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Practice innovation and outcome evaluation in diabetes

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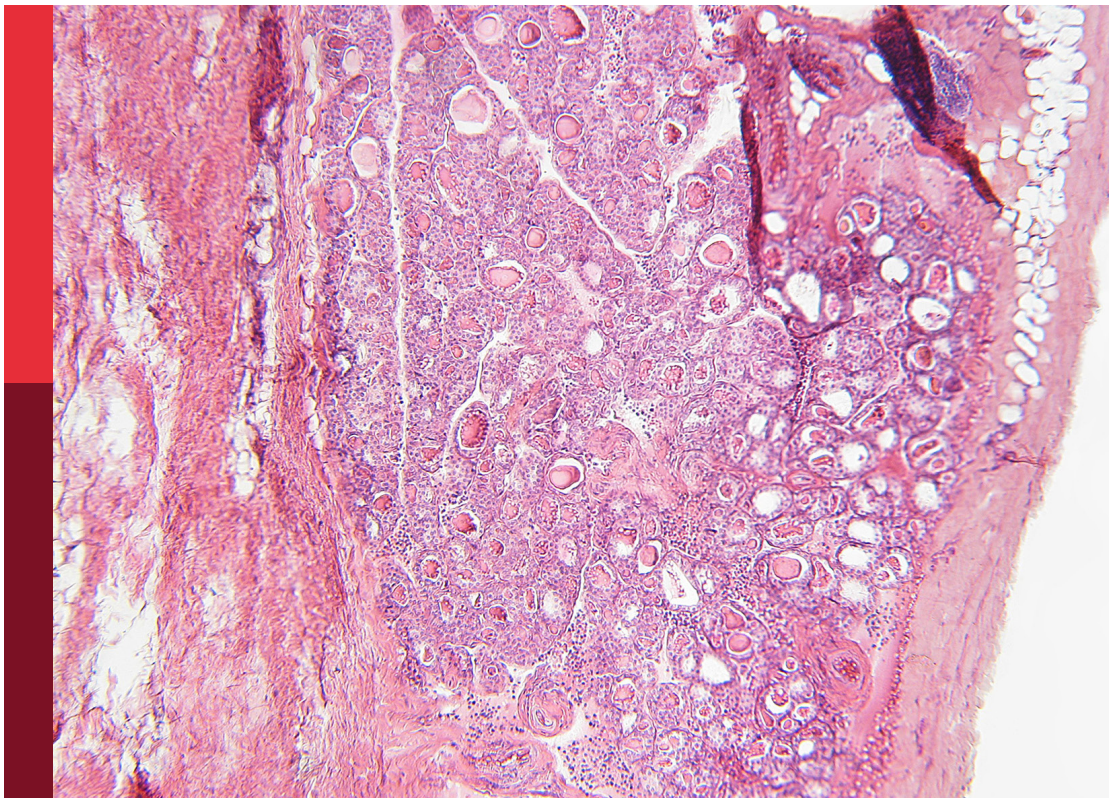

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Practice innovation and outcome evaluation in diabetes

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Pharmacoeconomic analysis (CER) of Dulaglutide and Liraglutide in the treatment of patients with type 2 diabetes

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Aim: To evaluate the treatment effect Fand pharmacoeconomic value of Dugaglutide in women with type 2 diabetes.

Methods: Women (n=96) with type 2 diabetes recruited from June 2019 to December 2021 were randomized into two equal groups. The control group was treated with Liraglutide, and the observation group was treated with Dulaglutide, both for 24 weeks. The blood glucose levels, biochemical index, insulin resistance index (HOMA-IR), cost-effect ratio (CER), and drug safety were determined and compared between the two groups.

Results: Blood glucose levels, the biochemical index, and HOMA-IR were lower in both groups after the treatment ($P < 0.05$), and there was no statistical difference in the blood glucose levels, biochemical index and HOMA-IR between the two groups ($P > 0.05$). The CER levels did not differ statistically between the two groups ($P > 0.05$). Both the cost and the incidence of drug side effects during solution injection were lower in the observation group than in the control group after 24 weeks of treatment ($P < 0.05$).

Conclusion: Both Dulaglutide and Liraglutide can reduce blood glucose levels, improve biochemical index, and HOMA-IR levels in women with type 2 diabetes. Dulaglutide is more cost-effective and safe.

Clinical trial registration: https://www.chictr.org.cn/index.aspx, identifier ChiCTR1900026514.

KEYWORDS

Dulaglutide, type 2 diabetes, treatment effect, pharmacoeconomics, insulin resistance index, cost-effectiveness ratio

1 Introduction

Type 2 diabetes tends to occur in adults because of a continuous increase in the blood glucose level, which is caused by insufficient insulin secretion or difficulty in the use of insulin for various reasons. Among all of the type 2 diabetes patients, evidence suggests that women experience a higher excess mortality than men (1, 2). Persistent hyperglycemia can cause pathological changes in the macrovascular, microvascular, and nervous systems and, in severe cases, damage to the heart and kidney (3). The pathology of type 2 diabetes is complex, and clinical symptoms can include polyphagia, polyuria, polydipsia, and weight loss (4, 5).

In the early 1980s, glucagon-like peptide 1 (GLP-1) was found to be the glucagon-stimulating enzyme cleavage product (6)produced in intestinal L cells. GLP-1, as an intestinal peptide mainly secreted after ingestion of glucose or mixed diet, increases glucose-stimulated insulin secretion at physiological plasma concentration, meeting all standards of incretin hormone (7, 8). The insulin-promoting effect of GLP-1 in type 2 diabetes patients shows that it has a potential role in drug treatment of the disease (9, 10). The most obvious physiological effect of GLP-1 is its insulin-promoting effect (6). It is worth noting that GLP-1 only increases insulin release in the case of hyperglycemia, so it will not lead to hypoglycemia. In addition, GLP-1 inhibits the pancreas α The cells release glucagon, which may be through the islets δ Somatostatin is locally released from the cells to mediate the release of (10, 11). In addition, GLP-1 has many other functions: the central nervous system (CNS) induces satiety and satiety (12), reduces blood pressure (13), and reduces postprandial triglyceride and free fatty acid concentrations. Lilalutide is a GLP-1 receptor agonist. The standard therapeutic dose of liraglutide is 1.2mg once a day. However, if the patient has insufficient blood glucose response to the drug, it is recommended to titrate to 1.8mg once a day. In phase III clinical trial of liraglutide in patients with type 2 diabetes, HbA1c levels were reduced by 1.1 – 1.8% (14, 15).

Liraglutide is a commonly used glucagon-like peptide 1 (GLP-1) receptor agonist. *In vivo*, Liraglutide can bind to GLP-1 receptors on pancreatic beta cells and then stimulate the synthesis and secretion of insulin, which can increase insulin sensitivity in peripheral tissues, enhance insulin-mediated glucose utilization, inhibit hepatic glycogen callogenesis, reduce glucose uptake by intestinal cells and decrease hepatic glucose output (16). Liraglutide also increases satiety by acting on the central nervous system (17) and slowing gastric emptying time, which reduces the total energy intake (18, 19).

Unlike short-acting compounds, long-acting GLP-1 receptor agonists do not appear to substantially affect gastric motility when taken for a long time. Long-acting GLP-1 receptor agonists lack influence on gastric emptying rate76. Dulaglutide is a GLP-1 peptide fused with IgG. Compared with natural GLP-1, Dulaglutide shows extended biological activity due to its extended half-life (~90 hours), which supports the weekly administration of the drug (15). A weekly dose of 0.05 – 8.0mg resulted in a decrease of 0.2 – 1.2% in HbA1c levels after 5 weeks. Compared with short-acting drugs that require more frequent administration, the convenience of injecting long-acting compounds once a day or once a week is an obvious advantage. Patients with frequent changes in daily activities, such as business travelers and shift workers, may prefer long-acting compounds, which can improve patient compliance (20).

Dulaglutide is one GLP-1 Fc fusion protein that activates GLP-1 receptors and promotes glucose-dependent insulin secretion, which helps reduce fasting and postprandial glucose levels (21). Dulaglutide improves the insulin secretion index and helps regulate the body's blood glucose level (22). Growing evidence suggests that Dulaglutide has the potential to treat diabetes-related neurodegenerative diseases (23, 24). The mechanism of action for how the drug decreases blood glucose is shown in (Figure 1). However, few clinical studies have focused on the economic value of Dulaglutide injection in type 2 diabetic patients (25). This study was conducted to investigate the therapeutic effect and pharmacoeconomic value of Dulaglutide in female patients with type 2 diabetes.

2 Materials and methods

2.1 Patient recruitment

Female patients (n=96) with type 2 diabetes were recruited from June 2019 to December 2021, ranging in age from 23 to 69 years, and the average age of patients was 46.14 ± 5.78 years (mean ± S.D.); the mean body mass index (BMI) was 22.62 ± 3.71 kg/m^2 (the BMI levels ranged from18.31 to 29.34 kg/m^2); and the disease duration was an average 5.73 ± 0.92 years (the range was 1-12 years). We randomly assigned participants at a ratio of 1:1. Using an interactive voice response system, all patients were randomly divided into two groups according to a computer-generated random sequence.

2.2 Inclusion and exclusion criteria

The study design included the following inclusion criteria (26): (1) The type 2 diabetes patients were diagnosed based on the American Diabetes Association criteria which specify that the FPG ≥ 126 mg/dL (7.0 mmol/L), 2-h PG ≥ 200 mg/dL (11.1 mmol/L) during OGTT (Oral Glucose Tolerance Test), an A1C level ≥ 6.5% (48 mmol/mol), or in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥ 200 mg/dL (11.1 mmol/L). Fasting is defined as no caloric intake for at least 8 h. The fasting glucose test should be performed as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water, and the test should be performed in a laboratory using a method that is NGSP (National Glycohemoglobin Standardization Program) certified and standardized to the DCCT (Diabetes Control and Complications Trial) assay.(2) No history of allergy and contraindication to Dulaglutide and Liraglutide, and be able to tolerate the treatment. Exclusion criteria included: (1) Patients with other types of diabetes mellitus (DM) rather than T2DM; (2) Patients who have used weight reduction drugs within 24 weeks; (3) Patients with clinically significant hepatobiliary, renal, cardiovascular, gastrointestinal or autoimmune system disease; (4) Coagulation disorders; (5) Patients who are judged by the investigator as unlikely to comply with the protocol, or patients with serious physical or psychological illnesses that could affect the effectiveness or safety of the study.

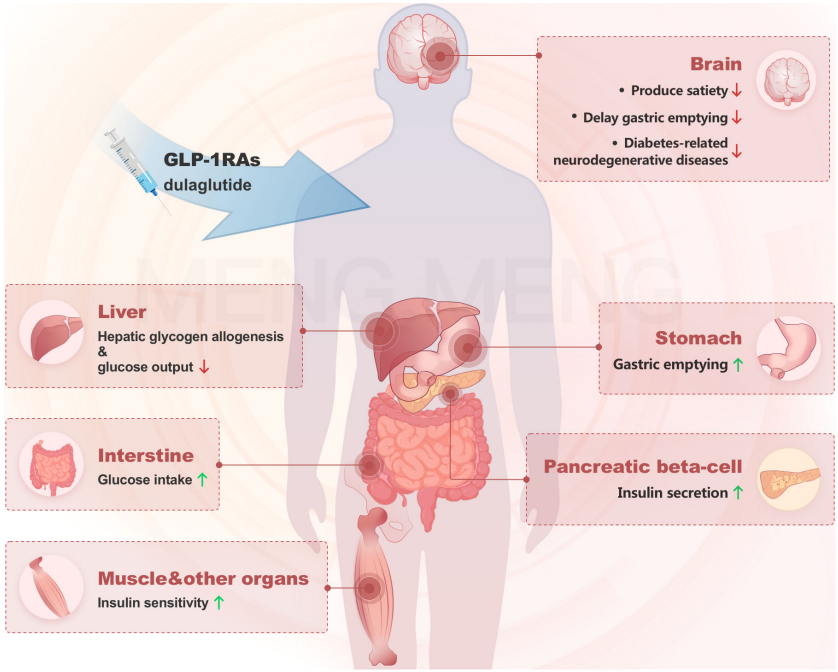

FIGURE 1
The mechanism of action of Dulaglutide in decreasing blood glucose.

2.3 Methods

Both groups of patients were admitted to the hospital and underwent stringent blood glucose monitoring. All patients did not use other hypoglycemic drugs. During the study period, the patients' exercise intensity was medium to low, and they followed the diabetes diet. The control group was injected subcutaneously with Liraglutide (Novo Nordisk Pharmaceutical Co., Ltd., China; at the specification 3 mL: 18 mg/stick) at the following regimen: 0.6 mg once a day during the first week before breakfast; then 1.2 mg once a day, from the 2nd to 24th week. The observation group was treated with Dulaglutide. Patients were given a subcutaneous injection of Dulaglutide every week. The dose of Dulaglutide injected was 0.75mg in the first week, if the blood glucose is not well controlled, the dose can be increased according to the patient's actual situation, where the range of injection was generally 0.75-1.5 mg for 24 weeks (1 course of treatment).

2.4 Endpoints

(1) Glucose metabolism indices. Fasting postprandial glucose (FPG) and 2-hour postprandial glucose(2HPG) levels were measured using a glucose meter before treatment and after 24 weeks of treatment in both groups. Patients' glycosylated hemoglobin (HbAlc) levels were measured using an automatic biochemical analyzer (27).

(2) Biochemical index and insulin resistance index (Homa-IR). The levels of visceral adiponectin were measured by enzyme-linked immunosorbent assay (2). Leptin (Lp) levels were measured by radioimmunoassay. Fasting-insulin (FINS) was measured by a fully automated immunoluminescence analyzer and HOMA-IR (HOMA-IR=FPG*FINS/22.5) levels were calculated (17).

(3) Cost-Effectiveness Ratio (CER). The economic value analysis contained two aspects: cost determination and efficacy analysis. A cost-effectiveness ratio (CER) was performed, where the lower CER indicates the better economic value (28). Safety was assessed based on adverse events: the incidence of nausea and vomiting, hypoglycemia, cholecystitis, allergic reactions, and liver and kidney abnormalities (29).

2.5 Statistical analysis

Unpaired Student's t-test for categorical variables was applied for comparison between observation and controlgroups. Results with a two-tailed p-value of <0.05 were considered significant (19 31). IBM SPSS Statistics 25 was used for thedata analyses.

3 Results

3.1 Changes in glucose metabolism indices

There was no statistical difference in glucose metabolism indices between the two groups before treatment (P> 0.05), though FPG, 2hPG, and HbAlc levels were lower in both groups after 24 weeks of treatment (P< 0.05). No statistical difference was found in glucose metabolism indices between the observation and control groups after 24 weeks of treatment (P> 0.05) (Table 1).

TABLE 1 Comparison of glucose metabolism indices between control and observation groups.

Group	n	FPG (mmol/L)		2hPG (mmol/L)		HbAlc(%)	
		W0	W24	W0	W24	W0	W24
Observation group	48	10.59 ± 2.31	6.23 ± 0.89#	13.21 ± 2.96	7.12 ± 1.42#	7.89 ± 0.93	5.67 ± 0.71#
Control group	48	10.62 ± 2.33	8.59 ± 1.52#	13.23 ± 2.99	10.97 ± 1.98#	7.92 ± 0.96	6.74 ± 0.82#
p value	/	0.950	0.000	0.974	0.0000	0.877	0.0000

W0, week 0, before the solution injection treatment; W24, week 24, after the solution injection treatment for the period of 24 weeks; #: P< 0.05 compared with before the solution injection treatment. Data are displayed as mean ± SD.

