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Review Article

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Review of Various Microbiota Modifications Due to Environmental Pollutant Exposure Causing Type 2 Diabetes in Rodent Model Experiments

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ABSTRACT

Introduction: In recent years, chemical-induced diabetes especially some endocrine disrupting chemicals (EDCs) are classified as diabetogen.

Aim/Objectives: The present meta-analysis aimed to investigate the diabetogenic nature and its resultant gut microbiome changes in mice exposed to EDCs.

Methods: This study was done by searching online database, PubMed using MesH terms; and gut microbiome/microbiota. Articles with EDC induced hyperglycemia and those performed meta-genomics analysis were included. All parameters including experimental set-up, biochemical tests, 16SrRNA sequencing were tabulated and grouped based on the type of EDC used.

Results: Out of 13 studies selected based on inclusion criteria, four administered POPs and three used emulsifiers. The meta-genomics analysis reported a total of 13 phylum and 64 genera. Only four studies reported F/B ratio in which two showed increased pattern. Some unique OTUs of 58/66, 42/38 and 43/38 were observed in diabetes mice when induced with glycerol monolaurate, PCB126 and PCB77 as compared to the control mice respectively. In induction group, majority of the studies have reported enhanced Gram's negative bacteria in which frequently documented organisms were *Akkermansia* and *Lactobacillus*, followed by Bifidobacterium, Bacteriodes and *Helicobacter*.

Conclusion: The outcome of this meta-analysis clearly indicated the diabetogenic nature of EDCs and its role in altering the gut microbiome. Hence, several such studies have to be carried out for understanding the cumulative incidence of chemical induced diabetes and thereby make necessary policies to reduce the burden of diabetes.

Keywords: Diabetes, EDCs, Mice, 16SrRNA, Microbiota, Meta-analysis

INTRODUCTION

Diabetes- A global and Indian scenario

Diabetes, a long-lasting multi-organ, multi-factorial, heterogenous metabolic disorder that accounts for 463 million adults who are at the age group of 20 to 79. [1] This alarming numbers will be predicted to rise further upto 700 million by 2045. Among the two diabetic types, the proportion of people (>79%) with type 2 diabetes is increasing in low- and middle-income countries. Apart from the above statistics, 1 in 2 people with diabetes were unnoticed or undiagnosed and this uplift the positivity by another 47% of the world's population. [2] WHO in 2019 warrants that 8.7% diabetic population were in the age group between 20 and 70. A study conducted by Indian Council of Medical Research - INdia DIABetes (ICMR-INDIAB) concluded that prevalence of diabetes and pre-diabetes in India population are higher compared with the results of previous studies.[3] Comparing the inter-state variations, Chandigarh ranked top with 13.6%, followed by Tamilnadu (10.4%) and Bihar (4.3%).

Diabetes is characterized by high blood glucose levels due to decreased insulin production or its action and/or increased insulin resistance. If not managed well, it leads to life threatening co-morbidities, including cardiovascular diseases, neuropathy etc. [4] In addition, one of the major causes of stroke, kidney failure, heart attacks, blindness and lower limb amputation is

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diabetes. Premature mortality from diabetes accounts for 5% between 2000 and 2016. According to the results of the Cost of Diabetes in India (CODI) study, ambulatory care constitutes 65% cost whereas an estimated 35% for hospitalization cost. The latter comprises of cost of general drug therapy cost (31%) of which 17% costs for specific antidiabetic medications. [5]

Anti-diabetic drugs

Many anti-diabetic drugs normalize blood glucose by targeting gut hormones such as glucagon-like protein-1 (GLP-1), DPP-4 (GLP-1 degrading enzyme) inhibitors or gliptins, sodium-dependent glucose transport-2 (SGLT2), metformin (a biguanide), thiazolidinediones (TZDs; PPAR-γ agonists), sulfonylureas (SU).[6] Newly diagnosed T2D patients are most preferably treated with first line anti-diabetic drug called 'metformin' that ameliorates glycemic control, cardiovascular mortality in overweight T2D patients and can even prevent T2D. Multiple evidences suggest that metformin modulates gut microbiota in humans and HFD-fed rodents and improves glycemia by enhancing the growth of mucus-degrading gut bacteria.[7]

