ANAÍSA MARTINS MARQUES

EFEITOS DE PROBIÓTICOS DO TIPO E DA QUANTIDADE DE CARBOIDRATOS EM DIETAS DE ANIMAIS COM DIABETES TIPO 2: UMA REVISÃO SISTEMÁTICA

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Biologia Animal, para obtenção do título de *Magister Scientiae*.

Orientadora: Mariella Bontempo Duca de Freitas
Coorientadora: Reggiani Vilela Gonçalves

VIÇOSA - MINAS GERAIS

2020

Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Câmpus Viçosa

T
M357e
2020

Marques, Anaísa Martins, 1993-
Efeitos de probióticos, do tipo e da quantidade de carboidratos em dietas para animais com diabetes tipo 2 : uma revisão sistemática / Anaísa Martins Marques. – Viçosa, MG, 2020.
94 f. : il. (algumas color.) ; 29 cm.

Texto em inglês.
Inclui apêndices.
Orientador: Mariella Bontempo Duca de Freitas.
Dissertação (mestrado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Diabetes. 2. Probióticos. 3. Carboidratos dietéticos. I. Universidade Federal de Viçosa. Departamento de Biologia Animal. Programa de Pós-Graduação em Biologia Animal. II. Título.

CDD 22 ed. 616.462

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APROVADA: 28 de fevereiro de 2020.

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RESUMO

MARQUES, Anaísa, M.Sc., Universidade Federal de Viçosa, fevereiro de 2020. **Efeitos de probióticos do tipo e da quantidade de carboidratos em animais com diabetes tipo 2: uma revisão sistemática.** Orientadora: Mariella Bontempo Duca de Freitas. Coorientadora: Reggiani Vilela Gonçalves.

Diabetes mellitus está entre as doenças mais prevalentes do mundo, afetando mais de 422 milhões de pessoas. Diabetes tipo 2 representa 90% dos casos e é marcado por hiperglicemia crônica que resulta em complicações microvasculares à longo prazo. A glicemia pós-prandial depende do conteúdo de carboidratos ingeridos, porém a quantidade e a fonte ideais deste macronutriente permanecem controversas. Ademais, diabéticos podem ter a microbiota intestinal alterada, o que piora a condição metabólica, pois estes micro-organismos interagem com o hospedeiro e influenciam o metabolismo. Assim, modificar a microbiota através de probióticos poderia regular o metabolismo da glicose nestes indivíduos. Seguindo o PRISMA, desenvolvemos duas revisões sistemáticas visando sintetizar as evidências disponíveis sobre probióticos em dietas de animais com diabetes tipo 2 e sobre os efeitos de diferentes fontes e quantidades de carboidratos em dietas de animais com diabetes tipo 2. Também avaliamos a qualidade metodológica das evidências, apontando as principais fontes de viés. Resultados mostram que sacarose piora condição diabética independentemente da quantidade de carboidratos ingeridos e que fibras, principalmente amido resistente, melhoram parâmetros glicêmicos e saúde de forma geral. Probióticos, principalmente *Lactobacillus*, melhoram parâmetros glicêmicos em 96% dos estudos. A avaliação da qualidade metodológica indica alto risco de viés devido à subnotificação de informações sobre os métodos. Concluímos que melhoras em parâmetros glicêmicos estão mais relacionadas ao tipo do que à quantidade de carboidratos da dieta, sendo o amido resistente a fonte mais adequada. Além disso, probióticos apresentam benefícios quando usados como tratamento de suporte em diabetes tipo 2.

Palavras-chave: Diabetes tipo 2. Modelos animais. Carboidratos dietéticos. Probióticos.

ABSTRACT

MARQUES, Anaísa, M.Sc., Universidade Federal de Viçosa, February, 2020. **Effects of probiotics, type and amount of dietary carbohydrates in animals with type 2 diabetes: a systematic review.** Adviser: Mariella Bontempo Duca de Freitas. Co-adviser: Reggiani Vilela Gonçalves.

Diabetes mellitus is currently one of the most prevalent diseases in the world, affecting over 422 million people. Type 2 diabetes accounts for 90% of the cases and it is marked by chronic hyperglycaemia, which can result in microvascular complications in the long-term. Postprandial glycaemia is reliant on the total carbohydrate content of a meal, however, the importance of the amount and the source of these carbohydrates remains controversial. Furthermore, the crosstalk between gut microbiota and metabolic processes has to be accurately regulated in order to maintain physiological homeostasis. Type 2 diabetes patients may have altered gut microbial composition and therefore, modifying gut microorganisms through probiotic treatment may be a way of regulating glucose metabolism. Following PRISMA guidelines, we developed two systematic reviews to assess the evidences on the impact of probiotics and the evidences on the effects of different types and amounts of dietary carbohydrates in type 2 diabetic animals. We also evaluated the methodological quality of evidence, pointing out the main sources of bias. Results show that sucrose deteriorates diabetic condition regardless of carbohydrate content and that fiber, particularly resistant starch, improves blood glucose parameters and overall health. Probiotics, particularly *Lactobacillus,* improve glycemic parameters in 96% of the studies. Methodological quality indicates a high risk of bias due to under-reported characteristics of experimental methods. We conclude that iimprovements in type 2 diabetes parameters in animal models are more closely related to the type of dietary carbohydrate than to its content on a diet, being resistant starch the most beneficial source for maintaining normoglycemia. In addition, probiotics should be used as a supporting alternative in type 2 diabetes treatment.

Keywords: Type 2 diabetes. Animal models. Dietary carbohydrates. Probiotics,

SUMÁRIO

Effects of the amount and the type of carbohydrates used in type 2 diabetes diets in animal models: a systematic review

Short title: Carbohydrates in T2DM diets

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Key words: carbohydrates; diabetes mellitus; glycaemia; insulin

Abstract

Type 2 diabetes is among the most prevalent diseases in the world, affecting over 420 million people. The disease is marked by a poor metabolic effect of insulin leading to chronic hyperglycaemia, which can result in microvascular complications. It is widely known that postprandial glycaemia is reliant on the total carbohydrate content of a meal. However, the importance of the amount and the source of these carbohydrates remains controversial due to mechanisms other than insulin secretion. Oxidative stress, inflammation, pyruvate production and the quality of the intestinal microbiota resulting in plasma lipopolysaccharides and short-chain fatty acids production play an important role in blood sugar control and consequently in type 2 diabetes. Thus, we systematically reviewed the preclinical evidences on the impact of the amount and type of carbohydrate found in different diets and its influence on blood glucose levels in diabetic animals. We used a comprehensive and structured search in biomedical databases Medline (Pubmed), Scopus and Web of Science, recovering and analyzing 27 original studies. Results showed that sucrose-rich diets deteriorated diabetic condition in animal models regardless of the total dietary carbohydrate content. On the other hand, fiber, particularly resistant starch, improved blood glucose parameters through direct and indirect mechanisms, such as delayed gastric emptying and improved gut microbiota. All studies used rodents as animal models and male animals were preferred over females. Improvements in T2DM parameters in animal models were more closely related to the type of dietary carbohydrate than to its content on a diet, i. e., resistant starch seems to be the most beneficial source for maintaining normoglycemia. Results show that current literature is at high risk of bias due to neglecting experimental methods.

Introduction

Diabetes mellitus has become one of the most common chronic diseases in the world, with 422 million people affected worldwide. Approximately 95% of people currently diagnosed with diabetes have type 2 diabetes mellitus (T2DM) [1]. The disease is marked by chronic hyperglycemia, which can impair pancreatic beta cell function and increase insulin resistance, deteriorating the metabolic condition [2] and causing microvascular complications in the retina, kidney or peripheral nerves [3]. Thus, glycemic control is essential for diabetes management and consequently to avoid complications in organs and systems, which is related with high morbidity and mortality rates [1, 4].

T2DM's causes are embedded in genetic and epigenetic elements interacting within a societal framework [5]. The genetic predisposition for T2DM is associated with increased risk of an individual developing T2DM when there is other affected family members [5]. On the other hand, epigenetic elements are those influenced by environmental factors. Thus they can be reversible and therefore can be manipulated in order to treat the disease [6, 7]. Regardless of the causes, the main targets of the treatment are to decrease insulin resistance and improve beta cell function with diet, exercise and oral hypoglycemic and anti-hyperglycemic agents [7, 8]. Based on this, given T2DM's high prevalence and the significant benefits of its management, it is of clinical importance to determine the best diet taking into account the amount and type of carbohydrates, as nutrition plays an important role in the disease treatment [9]. For T2DM patients, it is recommended an intake of 26-44% of total daily energy from carbohydrates, preferably from high-quality sources, such as vegetables, whole fruits and legumes [10], which are rich in fiber. Most fibers, which are unabsorbed carbohydrates, are insoluble and increase stool weight [11]. In contrast, starches are carbohydrates found in vegetables that are mostly broken down to sugars by digestive enzymes [11]. However, resistant starch, also found in vegetables and whole fruits, escapes digestion, being fermented in the intestine as well as dietary fiber [11].

Both the type and amount of carbohydrates in a diet may influence the glycemic index of a meal as some foods result in a marked rise followed by a more or less rapid fall in blood glucose, while others produce a smaller peak along with a more gradual decline in plasma glucose [12]. Based on this, it is particularly important to choose the ideal type and amount of carbohydrate for the glycaemic control and consequently for T2DM treatment.

Although the quantity and the quality of carbohydrates in the diet influence blood glucose levels [13], the most beneficial type, the ideal amount of dietary carbohydrates and the underlying mechanisms involved remain a matter of debate. Both the type and the amount of carbohydrates found in foods influence the glycemia by for changing insulin secretion and gastric emptying [13]. However, it is not clear what types of carbohydrates have the greatest benefits for the management of T2DM and what is the ideal amount of these carboydrates in the diet. In addition, it remains poorly understood the main mechanisms by which certain types and amounts of carbohydrates affect the management of T2DM.Thus, this study was designed to systematically review the *in vivo* preclinical effects of the type and amount of dietary carbohydrates in studies involving T2DM animal models, in order to clarify these aspects for improving T2DM management. Furthermore, this review also evaluated the methodological quality of current evidence, pointing out the main sources of bias.

Methods

Focus question

The main question to be answered in this systematic review was: What are the ideal types and amount of dietary carbohydrates in order to improving T2DM management in animal models? Secondly, what are the main mechanisms involved in the influence of dietary carbohydrates on T2DM animal models?

Literature search

This systematic review adhered to the PRISMA guidelines [14] (Preferred Reporting Items for Systematic Reviews and Meta-Analysis), including search strategy, selection criteria, extraction and data analysis. We completed a comprehensive bibliographic search using the electronic databases Pubmed/Medline (https://www.ncbi.nlm.nih.gov/pubmed), Scopus (https://www.scopus.com/home.uri) and Web of Science (https://www.webofknowledge.com). The studies were selected through an advanced search on the platforms MEDLINE, Web of Science and Scopus, on the 10th of January, 2020 at 1h10 pm. Based on two search parameters, we devised a comprehensive search strategy for the retrieval of all relevant studies: (i) direct

searches in electronic databases, and (ii) indirect screening of reference lists from all studies identified in the direct searches. The keywords for filters for three criteria were type 2 diabetes mellitus, dietary carbohydrates and animal studies (supplementary Table S1). The search filter for PubMed/Medline was based on standardized descriptors obtained from the hierarchical *Thesaurus* MeSH (Medical Subject Headings. In Pubmed/Medline, the commands MeSH and TIAB (title and abstract) were combined for the retrieval of indexed papers and those citations in the indexing process (*epub ahead of print*). The same research descriptors were structured according to the specific search algorithms required in Web of Science (TS=*descriptor*) and Scopus (TITLE-ABS-KEY[*descriptor*]) databases [15] . No chronological limits were applied in the primary search. All original full-text studies published up to 2020 were included in the systematic review. The search strategy is detailed in the supplementary file (Table S1).

Two reviewers (AMM and BSL) conducted the literature search, removed duplicate articles, and screened titles and abstracts with respect to eligibility criteria. After initial screening, full-text articles of potentially relevant studies were independently assessed for eligibility by two reviewers (MMS and MBF). The kappa test was done for the selection and data extraction (kappa=0.946). Selections were then compared, and inconsistencies were resolved in consultation with three other reviewers (RDN and RVG).

Study selection

Publication data (journal, volume, number, page and year), title and abstract of all studies identified in the primary and secondary searches were compared and duplicated registers were removed. This step was followed by a full-text screening stage in which authors reached a consensus to determine which study should be included or removed. In case of disagreements, another group of reviewers (RVG and MBF) decided whether the study fulfilled the inclusion and exclusion criteria. To discard the subjectivity in data collection and selection process, the information was independently extracted by two reviewers (RDN and RVG) and analyzed separately.

We retrieved only experimental studies performed *in vivo*, published in English, with full texts available. The selection was restricted to original studies, developed with animal models. We selected only studies that met all of the eligibility criteria listed below:

• Studies including glycemic control parameters;

• Studies targeting type 2 diabetic animals;

• Studies reporting the effects of different dietary carbohydrate content or different types of dietary carbohydrates

The exclusion criteria were: (i) papers without full-text available, (ii) secondary studies (i.e., literature reviews, comments, letters to the editor, and editorials), (iii) grey literature (studies not peer-reviewed or formally published in indexed journals). The flowchart indicating the process of study selection is presented in Fig. 1.

Data extraction

Considering a comprehensive description of the research models, data extraction was based on methodological features and the studies were synthesized admitting different descriptive levels as it follows: (i) Publication characteristics (authors, year, country of origin); (ii) characteristics of the animal models: species, strain, number of animals, sex, age/weight, intervention, total time of experiment); (iii) performed analyses, primary findings and secondary findings. In the absence of available data within the study, authors were contacted via e-mail to provide further information.

Studies were initially grouped into diet categories based on the degree of carbohydrate restriction of the intervention diet according to the parameters established by Sainsbury *et al.* (2018) [16]. A low carbohydrate diet was defined as <26% of total energy from carbohydrate per day. Moderate carbohydrate diets were defined as between 26% and 45% of total energy from carbohydrate per day. High carbohydrate diets were those >45% of total energy from carbohydrates per day [16]. Due to wide variations within high-carbohydrate diets in regards to carbohydrate amounts, we considered as very-high-carbohydrate diets those with >70% of total energy per day from carbohydrates. Studies were also divided according to the type of carbohydrate (sucrose, glucose, fructose, fiber, resistant starch) and glycaemic index, when available. Data were subsequently compared and conflicting information was identified and corrected after discussion among the reviewers [17].

Quality assessment

We assessed study quality through SYRCLE's risk of bias tool for animal studies (Systematic Review Centre for Laboratory animal) [18], adapted from Cochrane Collaboration. The assessment was made based on the following topics: 1. Random sequence generation. 2. Allocation concealment. 3. Blinding of caregivers and/or investigators from knowledge regarding interventions each animal received. 4. Blinding of outcome assessment. 5. Incomplete outcome data. 6. Selective outcome reporting [18]. The items in the RoB tool were scored with "yes" (low risk of bias); "no" (high risk of bias); or "unclear" (indicating that the item was not reported, and therefore, the risk of bias was unknown). Two review authors (AMM and BSL) independently assessed the risk of bias for each study using the adapted criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions. Any disagreements were resolved by discussion between authors. Risk of bias graph and summary figures were created using Review Manager 5.3 [19].

Results

Characteristics of publications

The initial search resulted in 1010 studies and 133 were duplicates. After reading the title and abstract, we excluded 823 studies that did not meet eligibility criteria. After this primary selection, the remaining 54 articles were completely read and 28 articles were excluded. The bibliographical references of the 26 selected articles were analyzed, and 1 study was added according to the inclusion criteria, resulting in 27 studies (Fig. 1). Most studies identified originated from the United States of America (37%, n=10), followed by Japan (26%, n=7) and China (15%, n=4). The remaining studies were from France (n=3), Australia (n=2) and Denmark (n=1) (Fig. 2).The country of origin of each study is found in Table 1.