3.2 Comparison of the biochemical index and Homa-IR index between control and observation groups

There was no statistical difference in the biochemical indices and HOMA-IR index between the two groups before treatment (P> 0.05). However, the levels of visceral adiponectin, Lipoprotein (LP), FINS. and HOMA-IR were lower than those before treatment in both groups after 24 weeks of treatment (P< 0.05). We found no statistical difference in biochemical indices and HOMA-IR levels between the observation and control groups after 24 weeks of treatment (P> 0.05) (Table 2).

3.3 Comparison of the Cost and CER between control and observation groups

Both groups completed the continuous treatment over 24 weeks and the clinical application value of the different drugs was assessed from an economic point of view. No statistical difference in CER levels was found between the two groups (P> 0.05); however, the cost was lower in the observation group than in the control group after 24 weeks of treatment (P< 0.05) (Table 3).

3.4 Comparison of safety between the two groups

The incidence of nausea and vomiting, hypoglycemia, cholecystitis, allergic reactions, and liver and kidney abnormalities was much lower in observation group compared to the control group (Table 4).

4 Discussion

Type 2 diabetic patients account for more than 90% of all diabetic patients. Many patients with type 2 diabetes might not have a complete loss of insulin secretion, and some might have excessive insulin secretion (30). However, type 2 diabetic patients are poor users of insulin, and the persistent hyperglycemic condition will have a negative impact on the ability of the body to metabolize glucose, leading to chronic elevation of blood glucose in patients. Liraglutide is one of the human glucagon plasmin-1 analogues, which belongs to a family of glucose-lowering drugs with a strong hypoglycemic effect. Liraglutide is an injection solution but not insulin, and it can promote insulin secretion and inhibits the secretion of hyperglycemic hormone and the feeding center in the brain.

In our study, both short-acting and long-acting GLP-1 receptor agonists can reduce the levels of FPG, 2hPG, and HbAlc, which is consistent with previous studies (31). Kapodistria's study (32) showed that Liraglutide could promote enterocytes to secrete insulin by elevating endogenous GLP-1 levels from a physiological dose to a pharmacological dose. Actually, long-acting GLP-1 receptor agonists can provide better blood glucose control than short-acting ones because patients with long-acting receptor agonists have higher fasting insulin levels (possibly at night) (33, 34). Persistent high plasma levels of long-acting GLP-1 receptor agonists lead to a decrease in plasma HbA1c levels, which is greater than the decrease observed in intermittent activation of GLP-1 receptor caused by the administration of short-acting compounds (13, 35). Moreover, long-acting GLP-1 receptor agonists have no substantial effect on gastric motility, 76 which may be due to rapid immune response, which means that the effect of these compounds on gastric emptying decreases rapidly over time because they continuously activate GLP-1 receptor (36). In addition, long-acting GLP-1 receptor

TABLE 2 Comparison of biochemical indices and Homa-IR index between control and observation groups .

Group		Visceral adiponectin (ng/mL)	Lp (μg/L)	FINS (IU/L)	HOMA-IR
Observation group (n=48)	W0	56.49 ± 5.69	5.97 ± 0.92	13.16 ± 1.41	4.34 ± 0.79
	W24	39.45 ± 4.31*	3.11 ± 0.49*	9.34 ± 0.67*	2.21 ± 0.42*
Control group (n=48)	W0	56.51 ± 5.72	5.99 ± 0.94	13.18 ± 1.43	4.36 ± 0.81
	W24	40.11 ± 4.34*	3.13 ± 0.51*	11.32 ± 0.98*	2.23 ± 0.44*

W0, week 0, before the solution injection treatment; W24, week 24, after the solution injection treatment for the period of 24 weeks; #: P< 0.05 compared with the other group; *: P < 0.05 compared with before the solution injection treatment. Data are displayed as mean ± SD.

TABLE 3 Comparison of the cost and CER between control and observation groups.

Group	n	Cost (RMB)	CER
Observation group	48	7515.69 ± 86.49	48.57 ± 4.31
Control group	48	24596.68 ± 453.69	48.91 ± 4.37
p value	/	0.000	0.702

Data are displayed as mean ± SD.

TABLE 4 Comparison of safety between control and observation groups [n(%)].

Group	n	Nausea and vomiting	Hypo-glycemia	Cholecy-stitis	Allergic reactions	Liver and kidney abnormalities	Total sum
Observ-ation group	48	0(0.00)	1(2.08)	0(0.00)	0(0.00)	0(0.00)	1(2.08)
Control group	48	2(4.17)	2(4.17)	2(4.17)	0(0.00)	1(2.08)	7(14.58);
p value	/	/	/	/	/	/	0.027

Data are displayed as number and percentage(%).

agonists do not reduce postprandial blood glucose fluctuations like short-acting compounds (37). by comparison, the clinical application of Liraglutide requires patients to inject the solution once a day. The drug is expensive with relatively low-cost performance, which limits its clinical use and makes it difficult to promote its application in primary hospitals.

In response to the expensive price and relatively low-cost performance of Liraglutide, Dulaglutide has begun to be used clinically (38). In our study, the levels of FPG, 2hPG, and HbAlc were decreased in both groups after the treatment for the period of 24 weeks. There was no statistical difference in blood glucose levels between the observation group and the control group after the treatment with Dulaglutide for the period of 24 weeks. However, the levels of visceral adiponectin, LP, FINS, and HOMA-IR were lower in both groups after treatment for 24 weeks than before the treatment. Cardiovascular disease caused by diabetes is one of the common complications of T2DM. Lipoprotein rich in cholesterol is an important risk factor for atherosclerosis, including coronary heart disease, myocardial infarction, stroke and peripheral vascular disease. Low density lipoprotein (LDL) and lipoprotein (a) Lp (a) are important components of cholesterol ester rich lipoproteins (39, 40). Kotani et al. found that endothelial dysfunction may be related to oxidized Lp (a) in T2DM patients (41). Saeed et al. studied the relationship between elevated Lp (a) and CVD risk in nearly 10000 male and female participants, including 1543 people with diabetes or pre diabetes (42). No statistical difference was determined in biochemical indices and HOMA-IR index levels between the observation group and the control group after the treatment for the period of 24 weeks. Dulaglutide can be applied to control blood glucose in type 2 diabetic patients. Because of the relatively high molecular weight of the injection solution, it is generally not easily absorbed and degraded by the body, thus the duration of drug activity is relatively long. Therefore, Dulaglutide can be used once a week to meet the clinical requirements. In addition, the solution can promote the release of insulin, delay gastric emptying, and control the total daily energy intake in a certain range by reducing the intake of food, to achieve a good hypoglycemic effect (43).

Pharmacoeconomics is the specific application of economic principles and methods in pharmaceuticals (44). By a broad generalized definition, pharmacoeconomics focuses on the study of the economic behavior of the supply and demand of drugs, the interaction between supply and demand of drug market pricing, and the measures of various intervention policies in the field of drugs (45). In a narrow sense, however, pharmacoeconomics is the application of the basic principles, methods, and analytical techniques of economics in the clinical treatment process of the drug, using the pharmacoepidemiological population as a guide and based on a society-wide perspective to seek maximum rational utilization (46). To analyze further the pharmacoeconomic value of Dulaglutide, we evaluated its use in this study from the perspective of cost and CER. We found no statistical difference between the two groups in terms of CER levels; the cost of the observation group was lower than that of the control group after the treatment for the period of 24 weeks.

In previous studies, it was found that the economic benefit of dulaglutide is higher than that of liraglutide in the short term, but the long-term economic benefit is still unclear. Our research results extend the previous results (12).Moreover, the economic value of Dulaglutide was higher, and the drug was relatively cost-effective compared with Liraglutide. Some researchers (47) gave Dulaglutide and Liraglutide to patients with type 2 diabetes and then evaluated the effect from the perspective of economics. Our research conclusion is consistent with the previous study. Their results also found that Dulaglutide has a price advantage for type 2 diabetic patients because Dulaglutide has a relatively low cost coupled with the fact that the solution is injected once a week and, therefore, is suitable for promotion in primary hospitals. In this study, the incidence of nausea and vomiting, hypoglycemia, cholecystitis, allergic reactions, and hepatic and renal abnormalities was lower in the observation group than in the control group during the treatment period, the complications of diabetes and its high hospitalization rate are

important factors for the increase in treatment costs of diabetes (48). The gastrointestinal reaction of dulaglutide is significantly reduced. The weekly injection rate can improve the compliance of patients, reduce the incidence of complications of diabetes (20), and reduce the cost of consumables such as injection needles and diabetes management (48).

Therefore, patients with type 2 diabetes should be treated to improve relevant glucose and insulin indices then, appropriate hypoglycemic drugs should be selected in combination with their economic status and family background, to improve the pertinent treatment.

5 Conclusions

Both Liraglutide and Dulaglutide can reduce blood glucose level and improve visceral adiponectin, Lp, and the HOMA-IR index level in type 2 diabetic patients. The effects of Liraglutide and Dulaglutide are similar. Because Dulaglutide is more cost-effective and safer with fewer adverse reactions, the application of Dulaglutide deserves further promotion.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

This study was approved by the Ethics Committee of Ethics Committee of Longhu Hospital, First Affiliated Hospital of Medical College of Shantou University (the registration number is: ChiCTR1900026514). The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization, KH and YS; methodology, KH; formal analysis, FWC and JC; investigation, ZZW and FYD; resources, DZ; data curation, SZ; writing-original draft preparation, SZ and KH; writing-review and editing, YS, ZZW, WL; supervision, WL, FWC; funding acquisition, KH; All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Distribution of microbes and antimicrobial susceptibility in patients with diabetic foot infections in South China

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Background: To investigate the distribution of microbes and drug susceptibility in patients with diabetic foot infections (DFI) and provide guidance for clinical empirical treatment and the rational selection of antibacterial drugs.

Methods: Retrospective analysis of the pathogenic bacterium distribution and antimicrobial susceptibility isolated from 581 DFI patients with different Wagner grades.

Results: The 534 positive samples included 473 cases (88.58%)) of monomicrobial infections and 61 cases (11.42%) of polymicrobial infections before antibiotic therapy. A total of 656 strains were cultivated, including 387 (58.99%) strains of gram-positive organisms (GPOs), 235 (35.82%) gram-negative bacilli (GNB), and 21 (3.20%) fungal strains. Polymicrobial infections mainly occurred in patients with Wagner grade 3-4 ulcers. GPOs were predominant in Wagner grades 1-3 (grade 1: 96.67%, grade 2: 76.52%, grade 3 62.81%), and the most common was Staphylococcus aureus (grade 1: 31.66%, grade 2: 33.04%, grade 3 35.53%). GNB were predominant in grades 4-5 (grade 4: 51.46%, grade 5:60%), and the most common GNB in Wagner grades 4-5 was *Proteus* (grade 4:27.88%, grade 5: 42.86%), while the most common GPO was *Enterococcus* (grade 4:34.48%, grade 5:25.00%). Staphylococcus (including MRSA) and Enterococcus were still highly sensitive to vancomycin, linezolid, and tigecycline. Most GNB were still highly sensitive to meropenem, tigecycline, ertapenem, and amikacin. *Proteus* was most sensitive to amikacin (97.14%), followed by meropenem (92%) and ertapenem (80%).

Conclusion: The distribution of microbes and antimicrobial susceptibility in DFI patients varied with different Wagner grades. The most appropriate antimicrobial therapy should be selected based on the pathogen culture and antimicrobial susceptibility.

KEYWORDS

diabetic foot infection, diabetic foot ulcer, microbes, antimicrobial susceptibility, diabetes

Introduction

Diabetes is an important public health problem. The overall standardized prevalence of total diabetes using the American Diabetes Association (ADA) criteria was 12.8% in 2017 (1). There are estimated to be 129.8 million diabetes patients in mainland China (1). Diabetic foot is the leading cause of diabetes-related hospitalization, which is characterized by longer hospitalization, difficulty in treatment, and high medical costs (2, 3).

Diabetic foot infection (DFI) is one of the most important causes of the deterioration, amputation and death of patients with diabetes and is also a common cause of increased hospitalization and medical expenses (4, 5). Patients with foot ulcers have a high incidence of infections, and 40% to 70% of them have had infections when they seek medical treatment (6). Studies from different countries have shown that different degrees of infection in patients with DFI lead to different pathogenic microorganism distributions and drug sensitivities (7–9). Current studies have found that the microbial distribution of diabetic foot infections varies in different seasons in different countries (10–13). China is a vast territory, and types of diabetic foot bacterial infections are different in different regions (14, 15). Nevertheless, no multicenter studies have been performed to assess the microbial distribution of patients with DFI in China. In this study, we analyzed the clinical characteristics, pathogen distribution, and antimicrobial susceptibility of different Wagner grades in diabetic foot patients to provide a reference for the antimicrobial treatment of DFI.

Methods

Patients

A total of 581 diabetic foot patients hospitalized in the Endocrinology Department of the Third Xiangya Hospital of Central South University from January 1, 2018, to December 31, 2021, were selected as the research subjects, and diabetic foot secretions were collected for microbial culture and drug sensitivity tests. Patients receiving antibiotics 7 days before admission were included. The author should ensure that the work described has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Specimen collection

Patients with DFI should be treated with sterile curette or scalpel before receiving antibiotic treatment, and then clean the wound with sterile saline. The process of wound sample collection was strictly aseptic. For patients with superficial ulcers, sterilized saline cotton swabs were dipped into the secretions or pus at the bottom of the ulcer for collection, and attention was paid to avoid contamination of the skin around the wound. For patients with deep ulcer and foot gangrene, the deep ulcer secretions or pus were collected by probe after debridement. The samples were immediately sent to the laboratory microbiology laboratory for aerobic bacteria, anaerobic bacteria, fungal culture and drug sensitivity tests.

Microbiological assessment

The secretions were inoculated with blood, chocolate, and MacConkey agars plates for aerobic culture. The inoculated medium was incubated in a 5% CO_2 incubator at 35°C for 24-48h. According to the morphology and gram staining characteristics of the colonies, the bacterial identification was carried out by the automatized VITEK 2 Compact system (bioMerieux, France). Kirby-Bauer, VITEK-2 Compact automatic system methods were used to test drug sensitivity. For anaerobic bacteria identification, samples were immediately inoculated on Columbia blood agars plates and Brucella agar plates, and were quickly incubated in an anaerobic incubator at 35°C for 48-72h. The distribution of specific strains was observed, and the strain types were determined with the aid of API20A kit. Drug sensitivity test was performed by using Kirby-Bauer method.

The selection of antimicrobial drugs for various types of bacterial susceptibility testing and the determination of the susceptibility results usually refer to the guidelines of the American Association for Clinical and Laboratory Standardization (CLSI) (16). MDR strains are defined according to the consensus issued by the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) in 2012 (17).

Diabetic foot ulcer grade

The Wagner grade classification is as follows (18): 1) Grade 0: there are risk factors for foot ulceration, but no ulcer; 2) Grade 1: superficial ulcers of the feet without signs of infection; 3) Grade 2: Ulcer extension to the ligament, tendon, joint capsule, or deep fascia without abscess or osteomyelitis; 4) Grade 3: deep infection, bone lesions or abscess; 5) Grade 4:localized gangrene (toe, heel or foot); and 6) Grade 5: fully infected foot. The course of ulcer disease is based on acute and chronic wounds. Chronic ulcers refer to ulcers that have not improved after 4 weeks of treatment or have not been cured within 8 weeks (19).