Linking anti-diabetic drugs and gut microbiome

Now-a-days treating diabetes has becoming regimen based rather than using a single drug. Drugs thus used can change the drug-microbiome-metabolism axis thereby enhance or decrease specific taxa or genus of microorganism. Till date, however, there is limited studies on the effects of different combination anti-diabetic therapies on the gut microbiome. Emerging discoveries indicate that the gut microbiota - bacterial communities inhabiting our intestine, play an important role in the development of diabetes, obesity, metabolic syndrome etc.[8] Gut microbiota and microbial synthesized metabolites may act as determinants for the proper action of anti-diabetic drugs. Decreased abundance of beneficial microorganisms provide room for increased amounts of opportunistic pathogens and abnormal microbiota often called 'dysbiotic' are also prevalent in T2D. Thus, formulating and prescribing therapeutic drugs should beneficially be modulate the gut microbiome to prevent and reduce progression of T2. [9]

MATERIALS AND METHODS

This retrospective study followed methods for conducting and reporting meta-analyses recommended by the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. Database searching was performed for relevant studies that primarily include diabetes and also provide information associated to gut microbiota and blood glucose level. [10]

Search Strategy

We systematically reviewed PubMed database for peer-reviewed articles published on 2021. Search terms included 'GUT MICROBIOME' to include all resources and consisted of 'DIABETIC' and 'MICE'. The detailed search strategy is depicted in flowchart. Two filters for study period '2021' and access to full length article 'free full text articles' were applied.[11]

Article Selection

The resultant search results were saved and each article was reviewed for its content. Initially, abstracts of all articles were screened while titles and objectives of each article were tabulated in excel sheet to identify potentially relevant articles.

Exclusion and Inclusion Criteria

The following were excluded: Studies published prior to 2021.[12] Paid articles were excluded.[13] Articles that belong to reviews,[14] meta-analyses, clinical trials and systematic reviews were removed. [15] Also, certain objectives with diabetes as an outcome of the study were excluded.

Inclusion Criteria: The interventional studies diabetic mice as animal model either it could be ready-to-use diabetic animal model or chemically induced diabetic model were included. Furthermore, we looked for information on 'sequencing of gut microbiota' in the methodology section and that would be considered as one of the primary inclusion criteria.[16] The search results were independently reviewed by two reviewers based on the inclusion and exclusion criteria. All references were downloaded for consolidation of methods used and parameters studied.

Data Extraction

The following data were extracted from the articles that met the included criteria: animal models used or animal models developed: chemicals used, dose, duration; intervention strategy in which chemicals or isolated compounds used: dose, duration, route of administration were used as intervention drug; glucose level: sample used, interval at which samples was taken, method adopted; Gut microbiome: samples taken, amount of sample, kit used for DNA extraction, platform used for 16s rRNA sequencing; insulin resistance or tolerance test: dose of insulin, interval studied. [17]

Meta-analyses

Upon thorough reviewing, three variables were chosen for meta-analysis based on consistent availability and their importance: gut microbiome analysis, glucose estimation and insulin tolerance.

RESULTS

Search Results

The search strategy resulted in 52 citations, of which 35 were eligible for full-text review and 22 studies were excluded in the systematic review. The detailed flow diagram of study selection is presented in Fig.1. Preliminary screening revealed that out of 35 two were review articles, one was clinical trial. After screening titles and abstracts of 32 studies, nine articles were retrieved for further analysis.

January – 3; feb -2; mar-2; apr -2; may-1. China -3; korea-2; USA-2; Australia-1; Denmark-1.