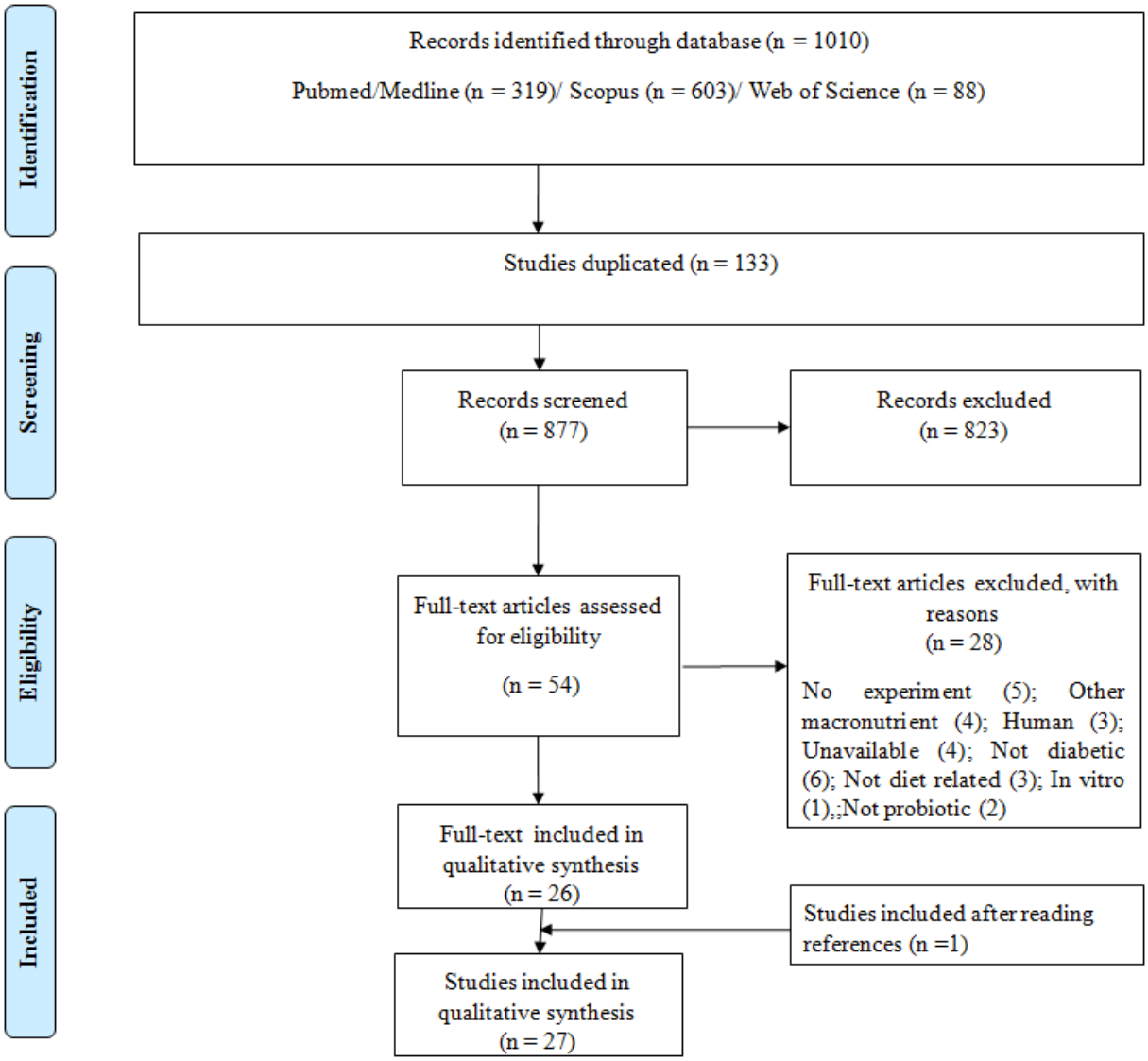


Figure 1. Flowchart detailing selection of studies included in systematic review. Based on PRISMA statement "Preferred Reporting Items for Systematic Reviews and Meta-Analyses". www.prisma-statement.org

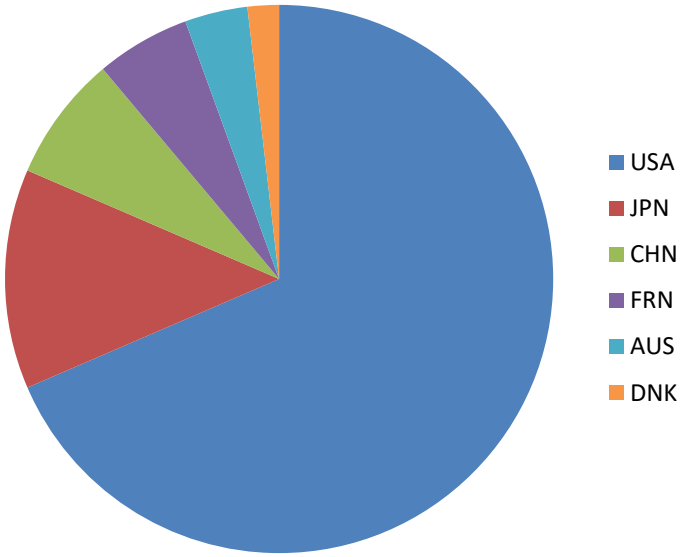


Figure 2. Country of origin of the studies included in this review. USA = United States of America; JPN = Japan; CHN = China; FRN = France; AUS = Australia; DNK = Denmark.

Characteristics of experimental animals

All studies reported the use of rodents as experimental models, being 63% (n=17) *Rattus novergicus*, 33% (n=9) *Mus musculus* and 4% (n=1) *Arvicanthis niloticus.* Male animals (96%, n=26) were preferred over females (4%, n=1). From the studies using *Rattus novergicus*, Wistar was the strain of choice (35%, n=6), followed by Sprague-Dawley (29%, n=5) and the age of experimental animals varied from 3 to 18 weeks. From the studies using *Mus musculus*, the strain of choice was C57BLKS (25%, n=3) and the age of experimental animals varied from 3 to 5 weeks. For those using *Arvicanthis niloticus,* the strain of choice was the Nile rat (100%, n=1) and the age for animals used in the study was 4 weeks. Three studies did not report the age of the experimental models [20, 21, 22]. Further details are found in Table S2 of supplementary material.

Characteristics of dietary strategies

Most studies reported high carbohydrate diets (48%, n=13), from which 15% (n=2) were high carbohydrate and high fiber diets. Low carbohydrate diets accounted for 30% of the studies (n=8), from which 13% (n=1) were low carbohydrate and high fiber diets. Diets with moderate amounts of carbohydrates accounted for 11% of the studies (n=3), from which 34% (n=1) were moderate carbohydrate and high fiber diets. Very high carbohydrate diets accounted for 15% (n=4) of the studies, from which 25% (n=1) were very high carbohydrate and high fiber diets. Sucrose was the main carbohydrate source reported on these articles, representing 30% (n=8) of the studied diets. Other carbohydrate sources reported in the selected studies were resistant starch (n=4, 15%), glucose and fructose (n=3, 11%) and high glycaemic index diets (n=2, 7%). The type of carbohydrate was not reported in 37% of the studies (n=10) and only the amount of dietary carbohydrates was evaluated in these studies. Most studies (59%, n=16) purchased their diets from feed manufacturers, from which the nutritional composition was approximately 23% crude protein, 4.5% crude fat, 6% crude fiber, 8% ash, 2.5% added minerals; provided it *ad libitum*. Further details are found in Table 1.

Main outcomes

Eighteen studies reported a worsening in blood glucose parameters, most of them (n=6) intervened with a high carbohydrate diet [23, 24, 25, 26, 27, 28]. One study intervened with a very high carbohydrate high fiber diet [29] and 3 studies intervened with a very high carbohydrate diet [30, 31, 32]. Six articles reported worsened blood glucose parameters under low carbohydrate diets [33, 34, 35, 36, 37, 38] and 2 under moderate carbohydrate diets [39, 40]. Most diets that deteriorated blood glucose parameters were rich in sucrose (n=8). Four studies with high dietary carbohydrate content reported no differences in glycemia in animals fed with different types of carbohydrates (corn starch on a high glycemic index diet, glucose and fructose) [41, 42, 43, 44]. One study improved blood glucose parameters combining a low carbohydrate diet and resistant starch as the carbohydrate type (low carbohydrate high fiber diet) [45] and one study improved T2DM parameters on a low carbohydrate high fiber diet [46]. Detailed analyzes of diets, experimental models and outcomes are found in Table 1.

Five of the 27 reviewed articles reported an improvement in T2DM control, from which 2 intervened with a high carbohydrate and high fiber diet, 1 with a moderate carbohydrate high fiber diet and 2 with a low carbohydrate high fiber diet. From these studies, 4 reported an improvement in blood glucose parameters on high resistant starch consumption [21, 22, 39, 45]. Results are detailed in Table 1. The impact of different types of diets on main parameters of T2DM is summarized in Table 2.

Table 1: Qualitative description of main negative‡ and positive* outcomes reported in all studies investigating the relevance of types and amount of carboydrates used in T2DM diet in animal models.

Study/Country	**Dietary strategy**	**Feed manufacturer**	**Negative outcomes‡**
Very High carbohydrate diet			
Parkman *et al*., 2016 [30] USA	70,8% CHO: sucrose	Purina 5001, BMI Nutrition, Brentwood, USA	↓glucose tolerance ↑BW
Arimura *et al.,* 2017 [31] JPN	71% CHO 59% CHO high protein	Wako Pure Chemical Industries, JPN	↑BG in both diets albumin excretion higher in High protein group No difference in BW or C-Peptide
Arimura *et al*., 2018 [32] JPN	71% CHO, 12% Protein 59% CHO, 24% Protein	-	High protein diet ↑HbA1c, ↑plasma insulin and retinal thickness No difference in BG, urinary glucose and BW

Author/Year/Country	Dietary strategy	Feed manufacturer	Negative outcomes‡
Very High carbohydrate **High fiber diet**			
Bolsinger *et al*., 2013 [29] USA	HC: 70% CHO MC: 40% CHO LC: 10% CHO HC+High Fiber: 70% CHO	-	Fasting BG higher in HC, followed by MC HC+High Fibre showed same results as LC

Study/Country	Dietary strategy	Feed manufacturer	Negative outcomes‡
High carbohydrate diet			
Bhathena *et al*., 1989 [23] USA	54% CHO: Sucrose or starch	Teklad test diet, Madison, USA #40060	↑BG in sucrose group compared to starch ↑TC, TG and BW in the sucrose-fed group
Velasquez *et al*., 1995 [24] USA	54% CHO: Sucrose or starch	Teklad test diet, Madison, USA #40060	↑urinary glucose, ↑BG Sucrose-fed: ↑protein excretion, abnormal glomeruli and ↑plasma insulin
Kazumi *et al*., 1997 [25] JPN	Chow + 10% glucose or fructose in water	CE-2, Oriental Yeast, Tokyo, JPN	Both glucose and fructose ↑BG Fructose ↑TG

Patel *et al*., 2009 [26] AUS	61% CHO: fructose or cornstarch	-	Fructose: ↑fasting BG and ↓glucose tolerance ↑arterial stiffness
Nojima *et al*., 2013 [27] JPN	47,8% CHO: 30% sucrose or 50% fat	CRF-1 Oriental Yeast, Tokyo, JPN	Sucrose-fed: ↓glucose tolerance and ↑BW Fat-fed: ↑BG
Zhuo *et al*., 2018 [28] CHN	61% CHO: sucrose	-	↑fasting BG, insulin, TC, TG, GLUT4 ↓GLUT2 in the liver

Author/Year/Country	**Dietary strategy**	**Feed manufacturer**	**Negative outcomes‡**
Moderate carbohydrate diet			
Noonan & Banks, 2000 [40] USA	35% CHO: sucrose	F2685, Bioserv Frenchtown, USA	↑fasting BG, BW and plasma insulin
Iwama *et al*., 2003 [20] JPN	30% CHO: sucrose	-	↑fasting BG, necrosis in pulpal tissue and alveolar bone reabsorption

Author/Year/Country	**Dietary strategy**	**Feed Manufacturer**	**Negative outcomes‡**
Low carbohydrate diet			
Pascoe *et al.,* 1992 [33]	20% CHO	Allied Feeds, Sydney, Australia	↑BG, BW, TC

AUS			
Surwit *et al*., 1995 [34] USA	25% CHO (High fat): HSHFD, LSHFD	Research Diets, New Brunswick, USA	High fat diet ↑BG, BW and plasma insulin (both HSHFD and LSHFD)
Kaneko *et al*., 2000 [35] JPN	40% CHO 20%CHO	CE-2 Nippon Clea, Tokyo, Japan	20% and 40% CHO: ↑fasting BG and ↓glucose tolerance 20% CHO: ↑BW and plasma insulin
Wang *et al*., 2003 [36] JPN	10% CHO, 65% Fat	-	10% CHO: ↑fasting BG, ↑BW, ↓plasma insulin and ↓glucose tolerance
Petro *et al*., 2004 [37] USA	26% CHO	Research Diets, New Brunswick, USA	↑fasting BG, BW and plasma insulin
Asghar *et al*., 2006 [38] CHN	12% CHO: sucrose	Research Diets, New Brunswick, USA	↑fasting BG ↑glucagon ↑plasma insulin
Study/Country	**Dietary strategy**	**Feed Manufacturer**	**Positive outcomes***
Very High carbohydrate **High fiber diet**			
Zhou *et al*., 2015 [22] CHN	80% CHO: resistant starch	-	↓BG, TC and TG ↑BW
High carbohydrate			

High fiber diet			
Hedemann *et al*., 2017 [21] DNK	52,95% CHO: Cornstarch, GLU, EMS or resistant starch	Altromin 1321, Brogaarden, DNK	↓Fasting BG in resistant starch and cornstarch-fed ↓HbA1c in resistance starch-fed All diets ↑TG
Study/Country	**Dietary strategy**	**Feed Manufacturer**	**Positive outcomes***
Moderate carbohydrate **High fiber diet**			
Shen *et al*., 2011 [39] USA	30% CHO: resistant starch	National Starch Food Innovation, Bridgewater, USA	↓fasting BG, ↑insulin sensitivity, ↑cecal short chain fatty acids and butyrate producing bacteria
Study/Country	**Dietary strategy**	**Feed Manufacturer**	**Positive outcomes***
Low carbohydrate **High fiber diet**			
Marsh et al., 2009 [46] USA	2% CHO	TestDiet, Richmond, USA	↓Fasting BG and HbA1c ↑arterial stiffness
Sun et al., 2018 [45] CHN	Resistant starch: Low dose (10%) Medium dose (15%) High dose (20%)	National Starch and Chemical Company, Shanghai, CHN	↓fasting BG, TC, TG and BW ↑plasma insulin

USA = United States of America; BG = blood glucose; TC = total cholesterol; TG = triglycerides; BW = body weight; CHO = carbohydrate; JPN = Japan; AUS, = Australia; HC = high carbohydrate; MC = moderate carbohydrate; LC = low carbohydrate; CHN = China; HbA1c = glycated hemoglobin A1c; - = missing info; HSHFD = high sucrose high fat diet; LSHFD = low sucrose high fat diet; LSLFD = low sucrose low fat diet; HSLFD = high sucrose low fat diet; DNK = Denmark; ♀ = female; GLU = glucidex; EMS = enzimatically modified starch; IHC = immunohistochemestry.

Table 2: Summary of the impact of different types of diets on main parameters of T2DM in animal models.

Carbohydrate type	**Diet[a]**	**Effect**
Sucrose (n=8)	VHC (n=1)	Worsened plasma blood glucose *vs.* control group (n=8)
	HC (n=4)	
	MC (n=2)	
	LC (n=1)	
Glu/Fru (n=3)	HC (n=3)	Worsened plasma blood glucose *vs.* control group (n=2) No difference (n=1)
	MC (n=0)	
	LC (n=0)	
Corn starch (HGI diet) (n=2)	HC (n=2)	No difference (n=2)
	MC (n=0)	
	LC (n=0)	
Resistant Starch (n=4)	VHC + high fiber (n=1) HC + high fiber (n=1)	Improved plasma blood glucose *vs.* control group (n=4)
	MC + high fiber (n=1)	
	LC + high fiber (n=1)	
NP (n=10)	VHC (n=2)	

HC (n=1)	Worsened plasma blood glucose *vs.* control group (n=8)
HC + high fiber (n=1)	
MC (n=0)	
LC (n=6)	Improved plasma blood glucose *vs.* control group (n=1)
	No difference (n=1)

VHC = very high carbohydrate; HC = high carbohydrate; MC = moderate carbohydrate; LC = low carbohydrate; BG = blood glucose; HbA1c = glycated hemoglobin A1c; Glu/Fru = glucose and fructose; HGI = high glycemic index; NP = not provided.