Statistics

All data were analyzed using SPSS 21.0 software (SPSS, Inc., Chicago, IL,USA). The measurement data are expressed as the mean ± standard deviation, and count data are expressed as a percentage [n (%)]. The chi-square test ($\chi 2$) was used for comparison between groups, and $p < 0.05$ was considered statistically significant.

Results

Basic characteristics of patients

We included 704 secretion culture results from 581 DFI patients, including 65.06% (378/581) male patients and 34.94% (203/581) female patients. Among these patients, there were 573 (98.62%) patients with type 2 diabetes, 7 (1.20%) patients with type 1

diabetes, and 1 (0.17%) patient with latent autoimmune diabetes in adults (LADA).

The mean age of the patients was 61.29 ± 11.5 years, and 57.48% (334/581) of patients were aged 60 years and older. The mean duration of diabetes was 10.45 ± 6.84 years. Patients with different Wagner grades and wound conditions had different average hospital stays. The duration of hospitalization was similar between acute ulcers and chronic ulcers (χ^2 = 0.352, p=0.425). Of the 571 patients, 109 (19.09%) had good glycemic control (HbA1c ≤ 7%), 90 (15.76%) had normal glycemic control (HbA1c 7.1-8), and 372 (65.15%) had poor glycemic control (Table 1).

Pathogen distribution

Before antimicrobial treatment, a total of 534 of the 581 secretion samples (91.57%) cultured pathogens (bacteria/fungi), of which 473 (88.58%) had monomicrobial infections and 61 (11.42%) had polymicrobial infections (number of microorganisms ≥ 2). A total of 20 positive bacteria and 12 negative bacteria were cultured after antibacterial treatment. A total of 656 microorganisms were cultured, including 387 (58.99%) gram-positive organisms (GPO), 235 (35.82%) gram-negative bacteria (GNB), and 21 fungal strains (3.20%). The detection rates of microorganisms were different in different Wager grades (χ^2 = 9.531, p = 0.049), and multi-pathogen infections mainly occurred in patients with Wager grade 3-4 ulcers. GPO were predominant in Wagner grades 1-3 (grade 1: 96.67%, grade 2: 76.52%, grade 3 62.81%), and the most common was Staphylococcus aureus (grade 1: 31.66%, grade 2: 33.04%, level 3 35.53%). GNB were predominant in grades 4 - 5 (grade 4: 51.46%, grade 5:60%). The most common GNB in Wagner grades 1 -5 was Proteus (100% in grade 1, grade 2: 36.36%, grade 3: 32.43%, grade 4: 27.88%, grade 5: 42.86%), and the most common GPO was *Enterococcus* (grade 4: 34.48%, grade 5: 25.00%). The detection rate of fungi was 3.36%, mainly distributed in Wagner grade 2, including Candida albicans (33.33%), Candida tropicalis (33.33%), Candida parapsilosis (19.05%) (Table 2).

The culture positivity rates of MDR, extended-spectrum-lactamase (ESBL), and methicillin-resistant Staphylococcus aureus (MRSA) were 41.31% (271/656), 12.16% (27/222), and 37.41% (52/139), respectively. The highest detection rate of MDR at Wagner grade 4 was 38.02%. The highest detection rate of ESBL at Wagner grade 2 was 18.18%. Wagner grade 1 had the highest incidence rates of MRSA at 16.67%.

The positive rate of pathogenic bacteria in the acute ulcer stage of diabetic foot was 89.45%, of which the positive rate of GPO was 59.63% and that of GNB was 23.39%. The positive rate was 93.34% in the chronic ulcer stage, of which the positive rate of GPO was 53.72%

TABLE 1 Clinical characteristics of patients.

Parameters	Variable	Values (%)
Gender	Male Female	378 (65.06) 203 (34.94)
Age(years)	<40 40-50 50-60 60-70 70-80 >80	16 (2.75) 74 (12.74) 157 (27.02) 183 (31.50) 126 (21.69) 25 (4.30)
Type of diabetes	Type 1 LADA Type 2	7 (1.20) 1 (0.17) 573 (98.62)
Duration of diabetes (years)		10.45 ± 6.84
Duration of hospital stay (days)	Wagner 1 Wagner 2 Wagner 3 Wagner 4 Wagner 5 Duration of ulcer ≤ 4 weeks Duration of ulcer > 4 weeks Average length of hospital stay	11.84 ± 4.62 14.07 ± 6.41 21.43 ± 13.28 24.13 ± 15.94 23.68 ± 13.08 20.02 ± 14.02 20.38 ± 12.99 20.22 ± 13.38
Complication*	Peripheral neuropathy Nephropathy Peripheral vascular Retinopathy	530 (91.22) 265 (45.61) 353 (60.75) 319 (54.90)
HbA1c (%)	≤7 7.1~8 8.1~9 >9	109 (19.09) 90 (15.76) 89 (15.59) 283 (49.56)
Site of ulcers	Two feet Left foot Right foot	115 (19.79) 249 (42.86) 217 (37.35)

*Peripheral neuropathy, nephropathy, peripheral vascular and retinopathy were defined as chronic microvascular complications of diabetes. LADA, latent autoimmune diabetes in adult.

and that of GNB was 35.81%; fungi accounted for 3.86%. The most common GPO in the acute and chronic ulcer period were Staphylococcus at 43.20% and 33.85%, followed by enterococci at 11.06% and 13.08%. Proteus was the most common GNB in the acute (8.40%) and chronic ulcer stages (12.82%) (Figure 1).

Drug sensitivity testing

Staphylococcus was still highly sensitive to vancomycin, linezolid, and tigecycline. Among the 137 strains of Staphylococcus aureus, 2 strains were resistant to vancomycin and 1 strain was intermediary. Two strains were resistant to vancomycin and 3 strains were intermediary in the 37 strains of hemolytic Staphylococcus. Among 33 strains of Staphylococcus epidermidis, 1 strain was intermediary to vancomycin, and 1 strain was intermediary to tigecycline (Table 3). Staphylococcus aureus maintains a high sensitivity to linezolid (100.00%) and tigecycline (100.00%), followed by vancomycin (97.81%), rifampicin (96.35%), sulfamethoxazole (91.24%), and moxifloxacin (83.94%). Other antibacterial drugs were more than 50% sensitive, including clindamycin (51.09%), tetracycline (66.96%), erythromycin (51.09%), ciprofloxacin (71.43%), and levofloxacin (70.80%).

TABLE 2 The distribution of pathogenic bacteria was detected in DFI with different Wagner grades.

		Before antibiotic therapy (%)					After antibiotic therapy
Wagner	1	2	3	4	5	Total	
Total samples	31 (5.50)	122 (21.63)	213 (37.77)	169 (29.96)	29 (5.14)	564	109
Positive samples	28 (90.32)	102 (83.61)	199 (93.43)	160 (94.67)	28 (96.55)	517 (91.67)	32 (29.36)
Total strains	30	115	242	202	35	624	32
single pathogens	27 (87.10)	91 (74.59)	173 (81.22)	138 (81.66)	27 (93.10)	456 (80.86)	32 (100)
Multiple pathogens	1 (3.23)	11 (9.02)	26 (12.21)	22 (13.02)	1 (3.45)	61 (10.82)	0
MDR	9 (3.42)	38 (14.45)	97 (36.88)	100 (38.02)	19 (7.22)	263 (42.14)	8 (25.00)
Gram-positive bacteria	29 (96.67)	88 (76.52)	152 (62.81)	87 (43.07)	12 (34.29)	368 (58.97)	19 (59.38)
Staphylococcus aureus	11 (36.66)	38 (33.04)	54 (35.53)	29 (14.36)	3 (8.57)	137 (26.36)	2 (10.53)
Other Staphylococcus	12 (40.00)	29 (25.22)	42 (17.36)	19 (9.41)	4 (11.43)	106 (28.80)	7 (36.84)
MRSA	5 (16.67)	8 (6.96)	21 (8.68)	15 (7.43)	2 (5.71)	50 (13.59)	2 (10.53)
MRSE/MRSH	3 (10.00)	13 (11.30)	32 (13.22)	12 (5.94)	3(8.57)	61 (16.58)	5 (26.32)
Streptococcus	2 (22.22)	8 (9.09)	15 (9.87)	8 (9.20)	1 (8.33)	34 (9.24)	0
Enterococcus	2 (22.22)	10 (11.36)	34 (22.37)	30 (34.48)	3 (25.00)	79 (21.46)	8 (25.00)
Gram-negative bacteria	1 (3.33)	22 (19.13)	74 (30.58)	104 (51.46)	21 (60.00)	222 (35.57)	13 (40.62)
Klebsiella	0	6 (27.27)	14 (18.92)	14 (13.46)	2 (9.52)	36 (16.21)	0
Escherichia coli	0	2 (9.09)	9 (12.16)	14 (13.46)	4 (19.05)	29 (13.06)	3 (23.08)
Proteus	1 (100)	8 (36.36)	24 (32.43)	29 (27.88)	9 (42.86)	71 (31.98)	2 (15.38)
Enterobacter	0	3 (13.64)	7 (9.46)	14 (13.46)	3 (14.29)	27 (12.16)	0
Citrobacter	0	1 (4.55)	2 (2.70)	5 (4.81)	0	8 (3.60)	0
Morganella	0	1 (4.55)	4 (18.92)	5 (4.81)	1 (4.76)	11 (4.95)	2 (15.38)
Pseudomonas aeruginosa	0	0	4 (5.41)	10 (9.62)	0	14 (6.31)	2 (15.38)
Acinetobacter baumannii	0	0	2 (2.70)	1 (0.96)	0	4 (1.80)	3 (23.0)
Serratia marcescens	0	0	2 (2.70)	3 (2.88)	0	5 (2.24)	1 (7.69)
Bacteroides	0	0	3 (4.05)	3 (2.88)	0	6 (2.69)	0
Stenotrophomonas maltophilia	0	0	0	1 (0.96)	1 (4.76)	2 (0.90)	1 (7.69)
Myroides spp.	0	0	1 (1.35)	1 (0.96)	1 (4.76)	3 (1.35)	0
Aeromonas	0	1 (4.55)	0	3 (2.88)	0	4 (1.79)	0
ESBLs	0	4 (18.18)	8 (10.81)	11 (10.58)	3 (14.29)	26 (11.66)	1 (7.69)
Fungus	0	2 (1.74)	13 (5.37)	5 (2.48)	1 (2.86)	21 (3.20)	0

MDR, multidrug-resistant; ESBL, extended spectrum beta lactamase; MRSA, methicillin-resistant S. aureus; MRSH, methicillin-resistant Staphylococcus *haemolyticus*; MRSE, methicillin-resistant Staphylococcus *epidermidis*.

A total of 111 methicillin-resistant Staphylococcus (MRS) strains were cultured, of which 51 strains were MRSA. MRS maintains had the highest sensitivity to linezolid (100.00%), followed by tigecycline (98.99%), vancomycin (92.98%), and rifampicin (87.72%), and had high resistance to clindamycin (35.96%), levofloxacin (30.70%), ciprofloxacin (34.31%), and erythromycin (22.21%) (Figure 2). A total of 79 cases of Enterococcus were detected, of which Enterococcus faecium maintained 100% sensitivity to linezolid, tigecycline, and vancomycin and had high resistance to ampicillin (11.11%), penicillin G (11.11%), high-concentration gentamicin (28.57%), ciprofloxacin (12.50%), and levofloxacin (33.33%). Enterococcus faecalis was highly sensitive to tigecycline (100.00%), followed by linezolid (98.08%), vancomycin (95.52%), ampicillin (95.59%), penicillin G (95.38%), and high-concentration gentamicin (97.14%), and maintained more than 75% sensitivity to quinolones. Among these, two strains of Enterococcus faecalis were resistant to vancomycin and two were intermediary, and one was resistant to linezolid (Table 3).

Most GNB still maintained high sensitivity to meropenem, tigecycline, ertapenem, and amikacin (Table 4). The Proteus genus had the highest detection rate among GNB and the highest sensitivity to amikacin (97.14%), followed by meropenem (92%), ertapenem (80%), ceftazidime (77.27%), piperacillin and tazobactam (73.91%), levofloxacin (72.46%), aztreonam (72.46%), cefepime (71.43%), and gentamicin (71.21%) and was still highly resistant to tigecycline. A total of 26 ESBL-producing bacteria were detected, which remained 100% sensitive to tigecycline and meropenem, followed by amikacin (88.46%), piperacillin and tazobactam (84.62%), cefoxitin (80.77%), while the sensitivity to quinol was below 40% (Figure 3). The sensitivities of Candida to amphotericin B, caspofungin, micafungin, voriconazole, flucytosine, fluconazole, and itraconazole were 100.00%, 95.00%, 95.00%, 95.00%, 94.44%, 80.95%, and 72.22%, respectively (Figure 4).

Discussion

Diabetic foot can seriously affect patients' daily life and work, reduce patients' health and quality of life, and even threaten patients' life safety. Diabetes increases the risk of cardiovascular disease, and diabetic foot ulcers (DFU) may further increase this risk. A meta-analysis found that DFU were associated with an increased risk of fatal myocardial infarction and fatal stroke (20). DFI is an important factor in the deterioration of diabetic foot (21). Treatment with antibiotics in DFI is imperative to improve outcomes. The initial treatment of diabetic infections is usually empirical and depends on the severity and extent of infection and any available microbiological data. With increase in age, S. aureus, Streptococci and Pseudomonas aeruginosa became more frequent. The proportion of mixed bacterial infection cases in elderly DFI patients was relatively high, and the drug resistance was higher than that in non-mixed infection patients (22). This may be related to the fact that elderly patients with DFI have underlying diseases, organ function decline, peripheral tissue oxygen supply and weak regeneration ability. In addition, some

TABLE 3 Drug sensitivity results of gram-positive bacteria from diabetic foot patients.

	Staphylococcus aureus	Staphylococcus haemolyticus	Staphylococcus epidermidis	Enterococcus faecalis	Enterococcus faecium	Streptococcus
Total strains	137	37	33	68	9	34
Oxacillin (%)	62.77	5.56	9.09	—	—	–
Ampicillin (%)	—	0.00	—	95.59	11.11	90.00
Penicillin G (%)	4.38	100.00	0.00	95.38	11.11	90.00
Macrodantin (%)	94.83	90.63	90.00	94.44	50.00	80.00
Moxifloxacin (%)	83.94	29.73	51.52	84.31	14.29	66.67
Levofloxacin(%)	70.80	13.51	27.27	79.10	33.33	70.59
Ciprofloxacin (%)	71.43	5.88	0.00	76.79	12.50	60.00
Sulfamethoxazole(%)	91.24	45.95	39.39	—	—	–
Tetracycline(%)	66.96	43.75	50.00	16.36	14.29	33.33
Erythromycin (%)	51.09	5.56	18.18	1.47	0	20.00
Clindamycin(%)	51.09	30.56	33.33	—	0	28.00
Gentamicin (%)	91.97	30.56	72.73	97.14	28.57	–
Rifampicin (%)	96.35	75.00	90.91	—	—	–
Tigecycline (%)	100.00	100.00	96.67	100.00	100.00	100.00
Vancomycin(%)	97.81	86.49	96.97	95.52	100.00	100.00
Linezolid (%)	100.00	100.00	100.00	98.08	100.00	100.00
Quinupristin/ dalfotristin (%)	99.21	93.94	9.09	100.00	71.43	83.33

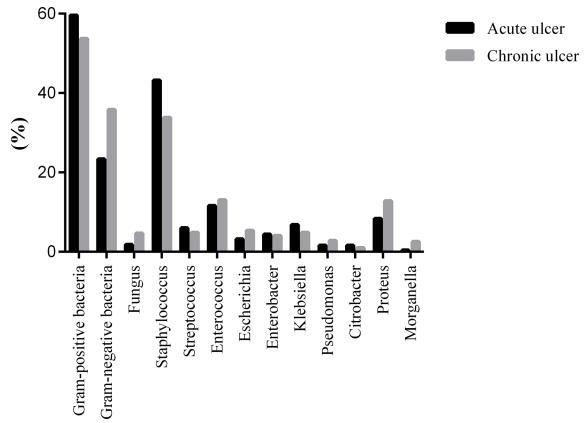

FIGURE 1
The distribution of pathogenic bacteria was detected in DFI with different duration of ulcer.