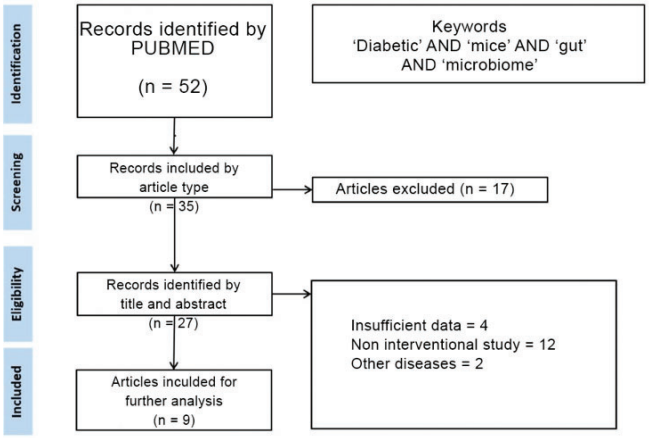

Figure 1: Flowchart representing article search

Characteristics of the Included Studies

Methods Adopted

Main characteristics of the studies included are shown in Table 1. Age of mice used for studies ranged from 4 to 14 weeks. All nine studies preferred male mice as the animal model. Between two and twelve groups of mice, with five to seventeen mice per group, were used. A minimum of 24 mice and up to 72 mice per study were sacrificed. Although all nine investigations preferred male mice, one study included mice of both sexes. Type 2 diabetes mellitus may be induced by STZ, a high-fat diet, or genetic manipulation. A total of nine studies using nine different models of diabetes that comprised of three ready-to-use diabetic mice, two by streptozotocin (STZ) injection and four with high-fat fed mice. Three mice models namely KKAy, B6. BKS(D)-Leprdb/J (db/db) and BTBR mice heterozygous for the Leptin^{ob} mutation were excellent diabetic mice models.

KKAy also known as KK.Cg-Ay/J due to its metabolic abnormalities make it appropriate for diabetes studies and B6. [24, 25] BKS(D)-Leprdb/J (db/db) also known as B6 db strain is an excellent model for type 2 diabetes and obesity related studies. BTBR with the ob/ob leptin-deficiency mutation develops severe type 2 diabetes. C57BL/6J bred, the mice model used in six cases to induce diabetes because of its proven versatility for development of severe obesity, hyperglycemia and hyperinsulinemia if exposed to a high-fat diet. [26, 27]One among the six studies that used C57BL/6L mentioned that 55% High Fat Diet (HFD) was orally given for 70 days for the induction of diabetes. Other two used streptozotocin (STZ) at 75mg/kg for three days and a single ip shot of 90mg/kg. The drug induced diabetic mice model was confirmed to be hyperglycemic or diabetic after induction period by Fast Blood Glucose (FBG) level. Most of the studies that account for 66.6% were considered the mice to be diabetic when FBG was >250mg/dl.[28]

Table 1: List of diabetic mice models used in different studies

Title	Mice per group	Age	Diabetic mice				Confirmation test	Groups	Total mice used
			model	chemical induced					
			Name	Name	dose	Duration	FBG level		
Characterization of local gut microbiome and intestinal transcriptome responses to rosiglitazone treatment in diabetic db/db mice. [12]	6	8 weeks	Diabetic B6.BKS(D)-Leprdb/J (db/db) mice				Model	2	12
QiDiTangShen granules modulated the gut microbiome composition and improved bile acid profiles in a mouse model of diabetic nephropathy. [14]	12	8 weeks	Male db/db mice in a C57BL/Ks background				>250mg/dl	5	60
Lactobacillus Sps in Reducing the Risk of Diabetes in High-Fat Diet-Induced Diabetic Mice by Modulating the Gut Microbiome and Inhibiting Key Digestive Enzymes Associated with Diabetes. [18]	5	4 weeks	C57BL/6J male mice	HFD-oral	45% kcal	8 weeks	180mg/dl	8	40

Table 1: ***(Continued)***

Title	Mice per group	Age	Diabetic mice				Confirmation test	Groups	Total mice used
			model	chemical induced					
			Name	Name	dose	Duration	FBG level		
Ramulus Mori (Sangzhi) Alkaloids (SZ-A) Ameliorate Glucose Metabolism Accompanied by the Modulation of Gut Microbiota and Ileal Inflammatory Damage in Type 2 Diabetic KKAy Mice. [21]	8	14-week	Male KKAy mice				model	3	24
Metformin Strongly Affects Gut Microbiome Composition in High-Fat Diet-Induced Type 2 Diabetes Mouse Model of Both Sexes. [23]	6	4-5-week	Male and female C57BL/6N mice	HFD-oral	60 kcal% fat	20 weeks	HOMA-IR index, and cut off above two	12	72
Gut Microbiome Prolongs an Inhibitory Effect of Korean Red Ginseng on High-Fat-Diet-Induced Mouse Obesity. [29]	15	eight-week-	C57BL/6 male	HFD-oral	55% kcal	70 days	>250mg/dl	4	60
Gut-Lung Dysbiosis Accompanied by Diabetes Mellitus Leads to Pulmonary Fibrotic Change through the NF-κB Signaling Pathway. [31]	10	8-week	C57BL/6 mice	Streptozotocin -ip	75 mg/kg	3 days	>288 mg/dL	3	30
A Newly Developed Synbiotic Yogurt Prevents Diabetes by Improving the Microbiome-Intestine-Pancreas Axis.[41]	8	10-12 weeks	C57BL/6J mice	Streptozotocin -ip	90 mg/kg	single	250mg/dl	3	24
Systemic Metabolic Alterations Correlate with Islet-Level Prostaglandin E 2 Production and Signaling Mechanisms That Predict β-Cell Dysfunction in a Mouse Model of Type 2 Diabetes.[42]	17		BTBR mice heterozygous for the Leptinob mutation				Model ≥300 mg/dL	3	51