[a]As established by Sainsbury *et al*. (2018) [14].

Secondary outcomes

In regards to secondary results, most frequent parameters reported were body weight and plasma insulin concentration (both 74% of the studies); followed by plasma triglycerides (37% of studies) and total cholesterol concentrations (44% of studies), all these related to sucrose intake. High carbohydrate diets increased body weight in all studies, particularly when the main carbohydrate source was sucrose. In the absence of fiber, both low-carbohydrate diets [34, 36, 37, 38] and high-carbohydrate diets [24, 28, 31, 32] led to increased insulin secretion. Similarly, both low- [35, 36] and high-carbohydrate diets [26, 27, 30] resulted in decreased glucose tolerance in the absence of fiber.

Lipid profile was improved by carbohydrate-rich diets, as longs as the main carbohydrate source was resistant starch [23, 33]. Ten out of the 27 selected studies reported an increase in plasma insulin and 40% of these intervened with sucrose as a carbohydrate source, with content varying between 12-61%.

All studies used fasting blood glucose, blood glucose, urinary glucose, HbA1c (hemoglobin A1c), fructosamine, oral GTT (glucose tolerance test), intraperitoneal GTT or a combination of these as parameters to assess T2DM management. In addition, most articles reported effects on body weight, blood insulin and lipid profile.

The main mechanisms involved with carbohydrate intake that impair T2DM management were related to increased pyruvate production leading to fatty liver and increased serum lipids [23, 24, 26, 27, 28, 29, 30, 33, 34, 35, 36, 37, 38, 40], leading to metabolic syndrome. Three studies reported a worsening in T2DM management, unrelated to these mechanisms [20, 31, 32]. Improvements in T2DM parameters were associated with prebiotic effects of fiber and resistant starch, increased satiety and increased gastrointestinal transit time [21, 22, 39, 45]. One study reported improved glycemic parameters without further analysis of underlying mechanisms [46].

Quality assessment

Overall, the assessment of key quality indicators resulted in high risk of bias for the selected studies. Most studies (62%, n =17) reported that animals were randomly allocated without providing further details on how allocation was designed, resulting in unclear risk of bias. The remaining 38% of the studies did not report information on random allocation, which resulted in high risk of bias. Blinding among groups was under-reported and resulted in high risk of bias in 93% of the studies (n=25). Only 6 studies (22%) reported blinding of personnel. Incomplete outcome data was adequately addressed in 85% (n=23) of the studies and most studies were free from selective reporting (n=25). Selected studies that are apparently free from other problems that could result in a high risk of bias accounted for 62%. Only 3 studies (11%) provided a conflict of interest statement and 24 studies (71%) mentioned approval by an ethical board. An overview of the main results of included articles was schematically shown in Fig. 3. Quality assessment at an individual level was reported in Fig. 4.

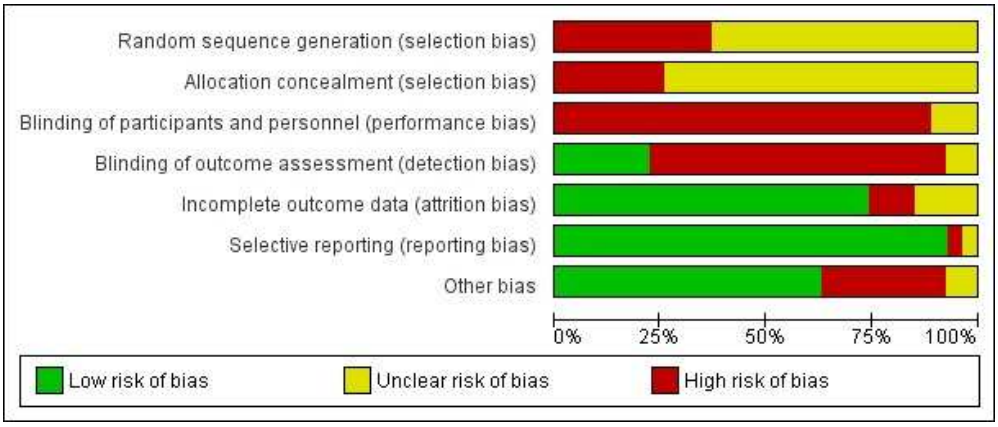
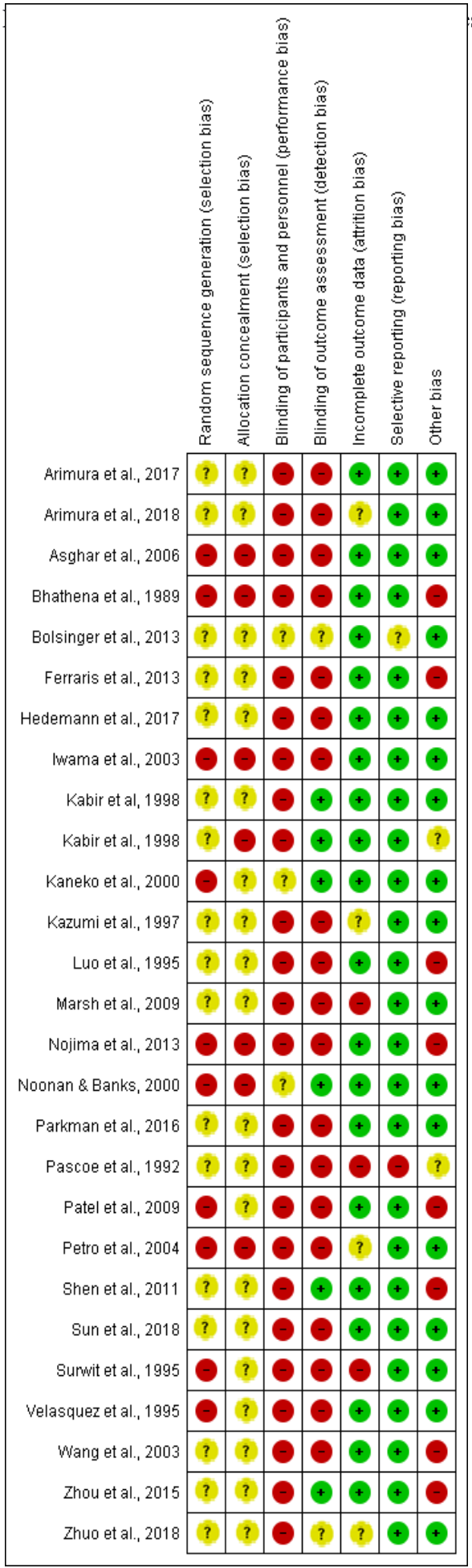


Figure 3. Risk of bias graph shows review authors' judgement about each risk of bias item presented as percentages across all included studies. The following methodological domains based on RoB were evaluated. Consider selection bias: "Was the allocation sequence adequately generated and applied?", "Were the groups similar at baseline or were they adjusted for confounders in the analysis?", "Was the allocation to the different groups adequately concealed?"; Consider performance bias: "Were the animals randomly housed during the experiment?", "Were the caregivers and/or investigators blinded from knowledge regarding which intervention each animal received during the experiment?"; Consider detection bias: "Were animals selected at random for outcome assessment?", "Was the outcome assessor blinded?"; Considers attrition bias: "Were incomplete outcome data adequately addressed?"; Considers reporting bias: "Are reports of the study free of selective outcome reporting?"; Considers other biases: "Was the study apparently free of other problems that could result in high risk of bias?"; The overall study quality indicators: "Was it stated that the experiment was randomized at any level?" and "Was it stated that the experiment was blinded at any level?". The items in the RoB tool were scored with "yes" (low risk of bias); "no" (high risk of bias); or "unclear" (indicating that the item was not reported, and therefore, the risk of bias was unknown) [12]. The items in the RoB tool were scored with "yes" (low risk of bias); "no" (high risk of bias); or "unclear" (indicating that the item was not reported, and therefore, the risk of bias was unknown) [17].

s' quality assessment at an individual level.



Discussion

In our study, we conducted a systematic revision to investigate the effect of the amount and the type of carbohydrates used in T2DM in animal models. Our results showed that sucrose deteriorates blood glucose regardless of its dietary content. On the other hand, resistant starch and dietary fiber seem to improve T2DM parameters even in high-carbohydrate diets.

Besides, our findings show that the studies investigating carbohydrate intake are concentrated in the United States, Japan and China. As the staple food in these countries is carbohydrate-based, rich in wheat and/or rice, the increased concentration of studies in these countries may be related to its populations' eating habits, in addition to the fast increase of fast food restaurants available worldwide. In The United States, convenience and/or high sugar foods are largely available in a wide variety of formats, which contributes to poor dietary habits. In addition, large portion sizes usually offered in restaurants also raise concern on the amount of extra calories ingested [47]. In Asian countries, a "nutrition transition" from a traditional vegetable-based diet to a processed, unhealthy diet has been occurring along younger generations. This change has been related to the rapid growth of non-communicable diseases in China and Japan, such as diabetes [48].

Although our search was not limited to rodents, all experimental models used in the selected articles were murines, which exclude other potentially useful animal models. While humans are undoubtedly the model of choice when studying the pathophysiology of human disease, using living humans as experimental models has several logistical and ethical limitations. Therefore, there is a need to develop *in vivo* animal models for this purpose. Frequently used genetic models of T2DM, such as db/db mice and Zucker fa/fa rats, have been useful in understanding mechanisms which contribute to disease development, however, they are not ideal models as these gene mutations are extremely rare in human populations [49]. Similarly, T2DM murine models induced by destruction or pancreatic ablation [50] are not representative of the etiology of T2DM in humans. As T2DM is linked to excessive accumulation of body fat, diet-induced obesity models are particularly relevant for investigating underlying mechanisms through which an excessive accumulation of body fat and/or an excessive dietary fat and/or sugar intake may result in insulin resistance and T2DM. Preclinical studies have shown that overfeeding

may induce obesity, low grade inflammation and insulin resistance in periods of time ranging from 8 weeks to 80 weeks [51, 52].

Nutritional manipulations to induce T2DM include changing the diet of the animal itself, or its mother during pregnancy and/or lactation; and involve either increases in dietary fat or carbohydrates. The study of diet-induced obesity and models of prenatal undernutrition and overnutrition has revealed several common mechanisms that contribute to the understanding of the physiological basis of reduced insulin sensitivity and provide some new insights into T2DM etiology in humans [49]. A low birth weight followed by a period of increased postnatal growth, or a high birth weight due to prenatal overnutrition, are both associated with a higher chance towards developing insulin resistance, glucose intolerance, and T2DM in adult life [53]. The two most widely used models for the study of T2DM found in this review were high-fat and high- sugar feeding in rodents. The high-fat feeding animal model C57BL/6 was the most used mice strain in the included studies, predominantly with *ad libitum* access to a high-energy diet from 2-16 weeks, developing glucose intolerance, obesity and hyperglycemia. Regarding the high-sugar feeding animal models, the most used strains were Wistar and Sprague-Dawley rats, fed *ad libitum* for 3-64 weeks, chosen for studying the metabolic effects of diet-induced obesity [49, 54], as shown by the results of our review. Considering the need to improve and standardize protocols of preclinical models for T2DM studies, it is important to highlight differences between commonly used rodent species. In rats, providing sucrose either in solid form or in drinking water (high-sugar diet) has been associated with both increased visceral fat accumulation and insulin resistance in both liver and skeletal muscle [55]. In mice, however, adding sucrose to drinking water fails to induce obesity, although this does lead to subtle metabolic changes, such as adipocyte hypertrophy, glucose intolerance, hyperinsulinemia, hyperlipidemia, fatty liver and increased levels of inflammatory cytokines [55]. Hence, further studies in the area of high-fat/high-sucrose feeding are required in order to establish the best model of T2DM in humans and to completely elucidate the effects of the diet.

In addition, most studies used only males as experimental models. Single sex studies still prevail in the biological literature [56], although studies limited to a single sex cannot yield a complete understanding of gender-related differences and underlying mechanisms, even if they only occur in certain environments, at specific ages or stages of the reproductive cycle. A partial list of sexually dimorphic rodent behavioral traits included wheel running behavior, open field

activity, aggression, taste preferences, food intake, performance on learning tasks, and responses to brain damage. Fluctuations in female hormones may affect pharmacokinetics of drugs used in studies and its efficacy [57], which may encourage the use of male animals in order to increase heterogeinity [57].

Animals in weaning age were more frequently used compared to older ones, which may be due to a better adaptation to the experimental diets [57]. Most diets (59%, n=16) were acquired from feed manufacturers, which allows controlled methodological standards and improves studies' reproducibility.

Sucrose, fructose, glucose and high glycaemic index diets

In regards to diets, carbohydrates are the first macronutrient to be broken into glucose, which is the main insulin secretagogue. Thus, one can assume that simply decreasing carbohydrate intake would lead to improved diabetes management. However, each individual may respond differently to a variety of diets. Most HC diets containing mono- and disaccharides resulted in a deterioration of the diabetic condition, shown on fasting blood glucose tests, HbA1c, fructosamine, intraperitoneal GTT or oral GTT, regardless of the percentage of carbohydrates in the diet and in the absence of fiber. At similar amounts of carbohydrates, low glycaemic index and high fiber meals tend to result in lower postprandial blood glucose compared to high glycaemic index meals [12, 58]. Controversially, the only 2 studies included in this review on high glycaemic index diets reported no difference in blood glucose parameters when high glycaemic index carbohydrates were added to the diet. It is suggested that an increase in GLUT4 at the cell surface compensates for the high glycaemic index food [42]. It is known that a sucrose-rich diet can induce upregulation of GLUT5 in the apical border of enterocytes in the small intestine, which increases the absorption of fructose [59]. High fructose consumption can lead to excessive pyruvate production and enhanced lipid biosynthesis as a consequence [60]. Hence, a sucrose rich diet could accelerate the development of metabolic syndrome and cause fatty liver disease. In addition, it may induce pancreatic inflammation with increased macrophage infiltration and islet injury associated with a reduction in insulin secretion [61], which may account for deterioration in blood glucose parameters. Similarly, diets containing only fructose and glucose showed worsened diabetes condition in 67% of the studies and the

other 33% showed no difference. However, only 3 studies used these carbohydrate types in their experimental diets from all studies included in this review. When the type of carbohydrate added was resistant starch, blood glucose parameters improved in all studies, regardless of the quantity.

Another mechanism that might be impairing well-managed diabetes is related to increased uric acid production, a product from sucrose and fructose metabolism due to the breakdown of adenine nucleotides [61]. Uric acid enters cells via specific transporters, such as URAT1, where it induces proinflammatory and prooxidative effects [62]. Sucrose-fed rats have been reported increased URAT1 expression in the islets, chronic hyperuricemia, hypertriglyceridemia and fatty liver [63], which supports the findings of the studies in this review.

In addition, very high carbohydrate diets, in which dietary carbohydrate content was 70% or more of total daily energy intake led to a pronounced deterioration of T2DM in animal models. Macronutrient composition modulates fatty acid deposition and inflammation in different tissues such as liver, brain and adipose tissue [64, 65]. In a study conducted by Antunes *et al*. [65], mice were fed a diet containing 73.8% carbohydrates for 2 months, resulting in increased lipid deposition and more intense inflammation due to increased proinflammatory prostaglandins and decreased anti-inflammatory mediators. This is in accordance with our findings, as both inflammation and lipid accumulation worsen metabolic syndrome [12], which is closely related to T2DM [1].