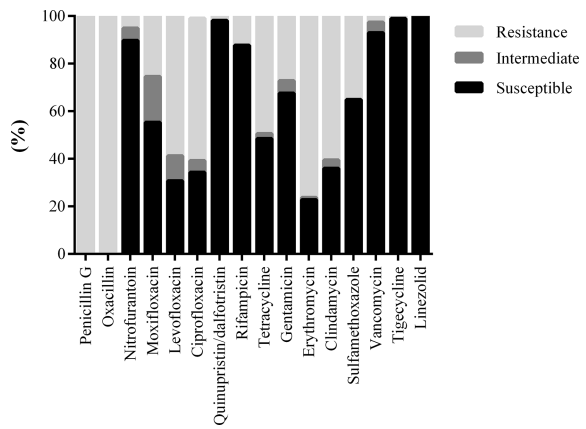

FIGURE 2
Drug sensitivity results of MRS from diabetic foot patients.

patients had received systemic or local antibiotics before admission, which further affected the distribution of pathogenic bacteria on the wound surface. Consistent with most studies (14, 23), DFI occurred in elderly male patients with type 2 diabetes, accompanied by some complications and poor blood glucose control.

The investigation found that GPO (59.75%) predominated in DFI, which was consistent with the survey results of DFI in southern China from 2009 to 2014, with GPO accounting for 54% (23), and different from studies in Southwest China, Beijing area and South India, where GNB accounted for 51%, 57.5%, and 51.4%, respectively (14, 24, 25). The most common GPO was Staphylococcus aureus, consistent with other reports (23, 24, 26–28). Proteus among the GNB was the most frequently isolated in our study, which was different from other reports, such as Pseudomonas aeruginosa (28–30) and Escherichia coli (15, 26). This study further demonstrated that DFI bacteria were different in different regions. Monomicrobial infections were the main cause, consistent with other studies from China (14, 24), accounting for 56.8% andw79.8%, and different from Pakistan and Kuwait, where polymicrobial infections accounted for 56.9% and 75%, respectively (26, 27).

In our study, The pathogen spectrum of DFI patients with high Wagner grade is mainly gram-negative bacilli and multimicrobial infection, especially in patients with Wager 3-4 ulcers, as previously reported (14, 15). Therefore, when using antibiotics empirically, DFI patients with Wagner ≥3 should use a combination of antibiotics or broad-spectrum antibiotics to ensure the simultaneous coverage of GPO and GNB.

In addition, the GPO mainly changed from Staphylococcus aureus to Enterococcus with the increased Wagner grade. Enterococci often appeared in patients with low immunity and could participate in the formation of biofilms (29, 31). The positive rates of pathogenic bacteria in the acute ulcer stage and the chronic ulcer stage of the diabetic foot were similar, and both were mainly GPO. After antimicrobial treatment, the positive rate of bacteria was significantly reduced, and GPO were still predominant, which was different from Southwest China (14).

Increased resistance of pathogenic bacteria is an important problem in the treatment of DFI. The study found that MDR organisms were very common in patients with DFI, which was in accordance with earlier studies (30, 32). In our research, Staphylococcus is the most common MDR organisms, similar to previous studies, followed by Proteus mirabilis (15, 24). The emergence of MDR organisms increases the risk of amputation, mortality, additional morbidity, hospital stay duration and costs of management in patients with DFI (30, 33–35). These instructions indicated that we should adjust antimicrobial drugs in a timely manner based on drug sensitivity and therapeutic effects.

In the present study, the culture positivity of MRSA infection was 37.41%, significantly higher than in previous studies from China (23, 27). Previous studies reported that MRSA is the main cause of suppurative skin and soft tissue infections (36). MRSA infections prolong wound healing times and hospitalization stays, increase the need for surgical procedures, and result in treatment failure (35). Long-term (more than 6 months) antibiotics, long foot wound duration, previous hospitalization history, high blood pressure, anemia, chronic osteomyelitis, and history of MRSA infection have been recognized as the predictive risk factors (37). Our region has a high infection rate of MRSA. According to IDSA guidelines, it has been necessary to cover MRSA regularly in patients with a previous history infection of MRSA, high local prevalence of MRSA, and very severe infection (38). Our research found that GPO, including staphylococcus, enterococcus, and streptococcus, were highly sensitive to vancomycin, linezolid, and tigecycline and were resistant to erythromycin and clindamycin. Staphylococcus aureus and Enterococcus faecalis were still more than 70% sensitive to fluoroquinolone, as previously reported from China (23, 25). Additionally, Bravo-Molina et al. found that fluoroquinolone antibiotics were the most sensitive antibiotics for GPO (19). MRS was highly sensitive to linezolid, tigecycline, vancomycin, rifampicin. For Enterococci, Enterococcus faecium had lower positive rates and higher resistance to ampicillin, penicillin G, high-concentration gentamicin, and fluoroquinolones compared with Enterococcus faecalis, which was consistent with the 2018 CHINET bacterial resistance monitoring results (33).

This study showed that GNB remained highly sensitive to meropenem, tigecycline, and amikacin, but previous studies showed a different pattern of susceptibility (14). Compared to other studies,

TABLE 4 The drug sensitivity results of gram-negative bacteria from diabetic foot patients.

	Escherichia coli	Serratia	Klebsiella	Enterobacter	Proteus	Pseudomonas	Acinetobacter baumannii	Morganella	Citrobacter
Total strains	32	6	36	27	73	17	6	13	8
Macrodantin (%)	64.29	25.00	35.71	12.50	3.17	0.00	0	0	–
Ampicillin(%)	9.38	0	0.00	0.00	15.63	0.00	0	0	100.00
Ampicillin-sulbactam (%)	22.22	0	50.00	100.00	53.85	11.11	0	0	–
Piperacillin-tazobactam (%)	75.00	25.00	91.67	44.00	73.91	53.33	20.00	58.33	83.33
Sulfamethoxazole(%)	34.38	100.00	58.82	54.17	47.06	0.00	50.00	41.67	0
Cefazolin (%)	66.67	0	100.00	0.00	25.00	0.00	–	0	100.00
Cefoxitin (%)	64.29	0	86.67	0.00	66.15	0.00	0	33.33	–
Ceftriaxone (%)	35.71	33.33	73.33	47.37	62.50	8.33	0	58.33	71.43
Ceftazidime (%)	57.14	74.00	86.96	45.45	77.27	100.00	0	50.00	12.50
Cefepime (%)	55.00	80.00	83.33	82.35	71.43	80.00	0	60.00	0
Levofloxacin(%)	40.63	50.00	75.00	51.85	72.46	81.25	33.33	83.33	0
Ciprofloxacin (%)	40.63	50.00	69.44	56.00	63.77	81.25	20.00	50.00	0
Aztreonam (%)	48.39	50.00	69.44	40.74	72.46	–	0	63.64	–
Amikacin (%)	90.63	100.00	91.43	96.00	97.14	100.00	100.00	100.00	71.43
Tobramycin (%)	64.52	80.00	73.53	55.00	69.23	80.00	40.00	50.00	0
Gentamicin (%)	62.96	100.00	74.19	75.00	71.21	76.92	33.33	63.64	0
Meropenem(%)	95.00	100.00	100.00	95.24	92.00	100.00	0	100.00	–
Imipenem (%)	68.75	25.00	63.89	44.00	0.00	62.50	16.67	50.00	100.00
Ertapenem (%)	80.00	100.00	100.00	88.89	80.00	–	–	100.00	71.43
Tigecycline (%)	100.00	100.00	100.00	95.65	5.80	6.67	50.00	0	–

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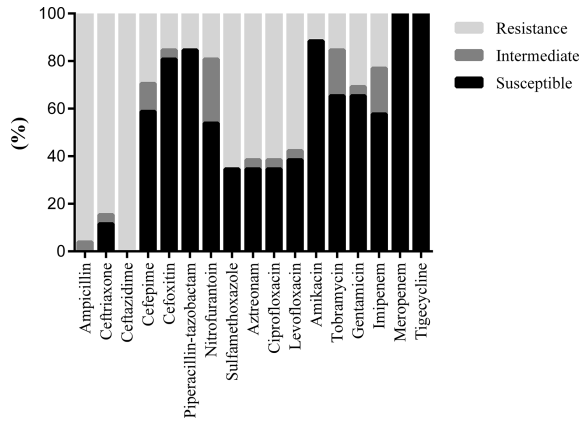

FIGURE 3
Drug sensitivity results of product ESBL bacteria from diabetic foot patient.

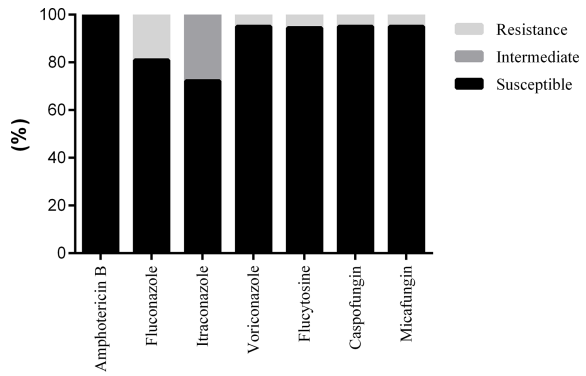

FIGURE 4
Drug sensitivity results of Fungus from diabetic foot patients.

we found that the prevalence of ESBL-producing isolates was higher in patients with DFIs (14). ESBL-producing Enterobacteriaceae showed higher susceptibility to meropenem, tigecycline, piperacillin-tazobactam, and amikacin and a high resistance rate to fluoroquinols. The ESBL-producing bacteria had fewer effective antibacterial drugs and could increase the length of hospital stay in patients with DFI (39).

Conclusion

In conclusion, this study provides a reference for the local bacterial distribution, antimicrobial sensitivity and empirical treatment. In the treatment of DFI, microbiology examination should be performed in a timely manner, and effective antibiotics should be selected to improve the clinical outcomes of DFI patients according to the severity of ulcers and infections, the risk factors of drug-resistant bacteria and antimicrobial susceptibility.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

WL and CW managed the study database, conducted the analysis, and wrote the first draft of the manuscript. LS, WS, WF, and CW edited the manuscript. WL, LS, WS, WF, and CW reviewed the last version of the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Systematic assessment of streptozotocin-induced diabetic metabolic alterations in rats using metabolomics

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Purpose: Type 1 diabetes is characterized by elevated blood glucose levels, which negatively impacts multiple organs and tissues throughout the body, and its prevalence is on the rise. Prior reports primarily investigated the serum and urine specimen from diabetic patients. However, only a few studies examined the overall metabolic profile of diabetic animals or patients. The current systemic investigation will benefit the knowledge of STZ-based type 1 diabetes pathogenesis.

Methods: Male SD rats were arbitrarily separated into control and streptozotocin (STZ)-treated diabetic rats (n = 7). The experimental rats received 50mg/kg STZ intraperitoneal injection daily for 2 consecutive days. Following 6 weeks, metabolites were assessed *via* gas chromatography-mass spectrometry (GC-MS), and multivariate analysis was employed to screen for differentially expressed (DE) metabolites between the induced diabetic and normal rats.

Results: We identified 18, 30, 6, 24, 34, 27, 27 and 12 DE metabolites in the serum, heart, liver, kidney, cortex, renal lipid, hippocampus, and brown fat tissues of STZ-treated diabetic rats, compared to control rats. Based on our analysis, the largest differences were observed in the amino acids (AAs), B-group vitamin, and purine profiles. Using the metabolic pathway analysis, we screened 13 metabolic pathways related to the STZ-exposed diabetes pathogenesis. These pathways were primarily AA metabolism, followed by organic acids, sugars, and lipid metabolism.

Conclusion: Based on our GC-MS analysis, we identified potential metabolic alterations within the STZ-exposed diabetic rats, which may aid in the understanding of diabetes pathogenesis.

KEYWORDS

streptozotocin (STZ), diabetes, gas chromatography mass spectrometry (GC-MS), metabolites, metabolomics

1 Introduction

Diabetes is an endocrine disease characterized by elevated blood glucose level, which can be divided into type 1 diabetes and type 2 diabetes according to the different pathogenesis. Among them, type 1 diabetes is caused by the destruction of pancreatic beta cells, insufficient insulin secretion, leading to hyperglycemia and even ketosis (1). Studies have shown that there will be approximately 8 million type 1 diabetes patients worldwide in 2021, and the prevalence of type 1 diabetes is predicted to increase by 60-107% to reach 135 million to 174 million in 2040. The substantial missing prevalence highlights the premature mortality from type 1 diabetes. Acute complications include diabetic ketoacidosis, which requires urgent management. Chronic complications include microvascular and macrovascular diseases (2). Type 1 diabetes can occur at any age, and while its onset peak incidence still in early adolescence, it is more common in adulthood, with the majority of incident and prevalent cases occurring in adult population. Macrovascular and microvascular complications are the greatest cause of excess morbidity and mortality in the adult population (3). The increasing global burden of type 1 diabetes, particularly in resource-limited low- and middle-income countries, calls for targeted interventions to optimize blood glucose control to reduce acute and chronic complications (4). Diabetes is also a metabolic disease, affecting the body's metabolism of matter and energy. It is speculated that the metabolomics findings of diabetes may reveal biological pathways, aid in the understanding of pathophysiological mechanisms, identify individuals at risk of disease, and benefit the development of novel treatments for type 1 diabetes (5).

Genomics and proteomics offer information about what is likely to happen. In contrast, metabolomics provides data on what is actually happening (Bill Lasley, UC Davis). Metabolomics employs high-throughput detection and data analysis to examine the cellular metabolome, which includes all low molecular weight metabolites (molecular weight <1000) within the cell, aims to realize information modeling and system integration in a branch of systems biology. Metabolomics is a research method based on mass spectrometry, and the common separation approaches are as follows: gas chromatography (GC), liquid chromatography (LC), capillary electrophoresis (CE), and so on (6). Metabolomics is a profitable tool for the identification of novel risk markers. Diabetes differential metabolites identification may highlight underlying mechanisms regulating diabetes pathogenesis. Moreover, it can screen patients at risk of disease, which can be a predictive platform for related warning signals (5, 7).