Interventions

Detailed interventions were presented in Table.2. The duration of intervention ranged from one to 12 weeks with a modal value of 8 weeks. Dosage depends on the chemicals used for the intervention with the highest of 3.37g/kg/day (QiDiTangShen granules) to as low as 3mg/kg/day (rosiglitazone). Oral gavage or intra-gastric administration of interventional drugs was followed in all studies undertaken. [30]

Table 2: List of interventions carried out in included studies

Title	Route of administration	Intervention drug		
		Name	dose	Treatment period
Characterization of local gut microbiome and intestinal transcriptome responses to rosiglitazone treatment in diabetic db/db mice. [12]	Per orally administered	Rosiglitazone	3 mg/kg	once daily for a total of 55 days
QiDiTangShen granules modulated the gut microbiome composition and improved bile acid profiles in a mouse model of diabetic nephropathy. [14]	Oral gavage	QDTS	3.37 g/kg/day and 10.29 g/kg/day	once daily 12 weeks
Lactobacillus Sps in Reducing the Risk of Diabetes in High-Fat Diet-Induced Diabetic Mice by Modulating the Gut Microbiome and Inhibiting Key Digestive Enzymes Associated with Diabetes. [18]	Oral gavage	*Lactobacillus sakei* Probio65 and *Lactobacillus plantarum* Probio-093	10^8 CFU/day	5 µL/g/day for 8 weeks
Ramulus Mori (Sangzhi) Alkaloids (SZ-A) Ameliorate Glucose Metabolism Accompanied by the Modulation of Gut Microbiota and Ileal Inflammatory Damage in Type 2 Diabetic KKAy Mice. [21]	Intragastrically	SZ-A powder	100 and 200 mg/kg	56 days
Metformin Strongly Affects Gut Microbiome Composition in High-Fat Diet-Induced Type 2 Diabetes Mouse Model of Both Sexes. [23]	Oral gavage	Metformin	50 mg/kg/day	week 20
Gut Microbiome Prolongs an Inhibitory Effect of Korean Red Ginseng on High-Fat-Diet-Induced Mouse Obesity. [29]	Oral gavage	Korean red ginseng extracts	235 mg/kg	two times a week for 4 weeks
Gut-Lung Dysbiosis Accompanied by Diabetes Mellitus Leads to Pulmonary Fibrotic Change through the NF-κB Signaling Pathway. [31]	Intragastric gavage	fecal microbiota transplant and baicalin treatment group	200 mg of stool and 40 mg/kg	1 week
A Newly Developed Synbiotic Yogurt Prevents Diabetes by Improving the Microbiome-Intestine-Pancreas Axis.[41]	Intragastric gavage	Synbiotic Yogurt	15% (w/v)	five-week
Systemic Metabolic Alterations Correlate with Islet-Level Prostaglandin E 2 Production and Signaling Mechanisms That Predict β-Cell Dysfunction in a Mouse Model of Type 2 Diabetes. [42]		Purina or Teklad diets		10 weeks

Gut microbiome

We analysed the composition of gut microbiota in the stool samples of mice that were taken in different groups namely, control, diabetic and treated. [31,32] Only one article studied the microbiota diversity in the gut segments of duodenum, jejunum, ileum, caecum, and colon. The remaining eight studies collected stool sample for 16s rRNA sequencing and its quantity taken for nucleic acid extraction ranged from 20 mg to 100 g. QIAamp and MN kit was most often used for extraction whereas 16S rRNA amplicon sequencing was carried out using 500 ng of extracted nucleic acid in Illumina platform. [33,34]