Fiber

The American Diabetes Association (ADA) emphasizes that nutrition therapy is fundamental for T2DM patients [66] and there is research on many food choices that assists people on improving blood glucose levels and overall health. Currently, there is no ideal macronutrient proportions that applies broadly, thus, the dietary macronutrient ratio should be individualized. The recommended amount of carbohydrates for healthy adults is 130 g/day and it is determined considering the brain's requirement for glucose, but this energy requirement is also fulfilled by other metabolic processes, such as glycogenolysis, gluconeogenesis, and/or ketogenesis [67]. A common dietary intervention is the Mediterranean diet [68] that emphasizes plant-based foods, seafood and olive oil as the main source of dietary fat. It includes moderate amounts of

dairy products, red meat and wine; and low or very low amounts of sugars. Benefits to T2DM patients include reduced HbA1c, lowered triglycerides and reduced risk of cardiovascular events [68]. The increasingly popular vegetarian and vegan dietary approaches emphasize plant-based eating and may include egg and/or dairy products (in case of vegetarian) or exclude all flesh foods and animal-derived products (in case of vegan) and both decrease HbA1c and body weight [69]. Low-fat diets emphasize vegetables, fruits, starches, lean protein sources and low-fat dairy products. Studies report weight loss as a common benefit for T2DM patients [70]. Low-carbohydrate diets emphasize vegetables that are low in carbohydrates and avoid starchy and sugary foods. Current diabetes reports consider 26-45% of total calories from carbohydrates as "low-carbohydrate" and fewer than 26% as "very low-carbohydrate" approach. These diets have been reported as a strategy for T2DM patients who are not reaching their glycemic goals with medication, as reported benefits include HbA1c reduction, weight loss, lowered blood pressure and lowered triglycerides [71]. Another dietary strategy that has been used for T2DM management is the Paleo diet, which emphasizes foods eaten during early human evolution such as meat, fish, shellfish, vegetables and nuts. Benefits of this diet remain unclear due to inconclusive evidence [72].

Increased fiber intake has been strongly recommended as part of T2DM management in humans due to its benefits in inducing satiety, increasing gastrointestinal transit time and improving overall blood glucose levels [73]. In addition, high-fiber diets are associated with lower all-cause mortality in people with T2DM [74], therefore, patients are encouraged to consume at least the amount of dietary fiber recommended for the general public (minimum of 14 g of fiber per 1,000 kcal) [66]. Thus, overall dietary recommendations include avoiding added sugars, preferring carbohydrates from fiber-rich sources [66, 73, 74]. This supports our findings in animal models, that show that increased fiber intake and using resistant starch as a the main carbohydrate source on a diet has benefits for maintaining normoglycemia and overall health. Furthermore, added sucrose is not recommended in any of the abovementioned diets, which corroborates our findings that sucrose intake leads to a worsening in lipid profile, fatty liver disease, development of metabolic syndrome and T2DM onset.

Resistance Starch

Regardless of the carbohydrate percentage in the diet, all studies included in this review resulted in an improvement of blood glucose parameters when using resistant starch. As resistant starch may escape digestion, a high carbohydrate diet in which the main source of carbohydrate is resistant starch may be considered a carbohydrate-restricted diet. Resistant starch physically inaccessible to digestive enzymes is referred to as the type 1, found in whole grains and seeds [11]. Type 2 resistant starch are those resistant to digestion due to the nature of the starch granule, found in raw potatoes and unripe bananas, for instance [11]. Resistant starch that results from food processing and from chemical modification are referred to as type 3 and 4, respectively [11]. Due to its natural features and benefits, it has received a lot of attention as a functional ingredient.

The main reason for that is that resistant starch is fermented and used by the microbiota in the large intestine [75], resulting in beneficial bacterial growth, such as Lactobacilli, Bifidobacteriacea, leading to increased SCFAs (short-chain fatty acids) production, which has anti-inflammatory properties. Therefore, resistant starch has been considered a prebiotic, being able to attenuate metabolic disorders [76], due to decreased decreased inflammatory status by increasing mucosal thickness and, as a result, reducing intestinal permeability to toxins. In addition, its presence can delay gastric emptying and the entrance of glucose into the bloodstream, decreasing postprandial glycaemia. It can also indirectly reduce insulin resistance and blood glucose levels due to reduced inflammation [75]. Different types of dietary carbohydrates on blood glucose parameters, considering the underlying mechanisms, are summarized in Fig. 5.

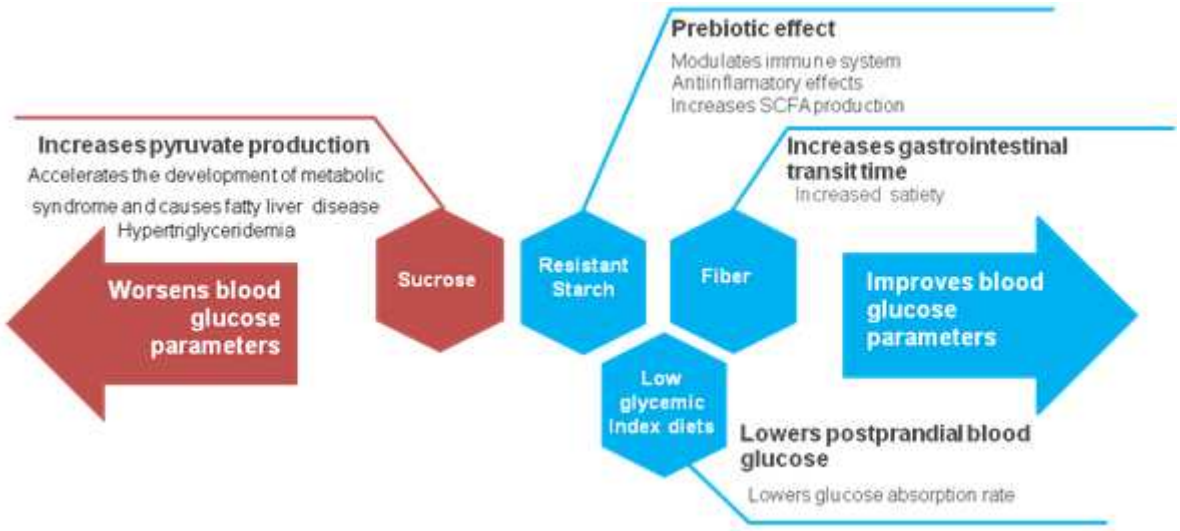


Figure 5. Effects of different sources of dietary carbohydrates on T2DM management in diets in animal models.

Fat

The National Academy of Medicine has defined an acceptable macronutrient distribution for total fat for all adults to be 20–35% of total calorie intake [53]. Diets with a high fat content and lower carbohydrate content have demonstrated important improvements in glycemia and cardiovascular risk factors compared with lower fat higher carbohydrate diets. While there is an association between cholesterol intake and serum cholesterol levels, there is no direct link between cholesterol intake and cardiovascular events [69]. More research is needed on T2DM, serum cholesterol, cholesterol intake and cardiovascular events.

Limitations

Systematic reviews are considered high-level studies that allow for the individual evaluation of studies in a blind manner using specific tools [77]. Such characteristics lead to a more inclusive and reliable approach, providing a broad understanding of the included studies.

One limitation of our review is that studies are grouped into 4 degrees of carbohydrate intake, being: very-high carbohydrate diets (>70%), high carbohydrate (45-70%), moderate

carbohyidrae diets (26-45%) and low carbohydrate diets (<26%). This could prevent definitive conclusions regarding the effect of carbohydrate amount in a diet as the range of carbohydrate intake is very wide among groups. Furthermore, commercial diets fed to the control gourps fit into the high carbohydrate diet group, which may hinder comparisons.

Another limitation is that dietary carbohydrate type was neglected in some studies, preventing a deeper understanding of the role of carbohydrates on a diet.

Conclusion

Improvements in T2DM parameters in animal models were more closely related to the type of dietary carbohydrate than to its content on a diet, i. e., resistant starch seems to be the most beneficial source for maintaining normoglycemia. Results show that current literature is at high risk of bias due to neglecting experimental methods.

Acknowledgements

The authors are grateful to the support provided by Fundação do Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, processes APQ-01895-16, PPM-00687-17 and PPM-00077-18), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, processes 303972/2017-3, 423594/2018-4, 305093/2017-7 and MCTIC 408503/2018-1), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES, finance code 001).

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Supporting information

Supplementary material 1 (S1): Table of descriptors of research, containing the filters used in each one of the platforms

PubMed-MEDLINE	***Date Recovered articles***
#1 Animal	
(("animal experimentation"[MeSH Terms] OR "models, animal"[MeSH Terms] OR "invertebrates"[MeSH Terms] OR "Animals"[Mesh:noexp] OR "animal population groups"[MeSH Terms] OR "chordata"[MeSH Terms:noexp] OR "chordata, nonvertebrate"[MeSH Terms] OR "vertebrates"[MeSH Terms:noexp] OR "amphibians"[MeSH Terms] OR "birds"[MeSH Terms] OR "fishes"[MeSH Terms] OR "reptiles"[MeSH Terms] OR "mammals"[MeSH Terms:noexp] OR "primates"[MeSH Terms:noexp] OR "artiodactyla"[MeSH Terms] OR "carnivora"[MeSH Terms] OR "cetacea"[MeSH Terms] OR "chiroptera"[MeSH Terms] OR "elephants"[MeSH Terms] OR "hyraxes"[MeSH Terms] OR "insectivora"[MeSH Terms] OR "lagomorpha"[MeSH Terms] OR "marsupialia"[MeSH Terms] OR	January 10th 2020 12h11 pm 363167

"monotremata"[MeSH Terms] OR "perissodactyla"[MeSH Terms] OR "rodentia"[MeSH Terms] OR "scandentia"[MeSH Terms] OR "sirenia"[MeSH Terms] OR "xenarthra"[MeSH Terms] OR "haplorhini"[MeSH Terms:noexp] OR "strepsirhini"[MeSH Terms] OR "platyrrhini"[MeSH Terms] OR "tarsii"[MeSH Terms] OR "catarrhini"[MeSH Terms:noexp] OR "cercopithecidae"[MeSH Terms] OR "hylobatidae"[MeSH Terms] OR "hominidae"[MeSH Terms:noexp] OR "gorilla gorilla"[MeSH Terms] OR "pan paniscus"[MeSH Terms] OR "pan troglodytes"[MeSH Terms] OR "pongo pygmaeus"[MeSH Terms]) OR(animals[tiab] OR animal[tiab] OR mice[Tiab] OR mus[Tiab] OR mouse[Tiab] OR murine[Tiab] OR woodmouse[tiab] OR rats[Tiab] OR rat[Tiab] OR murinae[Tiab] OR muridae[Tiab] OR cottonrat[tiab] OR cottonrats[tiab] OR hamster[tiab] OR hamsters[tiab] OR cricetinae[tiab] OR rodentia[Tiab] OR rodent[Tiab] OR rodents[Tiab] OR pigs[Tiab] OR pig[Tiab] OR swine[tiab] OR swines[tiab] OR piglets[tiab] OR piglet[tiab] OR boar[tiab] OR boars[tiab] OR "sus scrofa"[tiab] OR ferrets[tiab] OR ferret[tiab] OR polecat[tiab] OR polecats[tiab] OR "mustela putorius"[tiab] OR "guinea pigs"[Tiab] OR "guinea pig"[Tiab] OR cavia[Tiab] OR callithrix[Tiab] OR marmoset[Tiab] OR marmosets[Tiab] OR cebuella[Tiab] OR hapale[Tiab] OR octodon[Tiab] OR chinchilla[Tiab] OR chinchillas[Tiab] OR gerbillinae[Tiab] OR gerbil[Tiab] OR gerbils[Tiab] OR jird[Tiab] OR jirds[Tiab] OR merione[Tiab] OR meriones[Tiab] OR rabbits[Tiab] OR rabbit[Tiab] OR hares[Tiab] OR hare[Tiab] OR diptera[Tiab] OR flies[Tiab] OR fly[Tiab] OR dipteral[Tiab] OR drosphila[Tiab] OR drosophilidae[Tiab] OR cats[Tiab] OR cat[Tiab] OR carus[Tiab] OR felis[Tiab] OR nematoda[Tiab] OR nematode[Tiab] OR nematoda[Tiab] OR nematode[Tiab] OR nematodes[Tiab] OR sipunculida[Tiab] OR dogs[Tiab] OR dog[Tiab] OR canine[Tiab] OR canines[Tiab] OR canis[Tiab] OR sheep[Tiab] OR sheeps[Tiab]

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parrots[Tiab] OR donkey[Tiab] OR donkeys[Tiab] OR mule[Tiab] OR mules[Tiab] OR zebra[Tiab] OR zebras[Tiab] OR shrew[Tiab] OR shrews[Tiab] OR bison[Tiab] OR bisons[Tiab] OR buffalo[Tiab] OR buffaloes[Tiab] OR deer[Tiab] OR deers[Tiab] OR bear[Tiab] OR bears[Tiab] OR panda[Tiab] OR pandas[Tiab] OR "wild hog"[Tiab] OR "wild boar"[Tiab] OR fitchew[Tiab] OR fitch[Tiab] OR beaver[Tiab] OR beavers[Tiab] OR jerboa[Tiab] OR jerboas[Tiab] OR capybara[Tiab] OR capybaras[Tiab]) NOT medline[subset]))	
#2 Type 2 diabetes mellitus ("Type 2 diabetes mellitus" [Tiab] OR "Type 2 diabetes mellitus"[Tiab]) OR ("Type II diabetes mellitus" [Tiab] OR "Type II diabetes mellitus"[Tiab])	January 10th 2020 12h15 pm 45050
#3 Dietary carbohydrates ("Dietary carbohydrates"[Mesh Terms] or "Dietary carbohydrates"[Tiab])	January 10th 2020 12h17 pm 86585
SCOPUS	***Date Recovered articles***
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#2 Type 2 diabetes mellitus ((TITLE-ABS-KEY(type 2 diabetes) OR (TITLE-ABS-KEY(type II diabetes))	January 10th 2020 12h45 pm

	22384
#3 Dietary carbohydrates (TITLE-ABS-KEY (Dietary carbohydrates)	January 10th 2020 12h50 pm 49138
Web of Science	***Date Recovered articles***
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#2 Type 2 diabetes mellitus TS=Type 2 diabetes mellitus OR TS=Type II diabetes mellitus	January 10th 2020 12h59 pm 105431
#3 Dietary carbohydrates TS=Dietary carbohydrates	January 10th 2020 12h56 pm 21639

Supplementary material 2 (S2): Table of experimental models.

Table S2. General characteristics of the animal models used in all studies investigating the relevance of type and amount of carboydrates used in T2DM diet.

Study	Species	Sex	Weight (g)	Age (weeks)	Diet composition	Daily caloric intake (kcal)
High carbohydrate diet						
Bhathena *et al*., 1989	*Rattus norvegicus*	Male	-	5	54% sucrose 54% starch	-
Velasquez *et al*., 1995	*Rattus norvegicus*	Male	385-605	6	54% CHO: Sucrose or starch	-
Kazumi *et al*., 1997	*Rattus norvegicus*	Male	431	18	Chow and 10% glucose or fructose in water	153
Patel *et al*., 2009	*Rattus norvegicus*	Male	320-350	8-9	61% Fructose 61% Cornstarch	97
Nojima *et al*., 2013	*Mus musculus*	Male	15-20	4	47,8% CHO High sucrose group: 30% sucrose High fat: 50% fat	-

Study	Sex	Weight (g)	Age (weeks)	Diet composition	Daily caloric intake (kcal)

Very High carbohydrate diet					
Bolsinger *et al*., 2013	Male	36-46	4	HC: 70% CHO MC: 40% CHO LC: 10% CHO HC+High Fibre: 70% CHO	30-45
Arimura *et al*., 2018	Male	15-25	5	71% CHO CHO, 24% Protein	25,74-81,5
Parkman *et al*., 2016	-	22,8-34,3	3-4	High sucrose low fat: 70,8% CHO High sucrose high fat: 56,7% CHO	9,6-13,92
Arimura *et al*., 2017	Male	15-25	4	Low protein: 71% CHO High protein: 59% CHO	7,1
Study	**Sex**	**Weight (g)**	**Age (weeks)**	**Diet composition**	**Daily caloric intake (kcal)**
High carbohydrate diet					
Zhuo *et al*., 2018	Male	180-220	8	61% CHO being 20% sucrose	-

Zhou *et al*., 2015	Male	180-200	-	80% CHO being 8% Resistant starch	-
Hedemann *et al*., 2017	Male	-	5	52,95% CHO: Cornstarch, GLU, EMS or resistant starch	-

Study	**Sex**	**Weight (g)**	**Age (weeks)**	**Diet composition**	**Daily caloric intake (kcal)**
Moderate carbohydrate diet					
Noonan & Banks, 2000	Male	15	4	54% CHO, 5% fat 35% CHO (sucrose), 35% fat	62,4-114,8
Iwama *et al*., 2003	Male	230	-	30% CHO (sucrose)	-
Shen *et al*., 2011	Female	80-100	5	30% CHO (Resistant starch)	57,68

Study	**Sex**	**Weight (g)**	**Age (weeks)**	**Diet composition**	**Daily caloric intake (kcal)**
Low carbohydrate diet					
Pascoe *et al.*, 1992	Male	343-359	8	20% CHO (High fat) 69% CHO (Starch)	74
Surwit *et al*., 1995	Male	26,9-47,3	5	25% CHO (High fat): HSHFD, LSHFD 73% CHO (Low fat):	27-32

				LSLFD, HSLFD	
Kaneko *et al*., 2000	Male	470-800	10	80% CHO 60% CHO 40% CHO 20% CHO	-
Wang *et al*., 2003	Male	400	9	60% CHO, 15% fat 10% CHO, 65% fat	60
Petro *et al*., 2004	Male	15	4	73% CHO, 11% fat 26% CHO, 58% fat	7,1-8,7
Asghar *et al*., 2006	Male	10-15	5	12% CHO (sucrose), 58% fat	3,9-6,6
Marsh *et al*., 2009	Male	270-645	6	Control: 69% CHO High fat: 21% CHO Western diet: 45% CHO	-
Sun *et al*., 2018	Male	180-200	8	Resistant starch: 10%, 15% and 20%	-

g = grams; - = missing info; CHO = carbohydrate; HC = high carbohydrate; MC = moderate carbohydrate; LC = low carbohydrate; GLU = glucidex; EMS = enzimatically modified starch; HSHFD = high sucrose high fat diet; LSHFD = low sucrose high fat diet; LSLFD = low sucrose low fat diet; HSLFD = high sucrose low fat diet.