In recent years, there were multiple metabolomics investigations involving diabetic serum and other tissues, such as the heart (8), liver (9), kidney (10), and hippocampus (11). Based on our search of literature, the present article is the first to comprehensively analyze the metabolomics of STZ-treated diabetic rats. Herein, we employed GC-MS, along with univariate and multivariate analyses to elucidate STZ-based alterations in the serum, heart, liver, kidney, cortex, renal lipid, hippocampus, and brown fat metabolites of diabetic rats. Our findings further our understanding of STZ-based diabetes pathogenesis, and may lead to a new perspective on type 1 diabetes treatments and complications management.

2 Material and methods

2.1 Animals

8 week-old male Sprague-Dawley (SD) rats, averaging 200-230 gram in body weight, were maintained in typical polypropylene cages (three rats/cage), under regulated room temperature (RT) and humidity, with 12/12-hour light-dark cycle. All rats were given ample water as well pelleted regular chow diet for two weeks. Fasting glucose and body weight were measured weekly. Subsequently, the rats were arbitrarily separated into two groups, namely, control (n=7) and STZ-exposed diabetic rats (n=7). Following fasting for 12 hours, each rat in the STZ group was intraperitoneally injected daily with streptozotocin (STZ 50 mg/kg, resuspended in newly prepared 0.1 M, pH 4.5 citrate buffer, and kept on ice prior to usage, low dose STZ model) for two consecutive days to induce diabetes (12). The control rats were provided with the same volume and duration of citrate buffer alone. The STZ dosage was selected based on earlier report (13). In addition, the fasting glucose was measured on the second day after STZ injections to assess the success of the established type 1 diabetes model. Rats with blood glucose ≥11.1mmol/L were considered diabetic. The blood glucose and body weight assessments were performed once a week for 6 consecutive weeks. Our work received ethical approval from the Translational pharmaceutical laboratory, Jining First People's Hospital (protocol number JNRM-2022-DW-011), and followed the animal care and experimentation guidelines of the National Institutes of Health.

2.2 Reagents

STZ, citric acid, and sodium citrate were acquired from Yisheng Biotechnology Co., LTD (Shanghai, China). O-methylhydroxylamine hydrochloride (the purity of 98%) was acquired from J&K Scientific Ltd. (Beijing, China). Heptadecanoic acid (an internal standard, IS, purity ≥98%), N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethyl-chlorosilne (TMCS) were acquired from Sigma-Aldrich (Saint Louis, USA). Pyridine was provided by Macklin Biochemical (Shanghai, China). Chromatographic-grade methanol came from Thermo Fisher Scientific (Waltham, MA,USA). Water was bought from Wahaha Company (Hangzhou, China).

2.3 Sample preparation

Following euthanasia, we extracted blood samples and obtained serum samples using centrifugation (4500×*g*, 5 min). Next, 100 µL serum was combined with 350 µL methanol and 100 µg/mL heptadecanoic acid, prior to centrifugation at 20913 × *g* at 4°C for 10 min. The resulting supernatants were placed in 2-mL tubes, prior to drying at 37°C under nitrogen gas, then combined with 80µL O-methylhydroxylamine hydrochloride (resuspended in pyridine at 15 mg/mL), and maintained at 70°C for 90 min. Next, 100 µL BSTFA carrying 1% TMCS was introduced to the extracts, and maintained at 70°C for 60 min. This was followed by vortexing, centrifugation

(20913 × *g*, 4°C, 2 min), filter filtration using 0.22-μm membrane filter, and analysis using GC-MS.

Tissue samples (50mg; heart, liver, kidney, cortex, renal lipid, hippocampus, and brown fat) underwent homogenization in 1 methanol and 1 mg/mL IS, prior to transfer to 2-mL tubes and centrifugation at 20913 × *g* at 4°C for 10 min. The rest of the protocol mirrored that of the serum samples.

The quality control (QC) samples were described as a combination of control and STZ-treated diabetic rat samples.

2.4 GC-MS analysis

An Agllent 7890B 7000C MSD GC-MS was used to conduct GC-MS analysis. Samples were separated with an HP-5MS fused silica capillary column. Using the carrier helium, 1 μL derivative mixture was operated in split mode (50:1), with flow rates of 3 mL/min front inlet purge and 1 mL/min gas. The temperatures associated with the transfer line, administration, and ion source were 250°C, 280°C, and 230°C, respectively. The GC temperature regimen was as follows: 60°C for 5min, elevation to 300°C, at 8°C/min, then maintenance at 300°C for 5 min. The MS setting employed electron ionization with 20 spectra/s acquisition rate. MS identification was carried out in full scan mode using electrospray ionization (ESI) with m/z range of 50-800.

2.5 Multivariate analysis

MassHunter Workstation Unknown Analysis (Agilent, B.06.00) was employed for QC sample data analysis in GC-MS to generate a spectrum library. Next, the MassHunter Quantitative (Agilent, B.06.00) was employed to select the spectrum library to verify compounds.

SMICA-P 14.1 (Sartorius, Sweden) was employed for differential compound analysis. The original GC-MC-based data was preprocessed in Excel with arrangement and area ratio normalization, and the data set was sorted into a pattern that could be directly imported into SIMCA-P software for analysis. The principal component (PCA), partial least squares discriminant (PLS-DA), and orthogonal partial least squares discriminant analyses (OPLS-DA) were employed for model generation, and the model was fitted automatically. The score and load diagrams were viewed for data analysis, and permutation testing (200 permutations) was performed to verify whether the model was over-fitted. To further identify the differential compounds between control and experimental rats, IBM SPSS (Statistical Package for Social Science) version 26.0 (IBM Corp., Armonk, NY, USA) was employed to perform two-tailed Student's t-tests. Variable importance in projection (VIP) value >1.0 and computed p-value <0.05 were set as the significance threshold.

Pathway assessments were done *via* the MetaboAnalyst 5.0 (https://www.metaboanalyst.ca) and Kyoto Encyclopedia of Genes and Genomes (KEGG; https://www.kegg.jp) analyses. Raw $p < 0.05$ and impact > 0 were set as significance threshold.

3 Results

3.1 Assessment of diabetes in STZ-exposed rats

Following two intraperitoneal injections of STZ, the blood glucose of experimental rats was markedly enhanced, relative to control rats, and the body weight showed no gain during the entire experiment (Table 1).

3.2 Typical GC-MS total ion chromatograms

All chromatograms involving the QC serum and tissue samples displayed strong signals. Typical GC-MS total ion chromatograms (TIC) representatives of the serum, heart, liver, kidney, cortex, renal lipid, hippocampus, and brown fat are displayed in Figure 1.

3.3 Multivariate analysis

The GC-MS results were further verified with the SMICA software, and fitted *via* the OPLS model to identify significant metabolites. IBM SPSS version 26 (IBM Corp., Armonk, NY, USA) was employed for forecasting. Based on our analyses, 18, 30, 6, 24, 34, 27, 27, and 12 metabolites were DE between the control and STZ-treated diabetic rat serum, heart, liver, kidney, cortex, renal lipid, hippocampus, and brown fat tissues, respectively. These parameters (serum: R2X = 0.607, R2Y = 0.981, Q2 = 0.910; heart: R2X = 0.626, R2Y = 0.995, Q2 = 0.906; liver: R2X = 0.554, R2Y = 0.964, Q2 = 0.581; kidney: R2X = 0.734, R2Y = 0.991, Q2 = 0.929; cortex: R2X = 0.739, R2Y = 0.996, Q2 = 0.955; renal lipid: R2X = 0.629, R2Y = 0.993, Q2 = 0.824; hippocampus: R2X = 0.709, R2Y = 0.997, Q2 = 0.969; brown

TABLE 1 The metabolic parameters of control (CON) and STZ-treated diabetic (STZ) rats.

Parameter	Groups	Weeks of observation			
		week0	week2	week4	week6
Body weight	CON	233.70 ± 3.70	248.19 ± 4.02	265.44 ± 3.61	283.26 ± 4.07
(g)	STZ	231.96 ± 3.36	217.93 ± 2.56*	217.05 ± 4.04*	217.05 ± 3.81*
Blood glucose	CON	5.34 ± 0.06	5.54 ± 0.04	5.51 ± 0.05	5.37 ± 0.07
(mmo/L)	STZ	5.34 ± 0.04	15.89 ± 0.46*	15.14 ± 0.61*	14.94 ± 0.54*

values are presented as mean ± standard error (n=7) in each group, and were analyzed using repeated measures one-way ANOVA in the SPSS v26.0 software (IBM Corp., Armonk, NY, USA). P<0.05 was set as the significance threshold between CON (control) and STZ group. *indicates significant difference ($P < 0.05$) between CON and STZ group.

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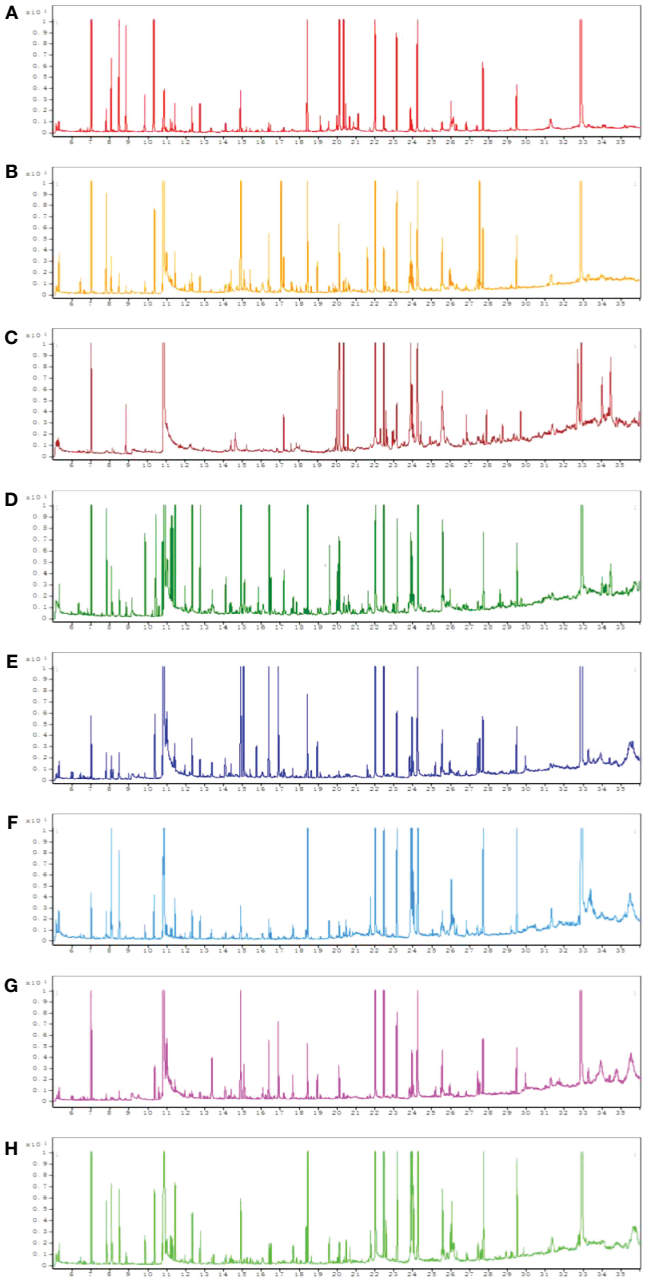

FIGURE 1
Typical gas chromatography–mass spectrometry (GC–MS) total ion chromatograms (TIC) of the quality control (QC) from serum **(A)**, heart **(B)**, liver **(C)**, kidney **(D)**, cortex **(E)**, renal lipid **(F)**, hippocampus **(G)**, and brown fat **(H)**.

fat: R2X = 0.605, R2Y = 0.996, Q2 = 0.815) clearly separated the control from the STZ-treated rats (VIP > 1, $p < 0.05$).

For all parameters, a value closer to 1 represented a better explanatory rate and prediction ability of the overall model. The permutation test carried out sequential replacement of samples, recalculated the statistical test quantity, constructed the empirical distribution, and lastly, computed the OPLS-DA model parameters for judgment. In total, 200 replacement tests were conducted for internal model validation, and to avoid the model from over-fitting. The judgment criterion was as follows: the Q2 intercept on the Y-axis was negative, indicating that over-fitting did not occur (Figure 2).

3.4 Identification of potential differential metabolites

Using SMICA 14.1 and SPSS 26.0 analyses, VIP > 1.0 and $p < 0.05$ were set as the significance threshold. The corresponding compounds were considered as differential compounds between the control and STZ-treated rats. There were 18, 30, 7, 24, 34, 27, 27 and 12 DE metabolites between the control and STZ-treated diabetic rat serum, heart, liver, kidney, cortex, renal lipid, hippocampus, and brown fat tissues, respectively (Table 2). The relative distributions of all differential metabolites in all tissues are expounded in Figure 3.

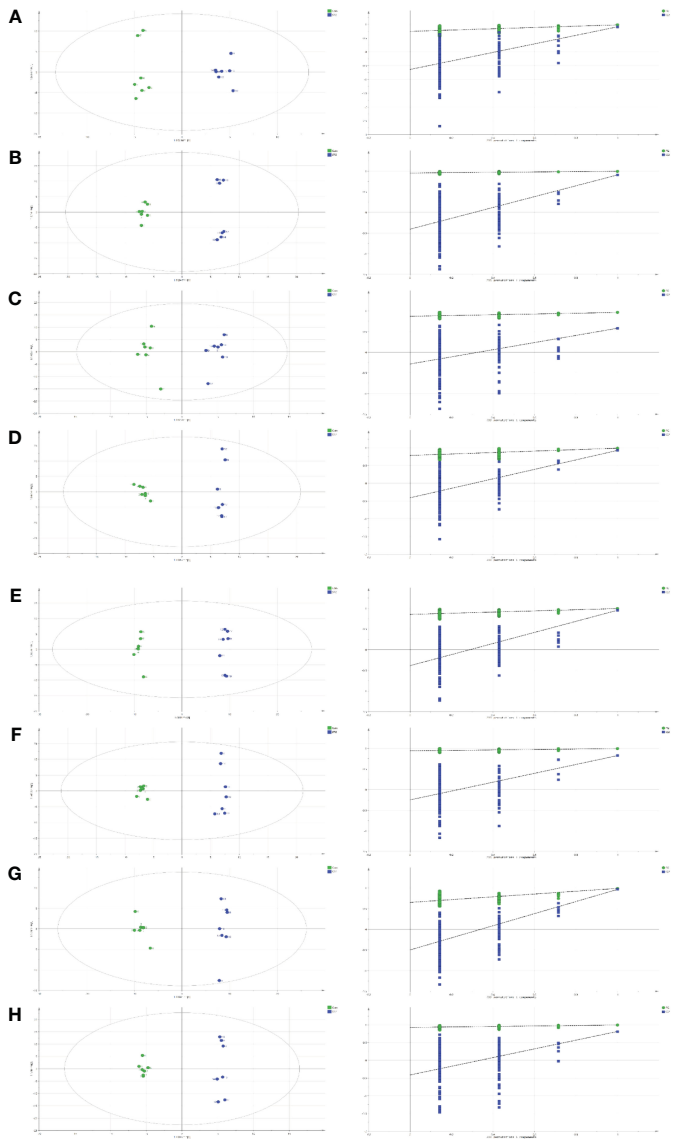

FIGURE 2
Orthogonal projections to the latent structural (OPLS) scores and 200 permutation tests for the OPLS-discriminant analysis (OPLS-DA) models: serum **(A)**, heart **(B)**, liver **(C)**, kidney **(D)**, cortex **(E)**, renal lipid **(F)**, hippocampus **(G)**, and brown fat **(H)**.

