and zonulin.

Probiotics alleviate intestinal immunity and body homeostasis by balancing the gut microbiota composition.[38] Furthermore, it has been mentioned that probiotics and prebiotics in combination provide beneficial advantages for the gut microbiota. This[26] Figure 4 illustrates the mechanism of action of probiotics, prebiotics, and synbiotics on the gut microbiota.

LPS, as a potential inflammatory mediator, can translocate through the damaged gut barrier and enhance insulin sensitivity while also distract the function of the innate immune system.[39-41] In diabetic patients, an enhanced LPS level is related to insulin production and metabolic pathways.[39] Experimental studies have shown that administering prebiotics to mice boosts the production of intestinal Bifidobacteria and declines LPS levels.[42,43] A significant decrease in LPS level was observed among all the included studies after administering prebiotics and probiotics. Prebiotics decrease inflammation by reducing intestinal permeability and reinforcing gut endothelial cell tight junctions. Moreover, the reduction in insulin levels improves Kupffer cell function, and LPS clearance reduces inflammation.[23] In the study by Toejing et al,[27] a significant decrease in plasma LPS level was observed after supplementing L. paracasei HII01 probiotic. This may be due to the decreased systemic inflammation and translocation of bacterial products. In the studies included, assessing the effect of probiotics, prebiotics and synbiotics supplementation, no significant differences were mentioned in the case of LBP. LBP is a marker for metabolic syndrome, and previous studies have shown a strong relationship between plasma level of IL-6 and LBP, and higher plasma LBP levels in obese T2DM patients or those with poor glycemic control. The studies assessing the effect of LBP had mainly used synbiotics for supplementation. One study claimed that it was not possible to evaluate the effect of synbiotics on the translocation level of live gut bacteria to the blood. Also, probiotic bacteria regulate intestinal permeability by TNF-α, which was not assessed.[26] Another study indicated that synbiotics are not able to affect glucose metabolism and markers of gut permeability such as LBP in treatment-experienced diabetic patients.[19]

Low-grade "metabolic" endotoxemia is the major determinant of metabolic syndrome and T2DM.[44] Accordingly, endotoxin levels are higher (76%) among T2DM patients compared to healthy ones.[39] In fact, prebiotics are able to moderate blood glucose homeostasis, oxidative stress, and inflammation by decreasing metabolic endotoxemia.[31] Two studies indicated that prebiotic supplementation was significantly efficient in declining endotoxin.[31,32] Researches have confirmed a positive association between increased endotoxemia and obesity.[45] Aliasgharzadeh et al,[31] indicated that resistant dextrin is effective in decreasing body weight by reducing metabolic endotoxemia, which subsequently improves glycemic status in obese diabetic patients. Another study showed that there was a significant association between endotoxins and sleep quality. Increased endotoxin levels in T2DM exacerbates quality of sleep, and resistant dextrin supplementation was effective in reducing metabolic endotoxemia.[32] Metabolic endotoxemia boosts the expression of pro-inflammatory cytokines including IL-1, IL-6, and TNF-α.[46] An increase in pro-inflammatory cytokine levels is attributed to the reduction of insulin action reduction via inhibitory phosphorylation mechanisms and reduced suppression of IRS-1 and IRS-2 by regulating c-Jun NH2-terminal kinase, IkB kinase, and p38 mitogen-activated protein kinase. Prebiotics such as dextrin decrease metabolic endotoxemia and are related to the modulation of glycemic status via body weight reduction and suppression of the production of inflammatory biomarkers.[47]

The SCFA (acetic, propionic, and butyric acids) produced by gut bacteria are considered as sources of energy for the colonocytes and components of the hepato-metabolic pathways. Prebiotic fibers escape the degradation process in the small intestine and are converted to SCFA during fermentation in the colon. This process enhances the abundance, growth, and activity of beneficial gut bacteria.[48] According to research, gut microbiota effectively affects glucose metabolism by producing SCFAs. Toejing et al. indicated that the significant increase in SCFA levels after L. paracasei HII01 administration in T2DM patients may be related to glucose metabolism regulation.[27] Indeed, SCFAs, as essential signaling molecules, are not only important for gut health but efficiently enter the systemic circulation and affect peripheral tissues via PI3K/Akt and AMPK activation directly. Subsequently, GLUT4 translocates to the membrane, facilitating glucose into cells.[49] Furthermore, SCFAs produced from dietary fiber fermentation are associated with incretin secretion, intestinal gluconeogenesis, insulin sensitivity in adipocytes, and insulin secretion by activating the parasympathetic nerve.[50] Few studies did not observe significant changes in SCFA concentrations. In the study by Gonai et al.,[30] GOS supplementation was implemented for only 4 weeks and with a limited sample size. In the study by McMurdie *et al*,[35] serum butyrate levels were relatively low at baseline and significantly increased at the end of the intervention; however, total SCFA levels did not significantly change. In the study by Tonucci et al.,[36] significant differences in acetic acid levels compared to butyrate and propionate were observed, but the total SCFA level change was not significant due to the short duration of the trial.

Zonulin, as a major biomarker for gut permeability, regulates endogenous tight junctions.[51] In the included studies assessing zonulin concentration, one study[28] observed a significant decrease, and zonulin concentration correlated with serum C-peptide, levels of LPS, and bacterial DNA. Zonulin concentration decrease was also observed in other studies, but the changes were insignificant.[19,37]

Several limitations can be attributed to the present systematic review. First, the simultaneous assessment of probiotics, prebiotics, and synbiotics. However, this was due to the small number of studies evaluating each treatment. Hence, statistical analyses were not possible. Second, it would have been beneficial if inflammatory markers were assessed as well. The strengths of the current study were administering the Cochrane Risk of Bias (RoB) tool for assessing the quality of studies and considering high-quality studies. Furthermore, assessing multiple gut permeability markers and including multiple randomized controlled trials were also strengths.

The present systematic review confirms the beneficial effects of probiotic, prebiotic, and synbiotic supplementation on gut microbiome biomarkers such as endotoxin and LPS in T2DM patients. However, multiple randomized controlled trials should be conducted to assess the efficiency of probiotic, prebiotic, and synbiotic administration on other markers of the gut microbiome. For future research, assessing the effects of antidiabetic agents on microbiome and microbiota-related parameters, as well as research on fecal microbiota transplantation treatment along with probiotic, prebiotic and synbiotic supplementation for boosting gut health in T2DM, is recommended.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: This study was approved by the Ethics Committee of Maragheh University (Approval No: IR.MARAGHEHPHC.REC.1403.014, Date: June 12, 2024).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – F.G.H.; Data Collection and/or Processing – M.F., L.P., A.E.; Analysis and/or Interpretation – F.G.H., L.P.; Literature Search – F.G.H., M.F., A.E.; Writing – F.G.H., L.P.; Critical Review – F.G.H.

Declaration of Interests: The authors have no conflict of interest to declare.

Funding: This study received no funding.

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