Supplementary material 3 (S3): PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as	4-5

		criteria for eligibility, giving rationale.	
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4-5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4-5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4-5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4-5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	N/A
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	N/A
Section/topic	**#**	**Checklist item**	**Reported**

			on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	N/A
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6-10
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	10
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8-9
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	N/A
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	10
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see	9-10

		Item 16]).	
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	12-14
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	N/A
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	14
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	N/A

Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Preclinical relevance of probiotics in type 2 diabetes: a systematic review

Short title: Probiotics in type 2 diabetic animals

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Key words: probiotic, diabetes mellitus, glycaemia, microbiota, nutrition, animal model.

Abstract

Context: Type 2 diabetes (T2DM) is amongst the most prevalent diseases in the world and may result in several long-term complications. The crosstalk between gut microbiota and host metabolism is closely related to T2DM. Currently, fragmented data hamper defining the relationship between probiotics and T2DM.

Objective: This systematic review aimed at investigating the effects of probiotics on T2DM in animal models

Data sources: We systematically reviewed preclinical evidences using Pubmed/Medline and Scopus, and recovering 24 original articles.

Data extraction: This systematic review was performed according to PRISMA guidelines.We included experimental studies with animal models reporting the effects of probiotics on T2DM. Studies were sorted by characteristics of publications, animal models, performed analyses, probiotic used and interventions.

Results: Probiotics improved T2DM in 96% of the studies. Most studies (96%) used *Lactobacillus* strains and all of them led to improved glycaemia. All studies used rodents as models and male animals were preferred over females.

Conclusion: Results suggest that probiotics have a beneficial effect in T2DM animals and should be used as a supporting alternative in T2DM treatment. Considering a detailed evaluation of reporting and methodological quality, the current preclinical evidence is at high risk of bias, and we hope that our critical analysis will be useful in mitigating the risk of bias in further studies.

Introduction

Diabetes mellitus is currently one of the most prevalent diseases in the world, affecting over 422 million people[1]. Type 2 diabetes (T2DM) accounts for 90% of the cases[1] and it may be caused by impaired insulin secretion by the pancreas, decreased insulin sensitivity in target tissues or a combination of these elements[2]. Consequently, there is a poor metabolic effect of insulin[2] leading to chronic hyperglycaemia, which can result in microvascular complication, particularly in the heart, kidneys and nervous system in the long-term[3]. Thus, its treatment is essential for preventing future complications in patients with T2DM[4]. It has been reported that patients with T2DM may show altered gut microbial composition[5], which suggests that modifying gut microorganisms through probiotic treatment may be a way of regulating glucose metabolism. Although many researchers have suggested the hypoglycaemic potential of probiotics, the underlying mechanisms remain a matter of debate, as T2DM has a complex etiopathology[6].

Several microorganisms, including bacteria, virus, fungi and archaea, reside in the gut and mutually interact with the host. This relationship is influenced by both host's genetics and the intestinal environment, including diet and diseases[7]. Important enteric residents include the genera *Lactobacillus, Bifidobacterium, Clostridium, Bacteroides, Faecalibacterium, Roseburia, Suterella, Akkermansia and Eubacterium*[7]. For general human health, probiotic manufacturers recommend 1 capsule (10 billion CFU) per day of *Lactobacillus rhamnosus* GG. The crosstalk between microbiota and metabolic processes has to be accurately regulated in order to maintain physiological homeostasis[8]. It has been suggested that probiotics contribute to attenuate several elements of metabolic syndrome, in many cases related to T2DM.[8] These improvements may be due to inhibition of potential pathogens by the maintenance of a variety of mucosal immune cells[7], decreased levels of pro-inflammatory cytokines (tumour necrosis factor-α and interleukin-6), delayed glucose absorption in the gut and suppression of rate-controlling enzymes of gluconeogenesis, such as glucose-6-phosphatase (G-6-Pase) and phosphoenol pyruvate carboxykinase (PEPCK)[4,6]. Uncontrolled gluconeogenesis leads to hyperglycaemia in T2DM patients. Therefore, a suppression of G6Pase and PEPCK by probiotic treatment improves glycaemia by decreasing glucose release from the liver to the bloodstream[4].

Another mechanism involved with T2DM and its complications is overproduction of reactive oxygen species (ROS), which can impair insulin sensitivity and directly damage β-cells[10]. Recent studies propose that oral administration of multi-strain probiotics enhances glucose tolerance, increases antioxidant activity and decreases chronic inflammation[10]. A better oxidative stress balance was also observed in diabetic animals treated with *Lactobacillus* strains, particularly in the liver[4].

Considering the extensive variation in methodologies in T2DM studies involving the role of probiotics, it important to compile data from various studies in order to clarify the underlying mechanisms and evaluate current evidence. In this context, this systematic review is a powerful tool to incorporate the variability between the studies and allow a conclusion on the effects of probiotic use in T2DM. Following PRISMA guidelines, we developed this systematic review to evaluate whether a rationale basis exists for probiotic use in T2DM treatment and to investigate the effects of probiotics on T2DM in animal models. This review also evaluates the methodological quality of current evidence, indicating the main sources of bias.

Methods

The systematic review was adhered to the PRISMA guideline (Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols)[11], including search strategy, selection criteria, extraction and data analysis.

Data Sources and research records

To identify relevant articles, we searched electronic databases Pubmed/Medline (https://www.ncbi.nlm.nih.gov/pubmed) and Scopus (https://www.scopus.com/home.uri). The studies considered eligible were identified until September 27th, 2019. The keywords for filters for three criteria were type 2 diabetes mellitus, probiotics and animal studies. Search filters were developed for PubMed by combining standardised descriptors and MeSH (Medical Subject Headings, www.ncbi.nlm.nih.gov/mesh) commands to retrieve indexed studies. In addition, the command TIAB (title and abstract) was applied to identify recently published records still in the indexing process. The logical operators “AND” or “OR” were used to combine the descriptors. To detect *in vivo* preclinical studies in PubMed, a standardised animal filter was applied[12]. We applied a standardised filter in the Scopus platform for animal studies

and the same PubMed search strategy was adapted and applied. The references of eligible studies were checked for additional articles not identified by the electronic search (Fig 1).

Records screening and eligibility

Studies had to attend several criteria to be included in this systematic review, following the PICOS strategy (Table S1 of supplementary material). The initial selection was based on title and abstract. After screening, the duplicate studies and the studies without experimental design were excluded. We considered only experimental studies performed *in vivo*, published in English and with full study text available. Studies *in vitro*, reviews, comments, notes, performed in not diabetic animals or that aimed to assess effects of a medicine, amongst other criteria, were excluded.

We selected only studies that met all of the eligibility criteria listed below:

• Studies with type 2 diabetic animals;

• Studies reporting the effects of probiotic use in T2DM.

The studies selected in this first screening were read in full and assessed for compliance with the established eligibility criteria. Requests to authors were made regarding the unavailable online studies. The selection was restricted to original studies, developed with animal models and published in English. An initial screening of search results was done by one reviewer to exclude clearly irrelevant records. The remaining records were screened by two reviewers independently to identify potentially relevant records meeting the inclusion/exclusion criteria based on title and abstract analysis. Full papers were obtained for these records and were assessed for relevance by two reviewers independently. Any disagreements were resolved by consulting a third reviewer. The kappa test was done to evaluate the agreement between two researchers (kappa=0.917).

Data extraction and studies characteristics

Data extraction was based on methodological features and the studies were synthesised admitting different descriptive levels as it follows: (i) Publication characteristics (authors, year, country of origin), (ii) characteristics of the animal models: species, strain, number of animals, sex, age and weight) (Supplementary material S2), (iii) performed analyses, probiotic used and intervention, type of diabetes induction, time of treatment, primary findings and secondary

findings (Supplementary material S3). In the absence of available data within the study, authors were contacted via e-mail to provide further information. Studies were initially grouped based on type/strain of probiotics used for treatment.

Risk of bias assessment

To assess the risk of bias in the studies included, SYRCLE's Risk of Bias (RoB) tool was used, with this tool being designed specifically for animal studies.[13] The following methodological domains based on RoB were evaluated. Consider selection bias: "Was the allocation sequence adequately generated and applied?", "Were the groups similar at baseline or were they adjusted for confounders in the analysis?", "Was the allocation to the different groups adequately concealed?"; Consider performance bias: "Were the animals randomly housed during the experiment?", "Were the caregivers and/or investigators blinded from knowledge regarding which intervention each animal received during the experiment?"; Consider detection bias: "Were animals selected at random for outcome assessment?", "Was the outcome assessor blinded?"; Consider attrition bias: "Were incomplete outcome data adequately addressed?"; Considers reporting bias: "Are reports of the study free of selective outcome reporting?"; Consider other biases: "Was the study apparently free of other problems that could result in high risk of bias?"; The overall study quality indicators: "Was it stated that the experiment was randomized at any level?" and "Was it stated that the experiment was blinded at any level?". The items in the RoB tool were scored with "yes" (low risk of bias); "no" (high risk of bias); or "unclear" (indicating that the item was not reported, and therefore, the risk of bias was unknown). Reporting quality was evaluated by complete screening of all manuscript sections (abstract to acknowledgements and funding) to evaluate the completeness of the scientific report.[13] The analysis of the individual studies and the relation with risk of bias and year of publication was done considering methodological items reported in Animal Research: Reporting of In Vivo Experiments guidelines[14].

Results

Characteristics of publications

The PRISMA diagram illustrates the selection process of the studies (Fig. 1). A total of 919 records were retrieved, with 250 from PubMed/Medline and 669 from Scopus. Out of these, 683 remained after removing duplicates. Following assessment based on title and abstract, 98 records were considered to be potentially included. Inclusion criteria were met by 21 articles. The bibliographical references of the 21 selected articles were manually analysed and 3 studies were added in accordance to the inclusion criteria, which brought us to 24 studies. The filters applied in each database and the flowchart, indicating the search structure, are shown in supplementary Table S4. Most studies originated from China (n=15, 63%), followed by Japan (n=2, 8%), Taiwan (n=2, 8%) and India (n=2, 8%). Other included studies were from South Korea (n=1, 4%), Iran (n=1, 4%) and Malaysia (n=1, 4%).

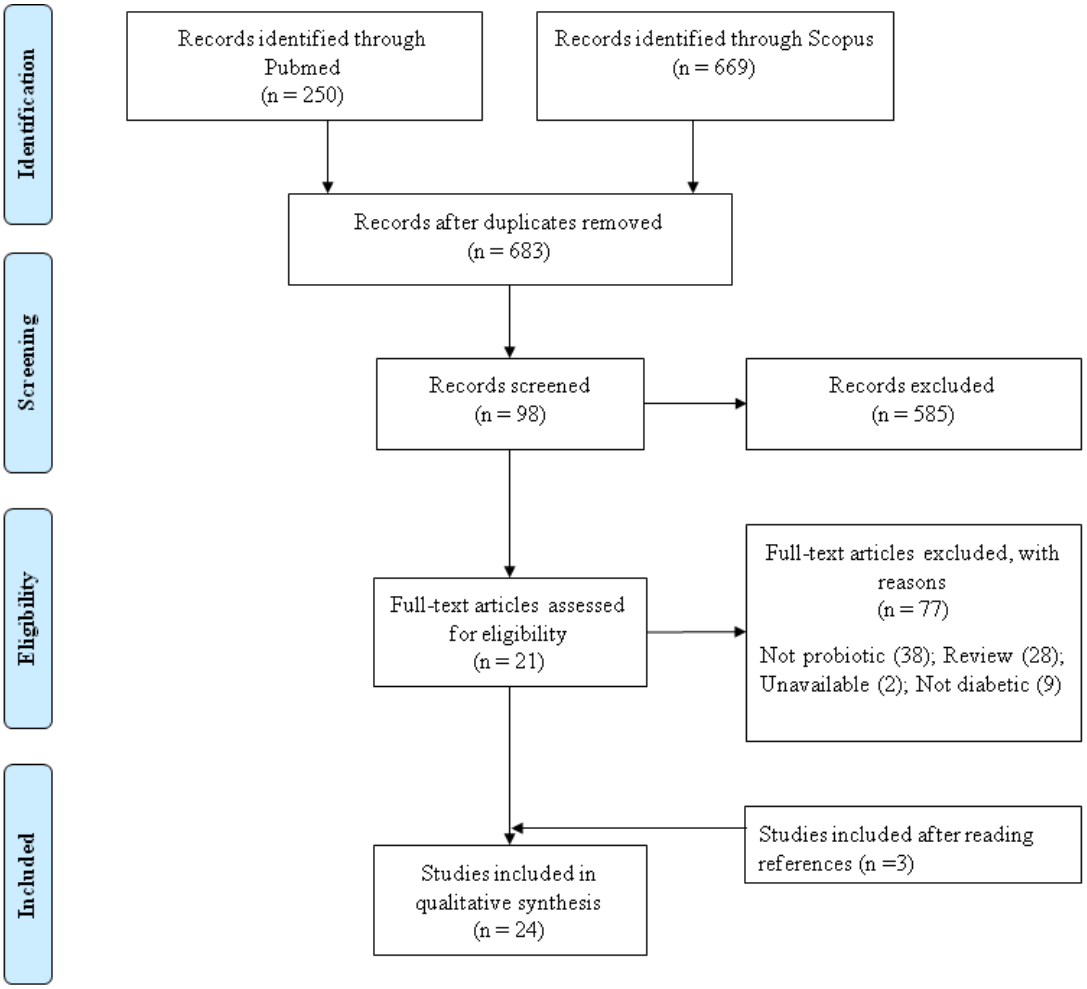

Fig. 1. PRISMA diagram. Different phases of selection of studies for conducting qualitative and quantitative analyses. Flow diagram of the systematic review literature search results. Based on 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement'. http://www.prisma-statement.org. From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009)[11].

Experimental animals and intervention characteristics

As shown in supplementary Table S2, rats (*Rattus norvegicus*) were the main animal model used (54%, n=13) and 46% (n=11) used mice (*Mus musculus*), being 87.5% (n=21) of the animals males and 12.5% (n=3) did not specify animal models' sex. There were no studies reporting the use of female animal models. In regards to mice lineages, 33% of the studies used C57BL⁄6J (n=8), 4% of the studies used C57BL⁄KS⁄J and KK-Ay mice (n=1). Amongst rat lineages, most studies preferred Wistar rats (29%, n=7), followed by Sprague-Dawley (16%,

n=4) and GK rats (4%, n=1). All animals were aged from 3 to 8 weeks. Mice weighted from 16 to 40 grams, rats weighted from 120 to 230 grams and 10 studies didn't report experimental animals' body weight. The number of experimental animals ranged from 18 to 105 and 14% of the studies (n=3) didn't provide this information.