3.5 Analysis of metabolic axes

Using metaboAnalyst 5.0, we identified 13 major pathways that were modulated by the DE metabolites (Raw $p < 0.05$, impact > 0). In the serum, they were phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp) biosynthesis; Phe metabolism; glycine (Gly), serine (Ser), and threonine (Thr) metabolism; as well as glyoxylate (Glyox) and dicarboxylate (DIC) metabolism. In the heart, they were glutathione (GSH) metabolism; Gly, Ser, and Thr metabolism; nicotinate and nicotinamide (NAM) metabolism. In the kidney, they were Gly, Ser, and Thr metabolism; Phe, Tyr, and Trp biosynthesis; GSH, Phe, glycerolipid (GL) metabolism; and pentose phosphate pathway(PPP). In the cortex, they were alanine (Ala), aspartate (Asp) and glutamate (Glu) metabolism; Phe, Tyr, and Trp biosynthesis; D-glutamine and D-glutamate metabolism; Phe, Glyox and DIC metabolism; and arginine (Arg) biosynthesis. In the renal lipid, they were Ala, Asp, and Glu metabolism; Arg, Phe, Tyr, and Trp biosynthesis; Phe metabolism; Glyox and DIC metabolism; Gly, Ser, and Thr, Arg and proline (Pro), and butanoate metabolism. In the hippocampus, they were Phe, Tyr, and Trp biosynthesis, Phe metabolism; Ala, Asp, Glu, Glyox, DIC, and GSH metabolism; and Arg biosynthesis.In brown fat, they were Phe, Tyr, and Trp biosynthesis. The network analyses are detailed in Table 3 and Figure 4, and the information is summarized in Figure 5. Furthermore, all network flowcharts are available at KEGG.

4 Discussion

There is an almost complete lack of insulin in type 1 diabetes (14), which acts as an anabolic hormone with multiple effects on sugar, lipid, protein metabolism, growth and development. Patients with type 1 diabetes need to use insulin throughout their life to maintain life, and even regular use of insulin cannot simulate the physiological

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TABLE 2 A summary of the statistically significant differential metabolites expressed in each tissue of the control and experimental rats.

Metabolites	HMDB	VIP	p-value	Fold Change
Serum				
Ethanolamine	HMDB0000149	1.29	1.31E-03	6.29E+00
Glucose	HMDB0000122	1.20	1.38E-02	5.54E+01
Glyceric acid	HMDB0000139	1.47	4.48E-04	3.37E+00
Glycine	HMDB0000123	1.05	3.82E-02	2.56E-01
L-Alanine	HMDB0000161	1.29	9.99E-04	7.95E+00
L-Isoleucine	HMDB0000172	1.29	1.86E-03	5.75E+00
L-Lysine	HMDB0000182	1.54	4.84E-05	1.17E+01
L-Methionine	HMDB0000696	1.54	4.67E-04	1.17E+01
L-Phenylalanine	HMDB0000159	1.44	5.25E-04	3.98E+00
L-Proline	HMDB0000162	1.32	9.39E-04	7.30E+00
L-Threonine	HMDB0000167	1.31	2.74E-03	3.79E+00
L-Tyrosine	HMDB0000158	1.39	2.42E-03	3.94E+00
L-Valine	HMDB0000883	1.30	9.99E-04	7.95E+00
Myo-Inositol	HMDB0000211	1.52	3.30E-05	4.59E-01
Serine	HMDB0062263	1.52	1.06E-02	3.57E+00
Tyrosine	HMDB0000158	1.16	2.53E-02	1.94E+00
Uracil	HMDB0000300	1.19	1.81E-02	2.09E+00
Urea	HMDB0000294	1.14	1.21E-02	3.96E+00
Heart				
4-Hydroxybutanoic acid	HMDB0000549	1.35	1.00E-03	1.35E+00
9H-Purin-6-ol	HMDB0000157	1.15	4.80E-02	4.63E-01
Arachidonic acid	HMDB0001043	1.04	3.10E-02	7.32E-01
Cholesterol	HMDB0000067	1.22	6.00E-03	7.32E-01
Dulcitol	HMDB0000107	1.11	2.30E-02	1.74E+00
Ethanolamine	HMDB0000149	1.30	3.00E-03	3.13E-01
Glyceric acid	HMDB0000139	1.31	1.00E-03	6.32E-01
Glycine	HMDB0000123	1.16	3.20E-02	4.29E-01
L-5-Oxoproline	HMDB0000267	1.16	1.60E-02	4.84E-01
L-Alanine	HMDB0000161	1.29	6.00E-03	3.48E-01
L-Aspartic acid	HMDB0000191	1.26	4.00E-03	3.20E-01
L-Isoleucine	HMDB0000172	1.24	2.10E-02	4.17E-01
L-Lysine	HMDB0000182	1.29	8.00E-03	3.61E-01
L-Proline	HMDB0000162	1.31	4.00E-03	3.29E-01
L-Threonine	HMDB0000167	1.28	1.10E-02	3.75E-01
L-Tyrosine	HMDB0000158	1.30	6.00E-03	3.74E-01
L-Valine	HMDB0000883	1.21	2.00E-02	3.92E-01
Myo-Inositol	HMDB0000211	1.12	8.00E-03	8.07E-01
Niacinamide	HMDB0001406	1.23	2.10E-02	4.85E-01
Oleamide	HMDB0002117	1.29	2.00E-03	3.08E-01

(Continued)

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TABLE 2 Continued

Metabolites	HMDB	VIP	p-value	Fold Change
Palmitic Acid	HMDB0000220	1.15	1.80E-02	7.98E-01
Phosphorylethanolamine	HMDB0000224	1.24	1.20E-02	4.00E-01
Propylamine	HMDB0034006	1.21	2.70E-02	4.63E-01
Putrescine	HMDB0001414	1.11	3.50E-02	4.28E-01
Serine	HMDB0062263	1.11	1.30E-02	3.83E-01
Stearic acid	HMDB0000827	1.25	3.00E-03	6.93E-01
Taurine	HMDB0000251	1.32	2.00E-03	2.78E-01
Trichloroethanol	HMDB0062438	1.32	2.00E-03	2.58E+00
Uracil	HMDB0000300	1.28	5.00E-03	4.23E-01
Urea	HMDB0000294	1.35	1.00E-03	2.76E-01
Liver				
2-linoleoylglycerol	HMDB0011538	1.37	1.70E-02	3.80E-01
Acetic acid	HMDB0000042	1.05	2.70E-02	2.43E-01
Arachidonic acid	HMDB0001043	1.34	3.10E-02	5.67E-01
Doconexent	HMDB0002183	1.34	1.80E-02	2.67E-01
Myristic acid	HMDB0000806	1.39	3.80E-02	5.40E-01
Octaneperoxoic acid, 1,1-dimethylethyl ester	HMDB0032827	1.28	3.70E-02	4.87E-01
Kidney				
4-Hydroxybenzyl alcohol	HMDB0011724	1.24	3.00E-03	5.25E-01
Arachidonic acid	HMDB0001043	1.31	3.00E-03	4.77E-01
Cadaverine	HMDB0002322	1.33	1.00E-03	2.95E-01
Cysteine	HMDB0000574	1.28	1.00E-03	1.88E-01
D-2-Aminobutyric acid	HMDB0000650	1.12	1.00E-03	3.74E-01
D-Gluconic acid	HMDB0000625	1.52	7.73E-11	2.10E-01
Doconexent	HMDB0002183	1.23	1.00E-03	4.22E-01
Ethanolamine	HMDB0000149	1.34	1.61E-04	1.96E-01
Glyceric acid	HMDB0000139	1.02	2.90E-02	4.48E-01
Glycerol	HMDB0000131	1.10	2.70E-02	1.29E+00
Glycine	HMDB0000123	1.12	1.30E-02	3.27E-01
Indole-2-carboxylic acid	HMDB0002285	1.41	7.00E-03	1.62E-01
L-(+)-Lactic acid	HMDB0000190	1.49	1.20E-07	1.72E+00
L-Alanine	HMDB0000161	1.42	4.20E-05	1.47E-01
L-Isoleucine	HMDB0000172	1.33	4.90E-04	2.06E-01
L-Lysine	HMDB0000182	1.37	2.03E-03	1.20E-01
L-Methionine	HMDB0000696	1.28	2.00E-06	2.82E-01
L-Phenylalanine	HMDB0000159	1.24	5.00E-03	3.19E-01
L-Proline	HMDB0000162	1.40	1.03E-04	1.60E-01
L-Threonine	HMDB0000167	1.21	1.00E-02	3.22E-01
L-Tyrosine	HMDB0000158	1.12	2.30E-02	3.41E-01
Octadecane	HMDB0033721	1.10	7.00E-03	3.13E-01

(Continued)

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TABLE 2 Continued

Metabolites	HMDB	VIP	p-value	Fold Change
Serine	HMDB0000187	1.16	1.80E-02	3.35E-01
Urea	HMDB0000294	1.29	1.00E-03	2.85E-01
Cortex				
1-Monopalmitin	HMDB0011564	1.14	4.63E-04	3.35E-01
2-Pyrrolidone-5-carboxylic acid	HMDB0000267	1.07	1.00E-03	1.44E-01
9H-Purin-6-ol	HMDB0000157	1.25	4.70E-05	1.09E-03
Anthranilic acid	HMDB0001123	1.18	4.78E-04	5.32E-03
Arachidonic acid	HMDB0001043	1.09	1.00E-03	3.75E-01
Cholesterol	HMDB0000067	1.16	2.32E-02	2.80E-01
Citric acid	HMDB0000094	1.19	3.13E-04	1.83E-02
Doconexent	HMDB0002183	1.22	5.00E-03	2.49E-01
Ethanolamine	HMDB0000149	1.27	5.00E-06	1.92E-03
Glycerol monostearate	HMDB0011535	1.20	1.50E-05	1.85E-01
Glycine	HMDB0000123	1.20	2.32E-04	1.08E-03
Inosine	HMDB0000195	1.14	1.00E-03	6.48E-03
L-Alanine	HMDB0000161	1.22	1.20E-04	1.13E-03
L-Glutamic acid	HMDB0000148	1.19	4.32E-04	1.34E-04
L-Isoleucine	HMDB0000172	1.25	4.30E-05	2.40E-02
L-Lysine	HMDB0000182	1.18	7.00E-06	2.40E-02
L-Phenylalanine	HMDB0000159	1.26	1.20E-05	1.47E-02
L-Threonine	HMDB0000167	1.26	1.90E-05	3.56E-03
L-Tyrosine	HMDB0000158	1.25	2.20E-05	1.57E-02
L-Valine	HMDB0000883	1.23	8.50E-05	4.27E-03
Malic acid	HMDB0000744	1.09	4.66E-08	3.74E-01
N-Acetylaspartic acid	HMDB0000812	1.02	3.00E-03	2.79E-03
Niacinamide	HMDB0001406	1.26	9.54E-07	2.20E-02
Octadecane	HMDB0033721	1.26	2.00E-03	4.00E-01
Oleamide	HMDB0002117	1.07	5.00E-03	3.08E-02
Palmitic Acid	HMDB0000220	1.17	1.46E-04	3.61E-01
Phosphorylethanolamine	HMDB0000224	1.12	1.00E-03	3.20E-02
Serine	HMDB0000187	1.26	2.40E-05	1.02E-03
Stearic acid	HMDB0000827	1.13	1.00E-03	3.71E-01
Tetrahydrofuran	HMDB0000246	1.25	3.08E-08	3.71E-02
Tromethamine	HMDB0240288	1.12	3.06E-04	2.12E-03
Tyrosine	HMDB0000158	1.17	1.00E-03	2.03E-02
Uracil	HMDB0000300	1.24	5.80E-05	1.58E-02
Urea	HMDB0000294	1.21	2.12E-04	1.58E-02
Renal Lipid				
4-Aminobutanoic acid	HMDB0000112	1.17	4.00E-03	1.53E-01
Acetyl valeryl	HMDB0031476	1.16	2.00E-02	5.67E-01

(Continued)

TABLE 2 Continued

Metabolites	HMDB	VIP	p-value	Fold Change
Anthranilic acid	HMDB0001123	1.30	1.67E-04	9.53E-02
Doconexent	HMDB0002183	1.05	6.00E-03	3.84E-01
Dodecanoic acid	HMDB0000638	1.02	1.40E-02	3.42E-01
Ethanolamine	HMDB0000149	1.32	1.81E-04	3.42E-01
Glyceric acid	HMDB0000139	1.09	7.00E-03	2.88E-01
Glycine	HMDB0000123	1.30	1.00E-03	1.29E-01
L-Alanine	HMDB0000161	1.29	1.00E-03	2.13E-02
L-Aspartic acid	HMDB0000191	1.32	1.99E-04	1.56E-01
L-Glutamic acid	HMDB0000148	1.33	1.36E-04	9.14E-02
L-Isoleucine	HMDB0000172	1.32	1.63E-03	9.40E-02
L-Lysine	HMDB0000182	1.33	3.70E-05	3.24E-02
L-Methionine	HMDB0000696	1.36	2.12E-04	5.68E-02
L-Phenylalanine	HMDB0000159	1.35	2.80E-05	5.30E-02
L-Proline	HMDB0000162	1.32	1.00E-04	7.22E-02
L-Threonine	HMDB0000167	1.33	1.22E-04	1.02E-01
L-Valine	HMDB0000883	1.30	2.50E-02	9.64E-02
Malic acid	HMDB0000744	1.22	3.00E-03	2.72E-01
Nonadecanoic acid	HMDB0000772	1.02	7.00E-03	4.18E-01
Palmitic Acid	HMDB0000220	1.17	1.80E-02	4.13E-01
Serine	HMDB0062263	1.36	3.20E-05	6.20E-02
Stearic acid	HMDB0000827	1.16	2.30E-02	4.06E-01
Tranexamic acid	HMDB0014447	1.28	3.78E-04	1.85E-01
Tyrosine	HMDB0000158	1.34	4.70E-05	3.92E-02
Uracil	HMDB0000300	1.30	1.00E-03	1.70E-01
Urea	HMDB0000294	1.28	2.00E-03	1.13E-02
Hippocampus				
1-Monopalmitin	HMDB0011564	1.20	4.20E-05	3.61E-01
1-Octadecanol	HMDB0002350	1.26	1.53E-08	9.16E-02
2-Pyrrolidinone	HMDB0002039	1.22	9.50E-05	5.78E-02
9H-Purin-6-ol	HMDB0000157	1.27	7.00E-06	7.29E-04
Arachidic acid	HMDB0002212	1.14	3.88E-04	3.11E-01
Arachidonic acid	HMDB0001043	1.23	7.00E-06	3.87E-01
Cholesterol	HMDB0000067	1.17	1.39E-04	3.71E-01
Citric acid	HMDB0000094	1.08	2.00E-03	8.85E-03
Diethanolamine	HMDB0004437	1.28	3.89E-07	2.30E-04
Doconexent	HMDB0002183	1.13	2.57E-04	3.53E-01
Glycerol monostearate	HMDB0011535	1.22	4.00E-06	3.00E-01
Glycine	HMDB0000123	1.25	3.20E-05	4.03E-04
Inosine	HMDB0000195	1.20	1.84E-04	1.23E-02
L-5-Oxoproline	HMDB0000267	1.19	1.70E-05	1.22E-01

(Continued)