Organisms Studied

A majority of 24 organisms were identified at genus level in nine articles and their per-study descriptions was presented

in Figure.2. In particular, bacteroides and firmicutes are the two organisms included in most of the studies either to compare them individually or to elucidate the ratio of bacteroides and firmicutes.[35] These were followed by *Lactobacillus*, Proteobacteria and Actinobacteria. The frequency of organisms studied across all articles falls in two modal value. In diabetic model, *Bacteroides*, *Firmicutes*, *Lactobacillus*, *Allistipes* were found to be increased, on the other hand, Bifidobacterium and verrucomicrobia reported as decreased microbial community. Limited data recorded in studies that use ready-to-use diabetic models made difficult to conclude the normal, dominant and non-commensal micro-organisms. Study by Gurung (2020) in human subjects revealed a negative associfation between Bacteroides, Verricomicrobia, Firmicutes and Bifidobacterium and T2D, while *Lactobacillus* showed most frequently associated with T2D followed by Fusobacterium. Discrepancies do exist among studies while reporting Lactobacillus in T2D patients. Bacteroidetes/Firmicutes ratio and indexes of diversity are the two inevitable parameters of microbiome studies. [36,37]

In most of the studies, gut microbiota data on normal mice has not been mentioned whereas comparison between diabetic and treated mice was done. The reported group-wise identified micro-organisms was more in diabetic group followed by treated mice. Differences observed among micro-organisms depend on intervention strategies used. [22] Out of nine, five studies showed lesser number of organisms in treated group than diabetic induced. Conversely, organisms in treated group reported higher in three studies. Very few studies have shown and compared data for all groups.[38]

Differences in species-level OTUs were disclosed in only two studies in which, one showed that intervention by SZ-A 200 group significantly reduced the OTUs numbers and the other concluded no differences was observed between diabetic and treated group. Three studies have shown diversity analysis on gut microbiome. Silamikele *et al*., expressed alpha and beta diversity as effective number of species (ENS) and clustering respectively.[21]

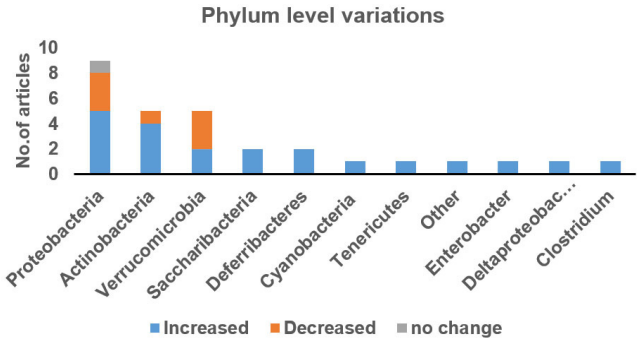

Figure 3: Distribution of Gut Microbiota Across 13 Phyla: Focus on Bacteroidetes/Firmicutes Ratio and Diversity Indexes from 10 Selected Studies.

Out of 10 articles scrutinized, four showed variations in gut microbiota in all levels including phyla, order, family, genera and species. In particular, bacteroides and firmicutes are the two organisms included in most of the studies either to determine their relative abundance or to elucidate their ratio. These were followed by Proteobacteria, Actinobacteria and verrucomicrobia. Bacteroidetes/Firmicutes ratio and indexes of diversity are the two inevitable parameters of microbiome studies. (Table 3) (Figure 3).

enhanced/increased | High | Low/decreased

ORGANISMS	PAPER 1			PAPER 2			PAPER 3			PAPER 4			PAPER 5			PAPER 6			PAPER 7			PAPER 8			PAPER 9			Total	Studywise
	N	DB	T	N	DB	T	N	DB	T	N	DB	T	N	DB	T	N	DB	T	N	DB	T	N	DB	T	N	DB	T		
Family																													
Lachnospiraceae																												6	3
Lactobacillaceae																												3	2
Genus																													
Lactobacillus																												9	6
Kineothrix																												3	2
Blautia																												2	1
Bacteroides																												16	7
Firmicutes																												11	6
Actinobacteria																												5	4
Proteobacteria																												11	4
Allistipes																												5	3
Eubacterium																												1	1
Bifidobacterium																												4	3
Alloprevotella																												1	2
Turicibacter																												1	1
Verrucomicrobia																												7	3
Tenericutes																												4	2
Deferribacteres																												4	2
Clostridium																												3	2
Desulfovibrio																												5	2
Aerococcus																												2	1
Mucispirillum																												3	2
Parabacteroides																												2	2
Prevotella																												3	2
Enterococcus																												2	2
Gram -ve bacteria																												1	1
Cyanobacteria																												4	2
Total	0	5	5	2	7	6	1	7	9	0	4	5	0	8	5	6	10	7	4	4	3	0	0	7	6	4	0		
	N	DB	T	N	DB	T	N	DB	T	N	DB	T	N	DB	T	N	DB	T	N	DB	T	N	DB	T	N	DB	T		

Figure 2: List of microorganisms identified in gut microbiome of T2D and treated mice.