The duration of probiotic treatment ranged from 3 to 18 weeks. *Lactobacillus* strains were investigated by 92% (n=22)[4, 6, 10, 14-27] of the studies, from which 21% (n=5) were in combination with other probiotics from the genera: *Bifidobacterium* (n=1)[28], *Pediococcus* and *Lactococcus* (n=1) [29], *Bacillus* (n=1)[30], *Rhizopus* (n=1)[31] and yeasts (n=1)[34]. Other reported probiotics were *Akkermansia muciniphila* (n=1)[33] and *Clostridium butyricum* (n=1)[34]. Oral administration of probiotics was used by 46% of the studies (n=11)[4, 6, 14-20, 24, 25] whereas 29% (n=7)[21, 23, 26-28, 33, 34] used oral by gavage and the remaining 25% used probiotics in foods (n=6). The foods used for probiotic administration were Shubat (n=1)[31], Tempeh (n=1)[30], fermented paste Xenji™ (n=1)[29], fermented juice (n=2)[19, 25] and fermented milk (n=1)[10]. The administered dose ranged from 10 to 10^{10} colony forming units (CFU) per mL regardless of the probiotic, although 3 studies[6, 25, 28] did not report the CFU per mL given as treatment for animals with T2DM. The time of probiotic treatment in the included studies ranged from 4 to 18 weeks.

Although it was not an eligibility criterion, all studies included here reported at least one of the following parameters for glycaemic control: fasting blood glucose (FBG), postprandial blood glucose (ppBG), glycated haemoglobin A1c (HbA1c) or oral glucose tolerance tests (oGTT).

Main Findings

Preclinical studies demonstrated that probiotic intervention present beneficial effects for T2DM treatment, as 96% (n=23) of the studies reported lower glycaemia. A dose-dependent benefit was reported in 13% (n=3) of the studies[4, 14, 16]. The remaining 4% (n=1) showed no difference in T2DM treatment. All studies that used *Lactobacillus* strains alone or associated with other microorganisms as probiotics (n=22.92%) resulted in better T2DM parameters after treatment. Underlying mechanisms involved increased glucose tolerance (n=16.67%), improved

inflammatory status (n=10.42%), increased antioxidant activity (n=12.50%), delayed gastric absorption of glucose (n=1.4%) and decreased gluconeogenesis (n=3.13%). The effects of different probiotics on T2DM parameters in animal models are summarised in Table 1.

Table 1. Summary of the effects of different probiotics on main parameters of T2DM in animal models. Data stratified by study are detailed in Table S2 of Supplementary material.

Probiotic	**Effect**	**Measure outcomes**
Lactobacillus sp. (n=17)[4, 6, 10, 14-27]	↓glycaemia (n=17)	Fasting BG ppBG HbA1c oGTT
Bifidobacterium bifidum *Lactobacillus casei* (n=1)[28]	↓glycaemia ↑glucose tolerance (n=1)	Fasting BG HbA1c oGTT
Lactobacillus sp. (n=11)[4, 10, 14, 16, 19, 20-25]	↑glucose tolerance (n=11)	oGTT
Lactobacillus sp. *Leuconostoc mesenteroides* *Pediococcus acidilactici* *Pediococcus pentosaceus* (n=1)[29]	↑glucose tolerance ↓antioxidant activity ↓cytokine production (n=1)	oGTT SOD, CAT Fasting BG PCR SOD, CAT
Lactobacillus sp. *Bacillus subtilis* (n=1)[30]	↓glucose tolerance ↓glycaemia (n=1)	oGTT HbA1c Fasting BG
Lactobacillus plantarum	↓glucose tolerance ↓glycaemia	oGTT HbA1c

Rhizopus oligosporus (n=1)[31]	(n=1)	Fasting BG
Lactobacillus sp. yeasts (*Kluyveromyces marxianus, Pichia membranifaciens, Candida ethanolica, Issatchenkia orientalis*) (n=1)[32]	↓glycaemia (n=1)	Fasting BG HbA1c
Akkermansia muciniphila (n=1)[33]	No difference in glycaemia ↓cytokine production ↑antioxidant activity (n=1)	Fasting BG Fasting serum levels of tumour necrosis factor-α, interleukin-6 SOD, GSH
Clostridium butyricum (n=1)[34]	↓glycaemia ↑glucose tolerance ↓cytokine production ↑antioxidant activity ↓expression of G6P genes (n=1)	Fasting BG HbA1c oGTT Fasting serum levels of tumor necrosis factor-α, interleukin-6 SOD, CAT, GSH PCR
Lactobacillus sp. (n=7)[10, 16, 19-21, 23, 25]	↓cytokine production ↑antioxidant activity (n=7)	Fasting serum levels of tumor necrosis factor-α, interleukin-6 SOD, GSH
Lactobacillus sp. (n=2)[4, 26]	↓expression of gluconeogenic genes (n=2)	PCR

Lactobacillus sp. (n=2)[24, 27]	↑antioxidant activity (n=2)	SOD, CAT
Lactobacillus sp. (n=1)[6]	↓absorption of glucose (n=1)	oGTT

Abbreviations: BG, blood glucose; ppBG, postprandial blood glucose; HbA1c, glycated haemoglobin A1c; oGTT, oral glucose tolerance test; SOD, superoxide dismutase; CAT, catalase.

Regarding secondary outcomes, 16% (n=4) of studies reported an increase in body weight, whereas 8% (n=2) reported weight loss with probiotic treatment. Plasma insulin was increased in 16% (n=4) of the included studies and it was decreased in 16% (n=4) of the studies. Food intake was decreased in 4% (n=1) of the studies. Further details are found in Table S3 of supplementary material.

Risk of Bias Methodological Quality Assessment

Quality assessment of the studies is summarised in Fig. 2. Quality assessment reported at an individual level is shown in Fig.3. No studies fulfilled all methodological criteria analysed. In relation to selection bias, the sequence generation process was not fully reported in 92% (n=22) studies (Q1). In terms of animals' characteristics, that is, their similarity to one another (Q2), 67% of the studies (n=16) did not reported this information clearly. Information about the allocation concealment (Q3) was not reported by 50% of the studies (n=12). None of the articles reported on random housing and blinding of caregivers. Only 13% of the studies (n=3) reported random animal housing (Q4). None of the studies reported blinding of personnel (Q5). Random selection for outcome assessment (Q6) was applied on 13% (n=3) of the studies. None of the studies reported blinding of outcome assessment (Q7). Incomplete outcome data (Q8) was addressed in 71% (n=17) of the studies. Selective reporting (Q9) was absent in 42% (n=10) of the studies. An important source of bias was the lack of information about experimental

models such as number of animals used, their sex and/or weight (Q10). Overall quality indicators, such as randomisation (Q11) or blindness (Q12) at any level were applied in 63% (n=15) and 8% (n=2) of the studies, respectively. None of the studies fulfilled all methodological criteria, and the mean quality score of all studies reviewed was 72.887±3.623. Seven studies (29%) did not reach the mean score (Fig. 3). Considering individually each criterion analysed, none of the studies reported information such as experimental blindness, a rational basis for the number of animals and details of the sample size calculation. The analysis of the individual studies found no relation between risk of bias and year of publication (Fig. 4).

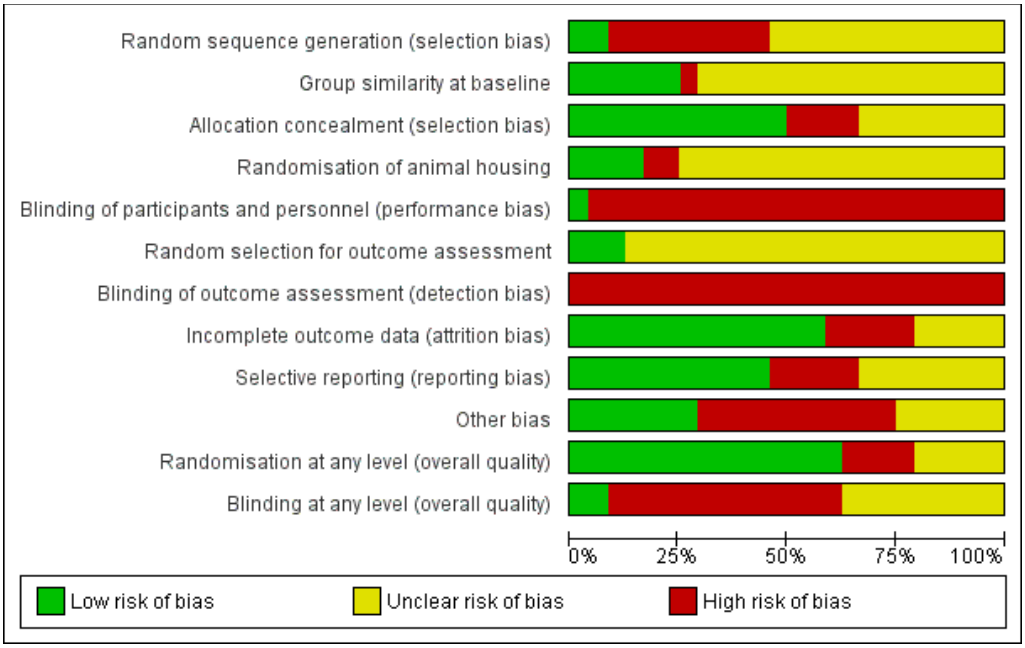

Fig. 2. Results of the risk of bias and methodological quality indicators for all included studies in this systematic review that evaluated the effect of probiotic treatment in T2DM. The items in the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) Risk of Bias assessment was scored with "yes" indicating low risk of bias, "no" indicating high risk of bias, or "unclear" indicating that the item was not reported, resulting in an unknown risk of bias. Consider selection bias: was the allocation sequence adequately generated and applied? Were the groups similar at baseline or were they adjusted for confounders in the analysis? Was the allocation to the different groups adequately concealed? Consider performance bias: were the animals randomly housed during the experiment?; Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment? Were animals selected at random for outcome assessment? Consider detection bias: was the outcome assessor blinded? Consider attrition bias: were incomplete outcome data adequately addressed? Consider reporting bias: are reports of the study free of selective outcome reporting? Consider other biases: was the study apparently free of other problems that could result in high risk of

bias? Consider overall quality: was it stated that the experiment was randomized at any level? Was it stated that the experiment was blinded at any level?

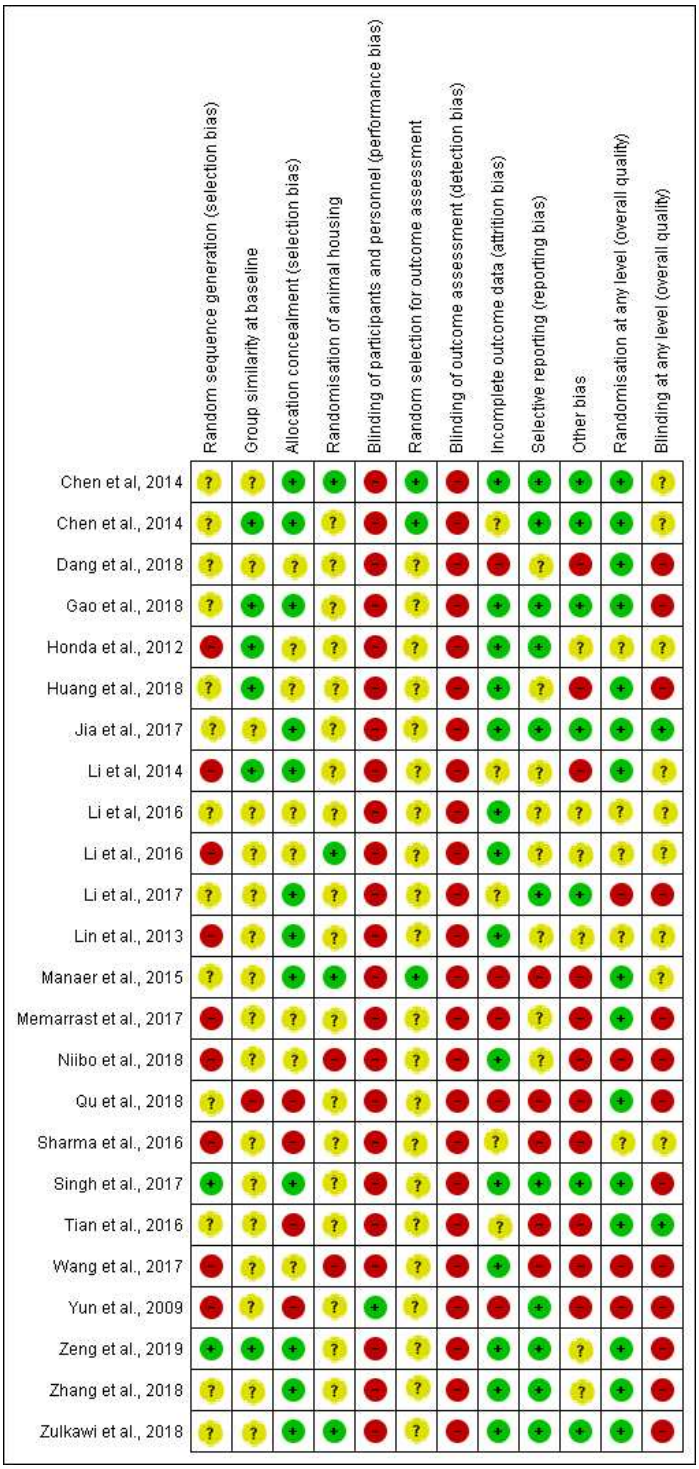

Fig.3. Risk of bias summary shows studies' quality assessment at an individual level.

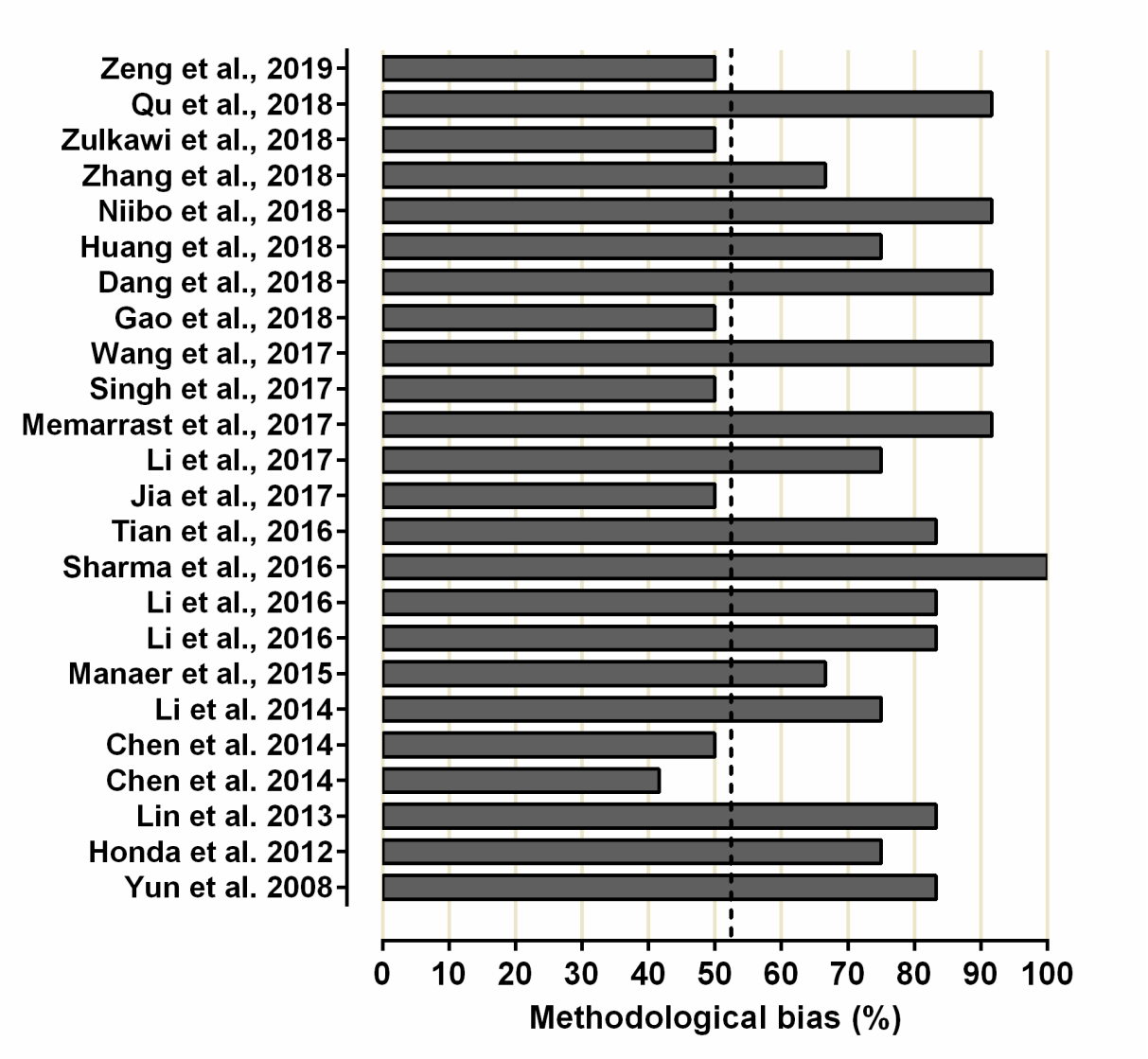

Fig. 4. Analysis of methodological bias (reporting quality) stratified by domains for each study included in the review. The dotted line indicated the mean quality score (%).