TABLE 2 Continued

Metabolites	HMDB	VIP	p-value	Fold Change
L-Alanine	HMDB0000161	1.19	3.35E-04	1.17E-03
L-Glutamic acid	HMDB0000148	1.27	1.00E-05	1.40E-04
L-Phenylalanine	HMDB0000159	1.28	7.86E-07	2.57E-02
L-Threonine	HMDB0000167	1.28	3.00E-06	1.26E-03
Niacinamide	HMDB0001406	1.28	4.55E-10	3.68E-02
Palmitic Acid	HMDB0000220	1.25	3.08E-07	3.23E-01
Phosphorylethanolamine	HMDB0000224	1.13	2.00E-03	1.30E-02
Pyroglutamic acid	HMDB0000267	1.06	1.00E-02	2.45E+01
Serine	HMDB0062263	1.28	4.00E-06	1.25E-03
Stearic acid	HMDB0000827	1.22	7.00E-06	3.62E-01
Tyrosine	HMDB0000158	1.24	4.90E-05	2.22E-02
Uracil	HMDB0000300	1.29	1.57E-07	1.34E-02
Urea	HMDB0000294	1.21	2.24E-04	6.90E-03
Brown Fat				
4-Hydroxybutanoic acid	HMDB0000549	1.56	4.00E-03	4.27E-02
Arachidonic acid	HMDB0001043	1.16	3.40E-02	5.90E-01
Doconexent	HMDB0002183	1.33	1.60E-02	4.62E-01
L-Lysine	HMDB0000182	1.27	3.20E-02	4.29E-01
L-Tyrosine	HMDB0000158	1.28	3.10E-02	4.23E-01
Mephobarbital	HMDB0014987	1.15	1.70E-02	4.23E-01
Oleamide	HMDB0002117	1.20	2.80E-02	3.72E-01
Pentadecanoic acid	HMDB0000826	1.53	1.00E-03	4.52E-01
Phenol	HMDB0000228	1.58	2.71E-04	4.20E+00
Phosphorylethanolamine	HMDB0000224	1.58	3.80E-02	4.17E-01
Tranexamic acid	HMDB0014447	1.22	3.50E-02	5.31E-01
Uracil	HMDB0000300	1.29	4.60E-02	4.60E-01

endogenous insulin secretion pattern (15), resulting in large fluctuations in blood sugar, enhanced health care costs (16), and reduced quality of life (17). STZ is an antitumor antibiotic produced from a certain type of streobasidium pullulans. It possesses a targeted destructive effect on pancreatic β cells, and it compromises insulin production to induce diabetes (18). Different dosages of STZ is known to induce different models of diabetes (19). Metabolomics involves an extensive analysis of metabolites within biological systems, namely, low-molecular weight biochemical compounds like sugars, lipids, amino acids (AAs), organic acids, and nucleotides (20). In this study, STZ-treated type 1 diabetic rats were employed for the analysis of compounds with varying performances in the serum and key target tissues of control and experimental rats, under conditions of hyperglycemia. Our goal was to better understand the type 1 diabetes pathogenesis in order to develop novel targets that prevent and/or treat type 1 diabetes and its complications.

As seen in Table 1, type 1 diabetic rat models were generated *via* two intraperitoneal injections of STZ (50mg/kg) in ice-cold citrate buffer (pH7.4). Roche blood glucose meter was used to monitor blood glucose weekly, and a blood glucose value greater than 11.1mmol/L was considered to indicate diabetes. The above data revealed that the rat models were successfully established.

We are the first to report an analysis of metabolic alterations in the serum, heart, liver, kidney, cortex, renal lipid, hippocampus, and brown fat of STZ-induced type 1 diabetic rats versus controls. This study revealed that, compared to controls, there were 18, 30, 6, 24, 34, 27, 27, and 12 DE compounds in the serum, heart, liver, kidney, cortex, renal lipid, hippocampus, and brown adipocytes of STZ rats, respectively. These compounds are related to one another, and they participate in metabolic networks associated with sugars, AAs, and energy metabolism. Based on our analysis of these DE compounds and related pathways, we gained a deeper understanding of the metabolic alterations caused by type 1 diabetes. These metabolites and metabolic networks are potential candidate for the indication of early alterations with diabetes, which may enable us to comprehensively understand type 1 diabetes pathogenesis, and provide new targets for its management.

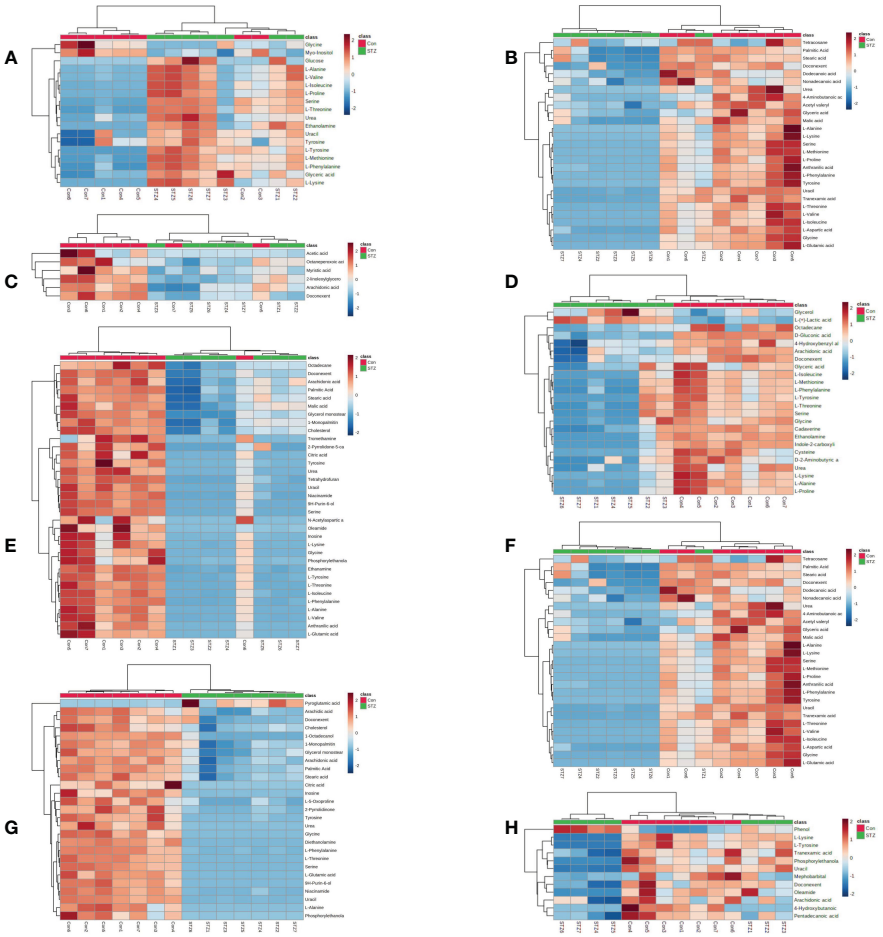

FIGURE 3
significance (red, elevated; blue, diminished). Rows denote samples, columns denote metabolites.The heatmap of differentially regulated metabolites from the serum **(A)**, heart **(B)**, liver **(C)**, kidney **(D)**, cortex **(E)**, renal lipid **(F)**, hippocampus **(G)**, and brown fat **(H)** of STZ and control rats. The color of each section signifies its alteration.

TABLE 3 List of metabolic pathways, as evidenced by MetaboAnalyst5.0.

Pathway Name	Raw p	Impact
Serum		
Phenylalanine, tyrosine and tryptophan biosynthesis	6.71E-04	1.00E+00
Phenylalanine metabolism	4.84E-03	3.57E-01
Glycine, serine and threonine metabolism	4.88E-03	2.70E-01
Glyoxylate and dicarboxylate metabolism	4.63E-02	1.85E-01
Heart		
Glutathione metabolism	1.41E-02	1.03E-01
Glycine, serine and threonine metabolism	2.20E-02	2.70E-01
Nicotinate and nicotinamide metabolism	3.05E-02	1.94E-01
Kidney		
Glycine, serine and threonine metabolism	9.52E-04	2.70E-01
Phenylalanine, tyrosine and tryptophan biosynthesis	1.13E-03	1.00E+00
Glutathione metabolism	6.46E-03	9.22E-02

(Continued)

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TABLE 3 Continued

Pathway Name	Raw p	Impact
Phenylalanine metabolism	8.08E-03	3.57E-01
Glycerolipid metabolism	2.05E-02	3.30E-01
Pentose phosphate pathway	3.74E-02	4.71E-02
Cortex		
Alanine, aspartate and glutamate metabolism	1.49E-03	2.84E-01
Phenylalanine, tyrosine and tryptophan biosynthesis	1.98E-03	1.00E+00
D-Glutamine and D-glutamate metabolism	4.84E-03	5.00E-01
Phenylalanine metabolism	1.39E-02	3.57E-01
Glyoxylate and dicarboxylate metabolism	2.03E-02	1.38E-01
Arginine biosynthesis	2.68E-02	1.17E-01
Renal Lipid		
Alanine, aspartate and glutamate metabolism	9.71E-04	5.07E-01
Arginine biosynthesis	1.35E-03	1.17E-01
Phenylalanine, tyrosine and tryptophan biosynthesis	1.59E-03	1.00E+00
Phenylalanine metabolism	1.12E-02	3.57E-01
Glyoxylate and dicarboxylate metabolism	1.51E-02	1.85E-01
Glycine, serine and threonine metabolism	1.64E-02	2.70E-01
Arginine and proline metabolism	2.40E-02	1.88E-01
Butanoate metabolism	2.49E-02	3.18E-02
Hippocampus		
Phenylalanine, tyrosine and tryptophan biosynthesis	1.35E-03	1.00E+00
Phenylalanine metabolism	9.59E-03	3.57E-01
Alanine, aspartate and glutamate metabolism	8.29E-03	1.97E-01
Glyoxylate and dicarboxylate metabolism	1.21E-02	1.38E-01
Glutathione metabolism	8.29E-03	1.15E-01
Arginine biosynthesis	1.87E-02	1.17E-01
Brown Fat		
Phenylalanine, tyrosine and tryptophan biosynthesis.	3.06E-02	5.00E-01

Our analyses uncovered that AAs serve critical functions in STZ-treated type 1 diabetes conditions. Herein, a variety of AA metabolites were DE in the STZ-treated diabetes rats, relative to the controls. These included Cysteine (Cys), Gly, L-5-Oxoproline, L-Ala, L-Asp, L-Glu, L-Isoleucine (Ile), L-Lysine, L-Methionine (Met), L-Phe, L-Proline (Pro), L-Thr, L-Tyr, L-Valine (Val), Pyroglutamic acid, Ser, and Tyr. The associated metabolic networks were as follows: Ala, Asp, Glu, Arg, Pro, Arg, D-glutamine, D-glutamate, Gly, Ser, Thr, Phe, Tyr, and Trp biosynthesis.

Type 1 diabetes occurs in the absence or insufficiency of insulin secretion (21). This often accompanies oxidative stress and alterations in the glucose and lipid metabolism (22–24). Branched-chain AAs (BCAAs) include Leucine (Leu), Ile, and Val, as well as aromatic AAs (AAAs) like Phe, Tyr, and Trp. Previous studies suggested that the serum concentration of BCAAs and AAAs are related to insulin resistance (25–28), and that they can predict future type 2 diabetes development (29). In addition to adverse effects on insulin sensitivity, elevated plasma BCAAs stimulate insulin secretion, deplete insulin reserves in early type 1 diabetes, impair pancreatic β cell function, and ultimately leads to insulin deficiency (30). The levels of BCAAs are also elevated in model animals with type 1 diabetes. The mechanism may be that in the state of insulin deficiency, the amination of BCAAs in the visceral tissue liver is activated, the absorption of BCAAs in the muscle tissue is reduced, the hydrolysis of total muscle protein is increased, the concentration of BCAAs is increased (31). In this study, the levels of BCAAs (Ile, Val) and AAAs (Phe, Tyr) in the serum of STZ rats were markedly elevated, compared to controls, thus corroborating the data from previous investigations. Some investigations also revealed that alterations in BCAAs and AAAs levels also precede increases in blood sugar (32).

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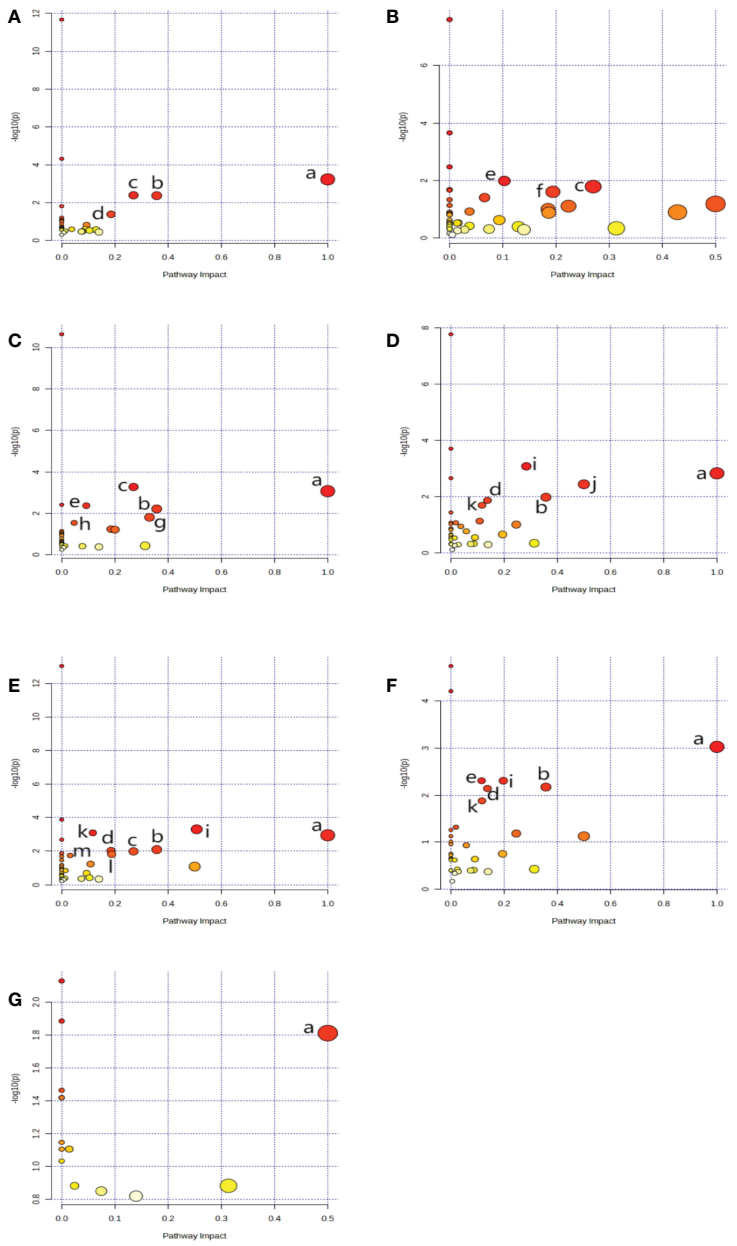

FIGURE 4
A network analysis summary, as evidenced by MetaboAnalyst 5.0. Serum **(A)**, Heart **(B)**, kidney **(C)**, cortex **(D)**, renal lipid **(E)**, hippocampus **(F)**, and brown fat **(G)**. Metabolisms or biosynthesis of (a) Phenylalanine, tyrosine, and tryptophan, (b) Phenylalanine, (c) Glycine, serine, and threonine, (d) Glyoxylate and dicarboxylate, (e) Glutathione (f) Nicotinate and nicotinamide, (g) Glycerolipid, (h) Pentose phosphate, (i) Alanine, aspartate, and glutamate, (j) D-Glutamine and D-glutamate, (k) Arginine, (l) Arginine and proline, (m) Butanoate.