Table 3: Strategies Used in 16s rRNA Sequencing

Title	Gut microbiome			
	Organs	Quantity	RNA extraction kit	platform used
Characterization of local gut microbiome and intestinal transcriptome responses to rosiglitazone treatment in diabetic db/db mice. [12]	Gut segments (duodenum, jejunum, ileum, caecum, and colon)	Not mentioned	Zymo BIOMICS DNA/ RNA Miniprep Kit	MinION Nanopore sequencer
QiDiTangShen granules modulated the gut microbiome composition and improved bile acid profiles in a mouse model of diabetic nephropathy. [14]	Fresh fecal samples (n = 50) were collected in the morning for two consecutive days	Not mentioned	QIAamp PowerFecal Kit	MiSeq platform (Illumina
Lactobacillus Sps in Reducing the Risk of Diabetes in High-Fat Diet-Induced Diabetic Mice by Modulating the Gut Microbiome and Inhibiting Key Digestive Enzymes Associated with Diabetes. [18]	Fecal samples	100 mg-200 mg freeze-dried	Stool DNA extraction kit (Bioneer	Not mentioned
Ramulus Mori (Sangzhi) Alkaloids (SZ-A) Ameliorate Glucose Metabolism Accompanied by the Modulation of Gut Microbiota and Ileal Inflammatory Damage in Type 2 Diabetic KKAy Mice.[21]	Feces	Not mentioned	Not mentioned	Illumina MiSeq sequencing
Metformin Strongly Affects Gut Microbiome Composition in High-Fat Diet-Induced Type 2 Diabetes Mouse Model of Both Sexes. [23]	Fecal n=48	Not mentioned	FastDNA®SPIN Kit for Soil (MP Biomedicals	DNBSEQ-G400RS next generation sequencing platform
Gut Microbiome Prolongs an Inhibitory Effect of Korean Red Ginseng on High-Fat-Diet-Induced Mouse Obesity. [29]	Fecal =15	Not mentioned	Not mentioned	Ilumina platform
Gut-Lung Dysbiosis Accompanied by Diabetes Mellitus Leads to Pulmonary Fibrotic Change through the NF-κB Signaling Pathway. [31]	Stool	Not mentioned	DNA Kit (Omega Biotek	Shanghai Majorbio Bio-Pharm Technology Co., Ltd (out-sourced)
A Newly Developed Synbiotic Yogurt Prevents Diabetes by Improving the Microbiome-Intestine-Pancreas Axis.[41]	Feces	100 g	Qiagen QIAamp PowerFecal Pro DNA Kit	Illumina MiSeq sequencer
Systemic Metabolic Alterations Correlate with Islet-Level Prostaglandin E 2 Production and Signaling Mechanisms That Predict β-Cell Dysfunction in a Mouse Model of Type 2 Diabetes. [42]	Feces	20–50 mg	Macherey-Nagel PCR Clean-up kit	Illumina's 16s Metagenomic Sequencing Library Preparation Protocol

Analysis of glucose level

The nine included studies reported FBG was performed in all groups of mice, of which, six reported monitoring additional diabetes-associated parameters such as insulin quantification, HbA1c estimation and insulin resistance or tolerance test. Some of the studies revealed data on fasting blood glucose in main text while others discussed it with the reference to the figures. Despite of its availability, the overall influence of interventional drugs significantly reduced FBG.[39]

Consolidated report on FBG

Rosiglitazone improved weekly FBG levels and terminal HbA1c levels whereas treatment with either QDTS or valsartan had no significant impact on the levels of FBG. Gulnaz et al., showed a normal blood glucose level of 130 mg/dL in naïve mice and once mice fed with HFD diet for 8 weeks, it has risen upto 180 mg/dL (27.7%).[18] Significant reduction of FBG level was achieved by administration of *L. plantarum* Probio-093 Live cells (15.0%) and *L. plantarum* Probio-093 SEL (11.9%). But mice treated both with metformin and *L. sakei* Probio65 SEL showed 20% decrease in FBG.[40]