Discussion

Preclinical evidences of the benefits of probiotics use in T2DM are of great relevance considering the high prevalence of T2DM and the possibility of a new alternative therapy to improve life quality. In addition, as probiotics are abundantly available and affordable, they may represent an interesting supporting alternative for T2DM treatment. Despite the great heterogeneity of the studies included in this review, results suggest beneficial effects of probiotic use in T2DM, mainly related to increased glucose tolerance, delayed gastric absorption of glucose, improved systemic inflammation, all due to increased SCFAs (short-chain fatty acids) production. Other effects are related to increased antioxidant activity and decreased hepatic gluconeogenesis.

In regars to the origin of the included studies, all of them were from Asian countries, most of them from China. These data were already expected since as the use of probiotics for fermentation is a widespread tradition with a big socio-economic importance in Asia[44]. In China, it has been documented by several historians as far back as 4000 BC[44]. Indeed, a typical diet of the Eastern World consists of boiled rice with many side dishes containing fermented foods, such as kimchi, soy sauce and miso[44]. The use of fermented foods in Asian countries has been related to the relevance of probiotics in alleviating lactose intolerance for Asian people compared to lactose-tolerant European people. Furthermore, the addition of *Lactobacillus* strains in foods is related to improved absorption of vitamins B2, B11 and B12, which is particularly relevant in developing Asian countries and ageing population[45].

Besides the origin of the included studies, our results show that all experimental animals were murine models. This could be due to their low cost, and easy handling and accessibility, allowing researchers to use a relatively large number of animals for their experiments, thereby generating a greater degree of evidence and reliability in the results. In addition, none of the studies reported the use of female animals. Single sex studies of males still predominate in the biological literature, and neglect of females is widespread in many disciplines. The preference for male animal models could be due to the fact that female murine models need more anti-inflammatory bacteria to maintain intestinal health[30], which could increase the amount of probiotics required or increase the time of probiotic treatment. These sex-related differences may underlie dissimilarities in the epidemiology, aetiology, and prognosis of diseases. Given these fundamental differences, it seems essential to investigate probiotic treatment in both

males and females. In addition, animals in weaning age were more frequently used compared to older ones, which may be due to better adaptation to treatments[46].

Regarding the main results of this review, our findings suggest that SCFAs production is responsible for several mechanisms that are reported to reduce blood glucose levels and improve overall health status.[32] SCFAs, such as butyrate, propionate and acetate, are metabolites produced by the gut microbiota as a result of microbial fermentation of oligo- or polysaccharides. SCFAs interact with host metabolism improving glucose tolerance due to increased glucagon-like peptide-1 (GLP-1) levels and peptide YY (PYY).[35] This is in accordance with our results as 67% (n=16) of the studies showed improved glucose tolerance after probiotic treatment. In addition, enhanced GLP-1 and PYY lead to increased satiety[26, 36], although decreased food intake was reported in 5% (n=1) of the studies[14]. Furthermore, the increased secretion of GLP-1 induced by SCFAs leads to delayed absorption of glucose, which results in better glycaemia[32,37]. This is consistent with the results reported in the included studies, as 4% showed slower glucose absorption and lower postprandial blood glucose levels. After a meal, GLP-1 is secreted and acts attenuating gastric emptying, modulating insulin production and reducing glucagon secretion.[38] Therefore, enhanced GLP-1 secretion due to probiotic treatment appears to benefit individuals with T2DM[38].

In addition to these beneficial effects, the SCFAs produced by the gut microbiota also boost the innate immune response against pathogens[9], inducing the activation, migration, proliferation, differentiation and maintenance of a variety of immune cells, as shown by 8% of the studies recovered in this review (n=2). The SCFA butyrate induces regulatory T cells through the activation of G protein-coupled receptors. Another SCFA, propionate, increases colonic regulatory T cells numbers by signalling via Ffar2. SCFAs also exert a direct bacteriostatic effect towards pathogens through the production of bacteriocins[39]. Moreover, microbial groups produce vitamins such as B2, B11 and B12[40], which have been reported as deficient in T2DM individuals[38], thus improving nutrient availability and overall health[41].

Another mechanism related to SCFAs production improving T2DM management is decreased inflammation. The onset and progression of metabolic diseases is closely related to increased inflammatory cytokines, which are mainly induced by the binding of lipopolysaccharides (LPS) and Tumour Necrosis Factor α (TNF-α) to the surface of innate immune cells[41-43]. SCFAs decrease cytokine production by preventing its gene expression[42], hence alleviating the chronic inflammation of T2DM[23], as reported in our results.[10, 16, 19-21, 23-25, 27, 29, 33, 34]

In addition to increased inflammation[41], T2DM patients often show high levels of oxidative stress indicators as a result of increased generation of free radicals, which may impair metabolic condition in T2DM[31]. Although the exact mechanisms remain unclear, improvements in antioxidant stress level through probiotic treatment may also indirectly affect insulin level and glucose homoeostasis[5, 27], as reported by 50% of the studies included in this review that showed increased antioxidant activity, mainly through increased SOD and CAT.[10, 16, 19-21, 23-25, 27, 29, 33, 34]

Finally, glucose homoestasis is directly influenced by gluconeogenesis, as an uncontrolled synthesis of hepatic glycogen may result in hyperglycaemia[4]. Rate-controlling enzymes of gluconeogenesis PEPCK and G-6-Pase were suppressed after probiotic use[4], which is consistent with the simultaneous increase in hepatic glycogen[4]. This is in accordance with our findings as 13% of the studies reported a downregulation of gluconeogenic genes and reduced blood glucose levels as a consequence.

The secondary results of this review, such as plasma insulin and body weight, varied widely.We suggest that the specific rodent model (age of the animals and the mode by which metabolic disorders are induced), the time of treatment and choice of probiotic could have a major impact on the outcomes of the included studies[43]. Both young and adult animals were used as experimental animals, even though animals have different nutritional requirements throughout their lives[43]. Furthermore, the time of treatment varied widely among studies (from 4-18 weeks), although some metabolic changes may take longer to emerge[43], which could have influenced the results. In addition, each individual have its own microbial population[43] and therefore, they may respond differently to the administered probiotics.

Main findings are summarised in Figure 4, that demonstrates the effects of probiotic use in different systems. In the liver, it shows decreased gluconeogenesis due to a decrease in the enzymes PEPCK and G-6-Pase. In the intestine, the figure illustrates the increase in microorganism number leading to increased vitamin production. Also in the intestine, it shows an increase in SCFAs production, resulting in decreased absorption of glucose, increased glucose tolerance, increased satiety, decreased inflammatory cytokines and a boost in the immune system. Finally, it illustrates an increase in antioxidant capacity, specially the enzymes catalase and superoxide dismutase, triggered by the use of probiotics.

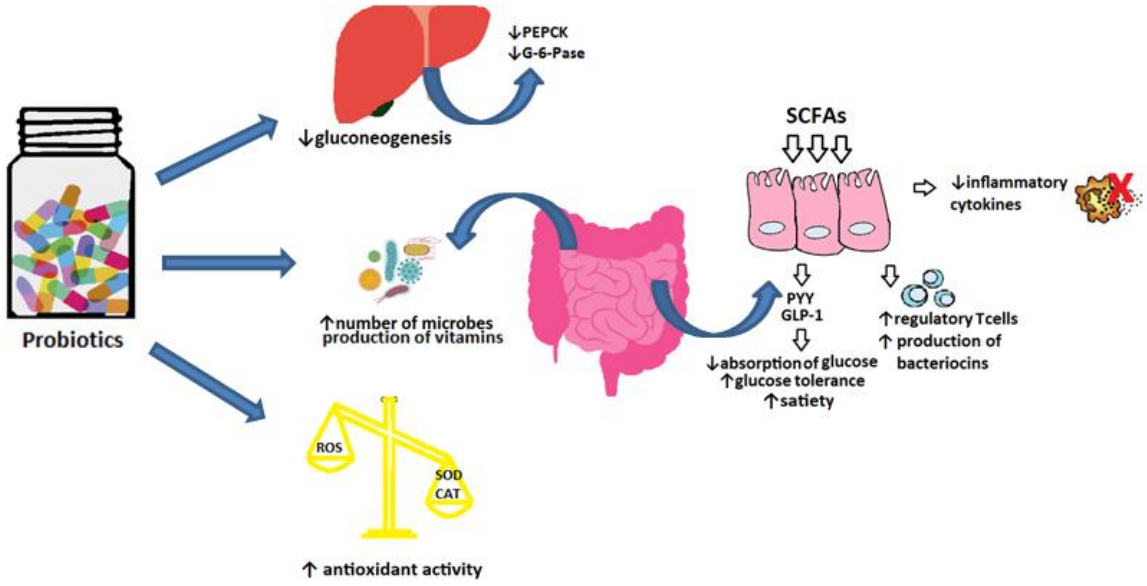

Fig. 4. Summary of the main findings on the effects of probiotic use in animals with T2DM. *Abbreviations:* PEPCK, phosphoenolpyruvate carboxykinase; G-6-Pase, glucose 6-phosphatase; SCFAs, short-chain fatty acids; PYY, peptide YY; GLP-1, glucagon-like peptide-1; ROS, reactive oxygen species; SOD, superoxide dismutase; CAT, catalase.

Methodological consistency is crucial for evidence reliability in clinical and preclinical studies[47]. Blindness of personnel and blinding of outcome assessment was not reported in the included studies, which impairs methodological quality in these studies. Other relevant sources of bias are under-reporting randomisation methods in the selected studies, data about experimental models such as age/weight/sex, number of animals used and the dose of probiotics used. Further, details in regards of group similarity at baseline were also under-reported. Neglecting these methodological aspects lead to bias and compromise the quality of evidence[47]. There are a number of guidelines on experimental design available in order to improve research quality and reliability, such as Approach Collaborative for Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES; www.camarades.info) and the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE; www.SYRCLE.nl). Following these guidelines improves research transparency and reproducibility, resulting in studies with a low risk of bias. In our review, we did not observe a direct relationship between the high risk of methodological bias and the year of publication of the studies, therefore there has been a systematic reproduction of methodological errors over the years. These findings show the need to enhance methodological quality of probiotic research

in order to reduce biases. Therefore, we expect that this review can be used as a guide to improve reporting for future research regarding probiotic treatment in T2DM.

Limitations

Systematic reviews are regarded as high level studies that allow for the individual evaluation of studies in a blind manner using specific tools [13]. Such features result in an inclusive and reliable approach that provides a broad understanding of the included studies. However, discrepancies between the included studies in regards to methods became clear considering the variety in charachteristics of animal models such as age, weight, total number of animals. Additionally, the variety in probiotic dose is a possible caveat that may hinder specific conclusions. Our risk of bias and methodological quality assessment showed that many studies fail in reporting their complete methodology, resulting in a high risk of bias. We did not find a direct relationship between the high risk of methodological bias and the year of publication of the studies, i. e., there has possibly been a systematic reproduction of methodological errors over the years, since the quality of the reports has not improved. For these reasons, we expect that this review may be used as a guide to improve reporting for future research in probiotic supplementation in T2DM.

Conclusion

Nevertheless, there are multiple studies suggesting that probiotic treatment has an effect in reducing blood glucose levels and improving different metabolic parameters that affect glycaemia. Some potential mechanisms underlying these effects are improved glucose tolerance, better inflammatory status, decreased oxidative stress, delayed gastric absorption of glucose and decreased hepatic glycogen synthesis. However, current evidence is at a high risk of bias. Although improving research methods is needed for future studies, probiotic treatment represents a potential supporting alternative for T2DM management.

Acknowledgements and Funding

The authors are grateful to the support provided by Fundação do Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, processes APQ-01895-16, PPM-00687-17 and PPM-00077-18), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, processes 303972/2017-3, 423594/2018-4, 305093/2017-7 and MCTIC 408503/2018-1), and

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES, finance code 001).

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Supplementary material 1 (S1): Table S1. PICOS criteria for inclusion and exclusion of the studies that evaluated probiotic use in type 2 diabetes

Parameter	**Definition**
Population	Type 2 diabetic animals (female and male).
Interventions	Use of probiotics as a treatment for type 2 diabetes
Comparison	PBS; rosiglitazone; pioglitazone; saline solution; standard diet for rodents with no supplementation.
Outcomes	Reduction of fasting blood glucose, postprandial blood glucose, glycated haemoglobyn A1c and improved glucose tolerance (oral glucose tolerance test).
Study design	Experimental studies were included. *In vitro* studies, reviews, consensus, letters to editor, theses and dissertations were excluded.

Supplementary material 2 (S2): Table of preclinical experimental models.
Table S2. General characteristics of preclinical experimental models used in all studies included in the systematic review.

Study	Species	Lineage	Sex	Weight (g)	Age (weeks)	Number of animals
Yun *et al*., 2009	*Mus musculus*	C57BL⁄KS⁄J db/db	Male	35-40	6	8 control 40 exp
Honda *et al*., 2012	*Mus musculus*	KK-A^{y}	Male	28	8	6 control 12 exp
Lin *et al.*, 2013	*Rattus norvegicus*	Sprague-Dawley	Male	346	-	6 control 18 exp
Chen *et al*., 2014	*Mus musculus*	C57BL/6J	Male	16-19	4	8 control 24 exp
Chen *et al*., 2014	*Mus musculus*	C57BL/6J	Male	-	3	8 control 24 exp
Li *et al*., 2014	*Rattus norvegicus*	Wistar	Male	120-150	-	10 control 30 exp
Manaer *et al*., 2015	*Rattus norvegicus*	Wistar	Male	160-200	-	10 control 38 exp
Li *et al*., 2016	*Mus musculus*	C57BL/6J	Male	-	3	8 control 42 exp
Li *et al*., 2016	*Mus musculus*	C57BL/6J	Male	-	3	8 control 16 exp
Sharma *et al*., 2016	*Rattus norvegicus*	Wistar	Male	150-200	-	12 control 18 exp
Tian *et al*., 2016	*Rattus norvegicus*	Wistar	Male	160-200	-	8 control 40 exp
Study	**Species**	**Lineage**	**Sex**	**Weight (g)**	**Age (weeks)**	**Number of animals**
Jia *et al*., 2017	*Mus musculus*	C57B1/6J	Male	-	3	8 control 32 exp
Li *et al*., 2017	*Mus musculus*	C57B1/6J	Male	-	3	8 control 24 exp
Memarrast *et al.*, 2017	*Rattus norvegicus*	Wistar	Male	200	-	18 control 30 exp
Singh *et al*., 2017	*Rattus norvegicus*	-	-	230	-	-
Wang *et al*., 2017	*Mus musculus*	C57B1/6J	Male	-	3	8 control 40 exp

Gao *et al*., 2018	*Rattus norvegicus*	Wistar	Male	160-200	3	30 control 50 exp
Dang *et al*., 2018	*Mus musculus*	C57B1/6J	Male	-	3	8 control 32 exp
Huang *et al*., 2018	*Rattus norvegicus*	Sprague-Dawley	Male	-	8	24 control 24 exp
Niibo *et al*., 2018	*Rattus norvegicus*	GK	Male	-	4	8 control 8 exp
Zhang *et al.*, 2018	*Rattus norvegicus*	Sprague-Dawley	Male	160-180	6-8	30 control 75 exp
Zulkawi *et al.*, 2018	*Mus musculus*	-	Male	80-100	6	-
Qu *et al*., 2018	*Rattus norvegicus*	Wistar	Male	160-200	-	8 control 32 exp
Zeng *et al*., 2019	*Rattus norvegicus*	Sprague-Dawley	Male	-	-	6 control 12 exp

Abbreviations: g, grams; exp, experimental.

Supplementary material 3 (S3): Table of interventions and results.
Table S3. General characteristics of the interventions and results of all studies investigating the effect of probiotics in type 2 diabetes in animal models.

Study	Probiotic	Intervention	Main results
Yun *et al*., 2009	*Lactobacillus gasseri* BNR1	Treatment with 10^7, 10^8, 10^9 or 10^{10} CFU orally twice a day	Improved FBG and ppBG in 10^{10} CFU and a dose-dependent improvement in glucose tolerance
Honda *et al*., 2012	*Lactobacillus rhamnosus* GG	Diet containing 2% viable GG cells or 2% heat-treated GG cells	Improved FBG in the viable GG group
Lin *et al.*, 2013	*Lactobacillus reuteri* GMN-32	Groups orally treated with 10^7 CFU/mL or 10^9 CFU/mL	Both treatments resulted in improved FBG
Chen *et al*., 2014	*Lactobacillus rhamnosus* CCFM0528	Groups orally treated with 10^9 CFU per day	Improved FBG and ppBG in the 10^{10} CFU group Dose-dependent improvement in glucose tolerance
Chen *et al*., 2014	*Lactobacillus casei* CCFM0412	Groups orally treated with 10^9 CFU per day	Improved FBG
Li *et al*., 2014	*Lactobacillus* plantarum NCU116	Groups treated with 10^9 CFU/mL per day in a juice	Improved FBG in all groups
Study	**Probiotic**	**Intervention**	**Main results**
Manaer *et al*., 2015	*Lactobacillus plantarum, Lactobacillus helveticus, Lactococcus lactis, Lactobacillus harbinensis, Lactobacillus hilgardii, Lactobacillus*	Shubat containing 6.97×10^6 lactic acid bacteria+2.20×10^4 yeasts CFU/mL, or 6.97×10^7 lactic acid bacteria+2.20×10^5 yeasts CFU/mL or 6.97×10^8 lactic	Improved BG in all groups

	rhamnosus, Lactobacillus mucosae, Lactobacillus par, Lactobacillus paracasei subsp.tolerans, Lactobacillus pentosus) and four yeasts (*Kluyveromyces marxianus, Pichia membranifaciens, Candida ethanolica, Issatchenkia orientalis)*	acid bacteria+2.20×10 ^{6} yeasts CFU/mL.	

Study	**Probiotic**	**Intervention**	**Main results**
Li *et al*., 2016	*Lactobacillus casei* CCFM419, *Lactobacillus plantarum* X1, *Lactobacillus rhamnosus* Y37, *Lactobacillus brevis* CCFM648, and *Lactobacillus plantarum* CCFM36	Groups orally treated with 8 × 10^{9} CFU/mL live bacteria 10 mg/kg body weight once a day	Improved FBG and glucose tolerance
Li *et al*., 2016	*Lactobacillus plantarum* CCFM0236	Groups orally treated with 9 × 10^{9} CFU/mL 0.25 mL/day	Improved FBG, ppBG and glucose tolerance
Sharma *et al*., 2016	Lactobacillus *casei* and *Bifidobacterium bifidum*	1 × 10 CFU/mL of *L. casei* and *B. bifidum* by gavaging	Improved FBG
Tian *et al*., 2016	*Lactobacillus. paracasei* subp *paracasei* and *Lactobacillus casei*	4×10^{9} CFU/mL by gavaging	Improved FBG and glucose tolerance

Jia *et al*., 2017	*Clostridium butyricum* CGMCC0313.1	2.5×10^8 CFU/kg per day by gavaging	Improved FBG, glucose tolerance and HbA1c
Li *et al*., 2017	*Lactobacillus casei* CCFM419	Groups orally treated with 8×10^{10} CFU/mL 0.25 mL/day	Improved FBG, ppBG and glucose tolerance

Study	**Probiotic**	**Intervention**	**Main results**
Memarrast *et al.*, 2017	*Lactobacillus reuteri* GMNL-263 *Lactobacillus crispatus* SJ3CUS and *Bacillus subtilis*	10^{10} CFU/mL by gavaging once daily	Improved FBG and glucose tolerance
Singh *et al*., 2017	*Lactobacillus rhamnosus* NCDC 17 and *Lactobacillus rhamnosus*	$10^{9.5\text{-}10}$ CFU/mL and $10^{8\text{-}8.5}$ CFU/mL in fermented milk	Improved FBG, HbA1c and glucose tolerance
Wang *et al*., 2017	*Lactobacillus casei* CCFM419	10^9 CFU by gavaging	Improved FBG, ppBG and glucose tolerance
Gao *et al*., 2018	*Lactobacillus plantarum* NCU116	10 mL/kg body weight of juice fermented with 0.01% (w/w) *L. Plantarum*	Improved FBG
Dang *et al*., 2018	*Lactobacillus paracasei* TD062	Groups orally treated with 10^9, 10^8 and 10^7 CFU/mL	Improved FBG, ppBG and a dose-dependent improvement in glucose tolerance
Huang *et al*., 2018	*Lactobacillus plantarum* and *Rhizopus oligosporus*	40 mg/kg body weight/day of tempeh with $10^{8.44}$ CFU/g of probiotics	Improved HbA1c and glucose tolerance

Study	**Probiotic**	**Intervention**	**Main results**
Niibo *et al*., 2018	*Lactobacillus gasseri* SBT2055	Groups orally treated with 6×10^7 CFU/g	Improved glucose tolerance ND ppBG
Zhang *et al.*, 2018	*Akkermansia muciniphila*	5×10^6 CFU/0.5 ml)	ND FBG

		and 5×10^8 CFU/0.5 mL by gavaging	
Zulkawi *et al.*, 2018	*Lactobacillus brevis, Lactobacillus casei, Lactobacillus curvatus, Lactobacillus paracasei, Lactobacillus pentosus, Lactobacillus plantarum, Lactococcus lactis, Leuconostoc mesenteroides, Pediococcus acidilactici* and *Pediococcus pentosaceus*	1 g/kg body weight of fermented food paste Xeniji™	Improved FBG and glucose tolerance

Study	**Probiotic**	**Intervention**	**Main results**
Qu *et al.*, 2018	*Lactobacillus casei* Q14	Treatment 2.3×10^9 CFU/mL in the diet	Improved glucose tolerance
Zeng *et al*., 2019	*Lactobacillus paracasei* NL41	Treatment with 10^{10} CFU/mL by gavaging	Improved HbA1c and glucose tolerance

Abbreviations: CFU, colony forming units; FBG, fasting blood glucose; ppBG, postprandial blood glucose; ND, no difference; mL, mililitre; kg, kilograms; -, no data; HbA1c, glycated haemoglobin a1c; g, grams.

Table S4. Complete search filters used in databases PubMed-Medline and Scopus.

Descriptors PubMed	*Items Found*	*Date/Time*
#1 Animal		
("animal experimentation"[MeSH Terms] OR "models, animal"[MeSH Terms] OR "invertebrates"[MeSH Terms] OR "Animals"[Mesh:noexp] OR "animal population groups"[MeSH Terms] OR "chordata"[MeSH Terms:noexp] OR "chordata, nonvertebrate"[MeSH Terms] OR "vertebrates"[MeSH Terms:noexp] OR "amphibians"[MeSH Terms] OR "birds"[MeSH Terms] OR "fishes"[MeSH Terms] OR "reptiles"[MeSH Terms] OR "mammals"[MeSH Terms:noexp] OR "primates"[MeSH Terms:noexp] OR "artiodactyla"[MeSH Terms] OR "carnivora"[MeSH Terms] OR "cetacea"[MeSH Terms] OR "chiroptera"[MeSH Terms] OR "elephants"[MeSH Terms] OR "hyraxes"[MeSH Terms] OR "insectivora"[MeSH Terms] OR "lagomorpha"[MeSH Terms] OR "marsupialia"[MeSH Terms] OR "monotremata"[MeSH Terms] OR "perissodactyla"[MeSH Terms] OR "rodentia"[MeSH Terms] OR "scandentia"[MeSH Terms] OR "sirenia"[MeSH Terms] OR "xenarthra"[MeSH Terms] OR "haplorhini"[MeSH Terms:noexp] OR "strepsirhini"[MeSH Terms] OR "platyrrhini"[MeSH Terms] OR "tarsii"[MeSH Terms] OR "catarrhini"[MeSH Terms:noexp] OR "cercopithecidae"[MeSH Terms] OR "hylobatidae"[MeSH Terms] OR "hominidae"[MeSH Terms:noexp] OR "gorilla gorilla"[MeSH Terms] OR "pan paniscus"[MeSH Terms] OR "pan troglodytes"[MeSH Terms] OR "pongo pygmaeus"[MeSH Terms]))) OR (((animals[tiab] OR animal[tiab] OR mice[Tiab] OR mus[Tiab] OR mouse[Tiab] OR murine[Tiab] OR woodmouse[tiab] OR rats[Tiab] OR rat[Tiab] OR murinae[Tiab] OR muridae[Tiab] OR cottonrat[tiab] OR cottonrats[tiab] OR hamster[tiab] OR hamsters[tiab] OR cricetinae[tiab] OR rodentia[Tiab] OR rodent[Tiab] OR rodents[Tiab] OR pigs[Tiab] OR pig[Tiab] OR swine[tiab] OR swines[tiab] OR piglets[tiab] OR piglet[tiab] OR boar[tiab] OR boars[tiab] OR "sus scrofa"[tiab] OR ferrets[tiab] OR ferret[tiab] OR polecat[tiab] OR polecats[tiab] OR "mustela putorius"[tiab] OR "guinea pigs"[Tiab] OR "guinea pig"[Tiab] OR cavia[Tiab] OR callithrix[Tiab] OR marmoset[Tiab] OR marmosets[Tiab] OR cebuella[Tiab] OR hapale[Tiab] OR octodon[Tiab] OR	6883153	September 27th 2019 11h00

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eels[Tiab] OR fish[Tiab] OR fishes[Tiab] OR pisces[Tiab] OR catfish[Tiab] OR catfishes[Tiab] OR siluriformes[Tiab] OR arius[Tiab] OR heteropneustes[Tiab] OR sheatfish[Tiab] OR perch[Tiab] OR perches[Tiab] OR percidae[Tiab] OR perca[Tiab] OR trout[Tiab] OR trouts[Tiab] OR char[Tiab] OR chars[Tiab] OR salvelinus[Tiab] OR "fathead minnow"[Tiab] OR minnow[Tiab] OR cyprinidae[Tiab] OR carps[Tiab] OR carp[Tiab] OR zebrafish[Tiab] OR zebrafishes[Tiab] OR goldfish[Tiab] OR goldfishes[Tiab] OR guppy[Tiab] OR guppies[Tiab] OR chub[Tiab] OR chubs[Tiab] OR tinca[Tiab] OR barbels[Tiab] OR barbus[Tiab] OR pimephales[Tiab] OR promelas[Tiab] OR "poecilia reticulata"[Tiab] OR mullet[Tiab] OR mullets[Tiab] OR seahorse[Tiab] OR seahorses[Tiab] OR mugil curema[Tiab] OR atlantic cod[Tiab] OR shark[Tiab] OR sharks[Tiab] OR catshark[Tiab] OR anguilla[Tiab] OR salmonid[Tiab] OR salmonids[Tiab] OR whitefish[Tiab] OR whitefishes[Tiab] OR salmon[Tiab] OR salmons[Tiab] OR sole[Tiab] OR solea[Tiab] OR "sea lamprey"[Tiab] OR lamprey[Tiab] OR lampreys[Tiab] OR pumpkinseed[Tiab] OR sunfish[Tiab] OR sunfishes[Tiab] OR tilapia[Tiab] OR tilapias[Tiab] OR turbot[Tiab] OR turbots[Tiab] OR flatfish[Tiab] OR flatfishes[Tiab] OR sciuridae[Tiab] OR squirrel[Tiab] OR squirrels[Tiab] OR chipmunk[Tiab] OR chipmunks[Tiab] OR suslik[Tiab] OR susliks[Tiab] OR vole[Tiab] OR voles[Tiab] OR lemming[Tiab] OR lemmings[Tiab] OR muskrat[Tiab] OR muskrats[Tiab] OR lemmus[Tiab] OR otter[Tiab] OR otters[Tiab] OR marten[Tiab] OR martens[Tiab] OR martes[Tiab] OR weasel[Tiab] OR badger[Tiab] OR badgers[Tiab] OR ermine[Tiab] OR mink[Tiab] OR minks[Tiab] OR sable[Tiab] OR sables[Tiab] OR gulo[Tiab] OR gulos[Tiab] OR wolverine[Tiab] OR wolverines[Tiab] OR minks[Tiab] OR mustela[Tiab] OR llama[Tiab] OR llamas[Tiab] OR alpaca[Tiab] OR alpacas[Tiab] OR camelid[Tiab] OR camelids[Tiab] OR guanaco[Tiab] OR guanacos[Tiab] OR chiroptera[Tiab] OR chiropteras[Tiab] OR bat[Tiab] OR bats[Tiab] OR fox[Tiab] OR foxes[Tiab] OR iguana[Tiab] OR iguanas[Tiab] OR xenopus laevis[Tiab] OR parakeet[Tiab] OR parakeets[Tiab] OR parrot[Tiab] OR parrots[Tiab] OR donkey[Tiab] OR donkeys[Tiab] OR mule[Tiab] OR mules[Tiab] OR zebra[Tiab] OR zebras[Tiab] OR shrew[Tiab] OR shrews[Tiab] OR bison[Tiab] OR bisons[Tiab] OR buffalo[Tiab] OR buffaloes[Tiab] OR deer[Tiab] OR deers[Tiab] OR

bear[Tiab] OR bears[Tiab] OR panda[Tiab] OR pandas[Tiab] OR "wild hog"[Tiab] OR "wild boar"[Tiab] OR fitchew[Tiab] OR fitch[Tiab] OR beaver[Tiab] OR beavers[Tiab] OR jerboa[Tiab] OR jerboas[Tiab] OR capybara[Tiab] OR capybaras[Tiab]) NOT medline[subset])		
#2 Type 2 diabetes (type 2 diabetes mellitus[MeSH Terms] OR type 2 diabetes mellitus[Title/Abstract])	140266	September 27th 2019 11h011h03
#3 Probiotics (gastrointestinal microbiome[MeSH Terms] OR probiotic[MeSH Terms] OR probiotics[MeSH Terms] OR lactobacillus[MeSH Terms] OR bifidobacterium[MeSH Terms] OR fermented milk product[MeSH Terms] OR fermented foods[MeSH Terms] OR gastrointestinal microbiome[Title/Abstract] OR probiotic[Title/Abstract] OR probiotics[Title/Abstract] OR lactobacillus[Title/Abstract] OR bifidobacterium[Title/Abstract]) OR fermented milk product[Title/Abstract] OR fermented foods[Title/Abstract])	88255	September 27th 2019 11h011h03
TOTAL: #1 AND #2 AND #3	250	September 27th 2019 11h011h03
Descriptors SCOPUS	*Items Found*	*Date/Time*
#1 Type 2 diabetes TITLE-ABS-KEY(type 2 diabetes)	217,461	September 27th 2019 11h011h03
#2 Probiotics (TITLE-ABS-KEY(probiotic) OR TITLE-ABS-KEY (probiotics) OR TITLE-ABS-KEY (gastrointestinal AND microbiome) OR TITLE-ABS-KEY(lactobacillus) OR TITLE-ABS-KEY(bifidobacterium) OR TITLE-ABS-KEY(fermented AND milk AND products) OR TITLE-ABS-KEY(fermented AND foods)	111,308	September 27th 2019 11h011h03

#3 Animal (LIMIT-TO (EXACTKEYWORD, "Nonhuman") OR LI MIT-TO (EXACTKEYWORD, "Animals") OR LIMIT-TO (EXACTKEYWORD , "Animal")	269	September 27th 2019 11h011h03
TOTAL: #1 AND #2	269	September 27th 2019 11h011h04

Supplementary material 5 (S5): PRISMA checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	2-3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	2-3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4-5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if	4-5

		applicable, included in the meta-analysis).	
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5-6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5-6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6-7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6-7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5

Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097.