Effect of SZ-A treatment for four weeks has decreased the FBG and postprandial blood glucose levels ($p < 0.01$, $p < 0.01$) compared to that of diabetic group. After 10 weeks of SGE and GE treatment, the HFD induced glucose was reduced in addition to insulin and leptin. FMT for up to 24 days showed poor outcome on FBG in STZ-Induced Diabetic Mice but gut microbiota could be restored as evidenced by Shannon index. Whist mice fed with symbiotic yogurt; a promising positive result was achieved as the FBG drops significantly to 200 mg/dL compared to 300 mg/dL in control DM mice. Surprisingly, control yogurt groups exhibited a significant increase (500 mg/dL) in FBG level.[41]

Similarly, a study by Schaid *et al.,* showed approximately 500 mg/dL of FBG level in T2D mice whereas normoglycemic obese mice had nearly identical (approximately 180 mg/dL) FBG level as WT mice. Many studies lack positive treatment arm in which mice treated with standard drug. Drug specific effect on FBG and gut microbiota shared a fruitful data. [42]

Sex specific Metformin changes in gut microbiome

Interestingly, influence of metformin on mouse gut microbiota has been well documented by Silamikele ., (2021). They studied metformin effect on both sexes. All analysis was carried out in mouse before and after metformin treatment. Female mice showed significantly altered taxa while in male not much differences were observed.[21]

Out of 53 altered species, Clostridia class such as *Faecalibacterium prausnitzii, Enterocloster clostridioformis and Anaerostipes sp.* 992A decreased along with Desulfovibrio fairfieldensis. An overall positive correlation in the relative abundance of Bacillus sp. LogFC upto 1.36, particularly B. ilei (LogFC = 2.85) was observed in HFD_Met+ group. Differential abundance of species that corresponds to taxa was higher in female treated mice where samples from both sexes are taken together. [43]

Sex specific functional analysis in Metformin treatment

Similar to what was observed in gut microbiome, functional analysis related to metabolic key indicators presented no significant change in male group. In females, 5 functions were significantly differentially represented by the read. All the analyzed hierarchies showed pronounced alterations in female HFD_met+ group and among all, phenylalanine metabolism, glyoxylate and dicarboxylate metabolism showed a minor variation. Although, combined analysis in both sexes showed increase in pyruvate metabolism.[44]

Other parameters studied

Insulin

Quantification of insulin was done in three studies while insulin tolerance analysis was reported in three studies. For latter assay, an insulin dose of 0.5U/kg or 0.75U/kg was intra-peritoneally injected and plasma collected at regular intervals of 15, 30, 60 and upto 120 min were analyzed. Only one study reported estimates of short fatty chain acid using gas chromatography. [45]

Insulin level maintained in rosiglitazone-treated db/db mice at 5718 ± 841 pg/mL. Similarly, SZ-A 100 and SZ-A 200 both significantly enhanced insulin secretion of nearly 6.5 ng/mL at 15 min after glucose stimulation. Another study highlighted the treatment with metformin significantly increased the insulin level in blood of male and female mice at 1666.22 pmol/L and 613.48 pmol/L respectively. HFD induced T2D mice had reduced blood insulin at 0.75 ng/mL if treated with SGE or GE. Only two studies discussed on insulin tolerance test in which SGE, GE and metformin reduced HFD-increased HOMA-IR. [46]

CONCLUSION

This analysis of nine studies on type 2 diabetes mellitus in mouse models highlights key insights into the disease's development, treatment strategies, and influencing factors. Various methods, including streptozotocin injection, high-fat diets, and genetic manipulation, were used to induce diabetes, with models like KKAy and B6.BKS(D)-Leprdb/J (db/db) proving particularly effective.

Alterations in gut microbiota, such as increased levels of Bacteroides and decreased Bifidobacterium, were noted in

diabetic models. Treatments like symbiotic yogurt and metformin showed potential in lowering fasting blood glucose (FBG) levels, though their effects on gut microbiota varied.

The efficacy of interventions like rosiglitazone, SZ-A, and metformin in improving FBG levels and insulin secretion, along with sex-specific effects, underscores the complexity of managing type 2 diabetes. These findings provide valuable direction for future research and therapeutic development.

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Conflict of interest

The author declare that they have no conflict of interest.

Authors' Contribution

Dr. G. Buvaneswari wrote various parts of the manuscript, while **Swetha Ethiraj** and **Dr.Frankline Noah Kanagaraj** were involved in proofreading and selecting articles for the study.

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