Gluconeogenesis is essential for glucose homeostasis (33). We observed elevated serum glycolic AAs (Ala, Met, Pro, Val, and Ser) levels in STZ-treated diabetic rats, which may induce hyperglycemia *via* hepatic or renal gluconeogenesis. Meanwhile, the circulating ketogenic AAs (Lys) and ketogenic and glycolic AAs (lle, Phe, Thr, and Tyr) concentrations increased, suggesting that the body weight of STZ rats was lower, compared to the control rats likely due to insufficient energy synthesis, reduced anabolism, and enhanced catabolism during diabetes. As previously revealed, Ile, Phe, Tyr, and Val all have "dual" identities, which further affect Phe, Tyr, and Trp biosynthesis, as well as Gly, Ser, and Thr metabolic networks. This causes a series of metabolic alterations mediated by diabetes. Our study supported the relationship between AA concentrations and diabetes risk observed in recent years (32, 34), and it facilitated the identification of a potential mechanism to explain this relationship in the future. Alterations in Ala concentration, observed in our study, were related to the metabolic pathways of Glyox and DIC. Using the Glyox cycle, fats are converted to sugars, and the resulting DIC is used as a supplement for compounds in the tricarboxylic acid (TCA) cycle, thereby affecting energy metabolism (4). Elevated Glyox levels may be a new metabolic feature of type 1 diabetes mellitus and its pathophysiology. These findings may help in treating STZ-induced type 1 diabetes.

Gly, one of the simplest AAs and a substrate for GSH biosynthesis, is known to enhance antioxidant defenses. Our study

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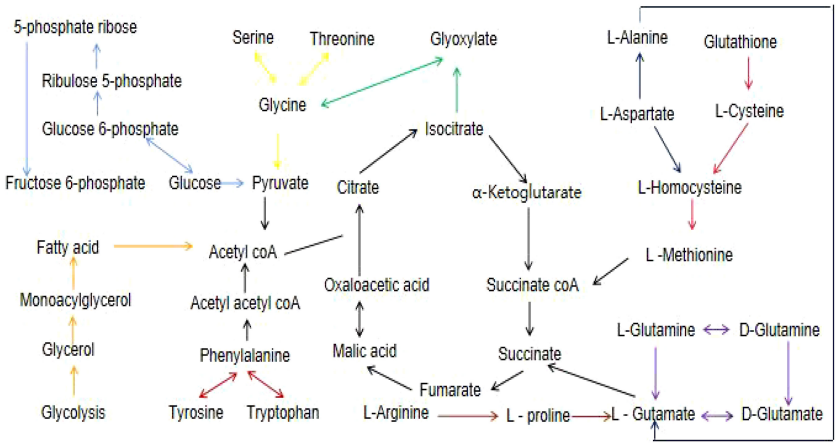

FIGURE 5
A flow chart of the metabolites and their associated networks in the target tissues of STZ-treated diabetic rats versus controls. Red arrows: phenylalanine, tyrosine and tryptophan biosynthesis; light blue arrows: pentose phosphate pathway; yellow arrows: glycine,serine, and threonine metabolism; green arrows: glyoxylate and dicarboxylate metabolism; blue arrows: alanine, aspartate, and glutamate metabolism; pink arrows: glutathione metabolism; purple arrows: D-glutamine and D-glutamate metabolism; orange arrows: glycerolipid metabolism; brown arrows: arginine and proline metabolism.

observed a decrease in Gly levels in the serum, heart, kidney, cortex, renal lipids, and hippocampus of STZ-treated type 1 diabetic rats, suggesting that the antioxidant defenses were weakened when the blood glucose was elevated (35). Asp serves as a carrier for K^+ and Mg^{2+} ions, and it delivers these electrolytes to the myocardium to improve myocardial systolic activity, while lowering oxygen consumption. This has a protective effect on the myocardium, particularly during hypoxia. A previous study revealed that Asp protects the heart (36). A decrease in the cardiac Asp levels in experimental rats suggests that the protective mechanism of the myocardia was affected in presence of elevated blood glucose. Glu provides energy to the brain tissue, and improves the maintenance of brain function (37). The levels of L-Glu in the hippocampal tissue of experimental rats diminished, suggesting that the energy metabolism of the brain was inhibited during hyperglycemia. In addition, a recent study revealed that the levels of plasma Glu also dropped significantly in people with type 1 diabetes (38). Taurine, a sulfurated amino acid derivative, alleviates hyperglycemia induced by STZ in type 1 diabetic mice, inhibits oxidative stress, and improves diabetes and its complications by upregulating glucose transporter (GLUT-2) expression (39). The reduction of taurine levels in the heart of STZ rats will lead to further studies on what role taurine plays.

Type 1 diabetes exhibits mild dyslipidemia (40), and vascular sclerosis (41). Changes in lipid metabolism have important effects on cell function, metabolism, inflammation and oxidative stress (42). Cardiovascular disease is the leading cause of morbidity and mortality in patients with type 1 diabetes (43). Free fatty acids (FFAs) have been concerned to replace glucose as the main energy source for the heart of patients with type 1 diabetes (44). FFAs are typically categorized as either saturated or unsaturated FFA. Unsaturated FFA are further separated into mono- and polyunsaturated FA (PUFA). Based on our analysis, relative to the control, no FFA alterations were detected in the serum of STZ-treated rats. However, the contents of PUFA arachidonic (ARA) and docosahexaenoic acids (DHA) were reduced in the heart, liver, kidney, cortex, renal lipid, hippocampus, and brown fat of STZ-treated rats. DHA is an omega-3 essential fatty acid, which is essential for brain structure and activity maintenance (45), and it is known to reduce the risk of heart disease (46). ARA is a PUFA that serves as a substrate for a range of bioactive compounds synthesis, such as, prostaglandins, thrombutane, and leukotrienes (47). Many brain disorders, such as, Alzheimer's disease and bipolar disorder (48, 49), appear to be related to PUFA metabolic disorders. The levels of ARA and DHA were reduced in the target organs of diabetic rats. The lipotoxicity hypothesis is widely accepted (50), and it may be related to the involvement of saturated FA. In this study, the levels of palmitic, stearic(also known as octadecanoic acid or C18:0), arachidonic, myristic (also known as tetracanoic acid or C14:0), and pentacarboxylic in the heart, liver, cortex, kidney lipid, hippocampus, and brown adipose tissue of STZ-treated diabetic rats were markedly diminished, compared to controls. Other lipids 1-monoflavin-MG (0:0/18:2(9Z,12Z)/0:0) and glycerin monostearate MG (0:0/18:0/0:0) in the above target tissues were also reduced, relative to controls. The serum exhibited no abnormality of lipid metabolism. It is speculated that in the early stages of type 1 diabetes, each key target organ actively self-regulates to maintain a normal lipid metabolism in the blood. Alterations in these tissue metabolites occur earlier than the detectable alterations in the blood. Thus, the tissue metabolites hold great potential as candidate differential metabolites for predicting type 1 diabetes-related vascular complications.

The TCA or citric acid cycle (51), is a robust biological system of producing energy *via* sugar or other substances oxidization. It is the final metabolic network of the three nutrients (sugars, lipids and AAs), and the hub of metabolic interactions among sugars, lipids, AAs, nucleic acids, and energy metabolism. Type 1 diabetes is often associated with energy metabolism disorders. In this study, we also revealed that the serum Glu levels in STZ rats were elevated, the citric acid levels in the cortex and hippocampus were diminished, and the lactic acid content in the kidney was increased, compared to controls. This likely involved the pentose phosphate pathway and Ala-Asp-Glu metabolism. The aforementioned data suggested that, when insulin deficiency causes hyperglycemia, glucose decomposition and utilization become impaired, Ala is transformed into pyruvate and

glutamic acid *via* joint deamination in the body, and pyruvate is acted upon by pyruvate dehydrogenase complex to generate acetyl coenzyme A, which enters the TCA cycle and generates ATP to energize the body.

Glu, Gly, and Cys combine to form GSH (52). GSH metabolism interacts with toxins or drugs to eliminate their toxic effects from the body, and being a strong reducing agent, GSH also participates in a variety of REDOX reactions *in vivo*. In STZ rats, we demonstrated diminished contents of Glu in the cortex, renal lipid, and hippocampus; Gly in the serum, heart, kidney, cortex, renal lipid, hippocampus; and cysteine in the kidney. The raw materials for GSH synthesis were thus reduced, therefore diminishing the antioxidant capacity of the body.

In addition, we also observed marked decreases in the levels of hypoxanthine (9h-Purin-6-ol), hypoxanthosine(inosine), and uracil in the heart, kidney, cortex, renal lipid, hippocampus, and brown adipocytes of STZ rats. These metabolites are strongly correlated with nucleotide synthesis and inflammation prevention. Thus, hypoxanthine, hypoxanthosine, and Ura are excellent candidates as indicators of early type 1 diabetes.

Among the limitations of our research are the following: we only employed a singular metabolomics platform, GC-MS. To better understand type 1 diabetes pathogenesis, additional investigations, based on a combination of metabolomics, proteomics, and transcriptomics, are required to validate our findings. In addition, it is critical to elucidate the effect of STZ on other tissues, such as, lungs, spleen, stomach, pancreas, skin, bladder, and nerves, to fully understand the mechanisms of STZ-induced diabetes.

5 Conclusion

Using GC-MS analysis, we extensively evaluated the metabolic alterations occurring within STZ-treated type 1 diabetic rat serum, heart, liver, kidney, cortex, renal lipid, hippocampus, and brown fat. We observed marked alterations related to the AAs, sugars, lipids, and energy metabolism in the STZ-treated rats versus controls. Overall, our data provided the first systematic analysis of STZ-induced type 1 diabetes, which may aid in the enhanced comprehension of STZ-induced type 1 diabetes and related complications pathogenesis. Type 1 diabetes has multifactorial etiology, and it demands further investigations based on the metabolic examinations in humans, animals, and cells.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by the Ethics Committeee of Jining First People's Hospital (No. JNRM-2022-DW-011, Jining, China).

Author contributions

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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The association between hemoglobin A1c and all-cause mortality in the ICU: A cross-section study based on MIMIC-IV 2.0

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Background: Hyperglycemia has been reported to be associated with the outcomes of patients in the intensive care unit (ICU). However, the relationship between hemoglobin A1c (HbA1c) and long-term or short-term mortality in the ICU is still unknown. This study used the Medical Information Mart for Intensive Care (MIMIC)-IV database to investigate the relationship between HbA1c and long-term or short-term mortality among ICU patients without a diabetes diagnosis.

Methods: A total of 3,154 critically ill patients without a diabetes diagnosis who had HbA1c measurements were extracted and analyzed from the MIMIC-IV. The primary outcome was 1-year mortality, while the secondary outcomes were 30-day mortality and 90-day mortality after ICU discharge. HbA1c levels were classified into four levels according to three HbA1c values (5.0%, 5.7%, and 6.5%). The Cox regression model was used to investigate the relationship between the highest HbA1c measurement and mortality. Finally, this correlation was validated using the XGBoost machine learning model and Cox regression after propensity score matching (PSM).

Results: The study eventually included 3,154 critically ill patients without diabetes who had HbA1c measurements in the database. HbA1c levels of below 5.0% or above 6.5% were significantly associated with 1-year mortality after adjusting for covariates in Cox regression (HR: 1.37; 95% CI: 1.02–1.84 or HR: 1.62; 95% CI: 1.20–2.18). In addition, HbA1c 6.5% was linked to 30-day mortality (HR: 1.81; 95% CI: 1.21–2.71) and 90-day mortality (HR: 1.62; 95% CI: 1.14–2.29). The restricted cubic spline demonstrated a U-shaped relationship between HbA1c levels and 1-year mortality. The AUCs of the training and testing datasets in the XGBoost model were 0.928 and 0.826, respectively, while the SHAP plot revealed that HbA1c was somewhat important for the 1-year mortality. Higher HbA1c levels in Cox regression were still significantly associated with 1-year mortality after PSM for other factors,

Conclusions: The 1-year mortality, 30-day mortality, and 90-day mortality rates for critically ill patients after discharge from ICU are significantly associated with HbA1c. HbA1c < 5.0% and ≥6.5% would increase 30-day, 90-day, and 1-year

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mortality, while levels between 5.0% and 6.5% of HbA1c did not significantly affect these outcomes.

KEYWORDS

hemoglobin A1c, mortality, the Medical Information Mart for Intensive Care, diabetes, cross-sectional study

Background

Currently, the mortality rate of critically ill patients in intensive care units (ICUs) remains high. According to previous studies, the global ICU mortality rate is approximately 15.5%–16.9% (1), and the ICU mortality rate in France is approximately 15% (2), while the ICU mortality rate in Norway is approximately 12.7% and 30-day mortality is approximately 21.2% (3). ICU or in-hospital mortality is significant; moreover, mortality following ICU discharge also has a significant impact on critically ill patients' quality of life (4, 5). To treat the patients' varied complications, several actions will be taken.

Even if these patients were previously normoglycemic, stress-induced hyperglycemia in an ICU is a typical consequence (6). In ICUs, stress and hyperglycemia are related. High quantities of counter-regulatory hormones such as glucagon, adrenaline, cortisol, and growth hormone are released in response to stress in the ICU, promoting hepatic gluconeogenesis and insulin resistance, which result in hyperglycemia (7, 8). Insulin resistance inhibits insulin-mediated glucose transport. Thus, in the internal environment, there is less glucose consumption and more gluconeogenesis, resulting in hyperglycemia (9). Hyperglycemia occurs more frequently in critical settings (10). According to a study, hyperglycemia is a sign of serious illness or trauma and a stand-alone predictor of poor outcomes in critically ill patients in the ICU (11, 12). Elevated blood sugar levels led to prolonged ICU and in-hospital stay and a higher incidence of infection or death (13, 14).

Previous studies suggested that hyperglycemia or hemoglobin A1c (HbA1c) might be associated with a poor prognosis in critically ill patients without diabetes (15). Several studies have found that HbA1c predicts ICU mortality in COVID-19 severe pneumonia, with patients with severe COVID-19 pneumonia having a higher 28-day mortality (16). Furthermore, compared to COVID-19 patients with a diabetes diagnosis, critically ill and COVID-19 patients with HbA1c levels above 6.5% and no prior diagnosis of diabetes had the highest risk of all-cause mortality (17). Furthermore, there was a study reporting that critically ill patients with unknown diabetes had higher mortality and higher glycemic variability (18), which were reported to be independently associated with in-hospital mortality and 30-day mortality in the ICU (19, 20). The relationship between HbA1c and long- or short-term outcomes following ICU discharge, on the other hand, has not been studied. One study showed that in-hospital control of glucose parameters reduced the length of stay and decreased 30-day and 1-year mortality in critically ill patients in cardiac ICU (21).

Based on the MIMIC-IV database and 3,154 patients without a diabetes diagnosis, this study assessed the relationship between the highest hypoglycemia A1c during ICU stay and long- and short-term outcomes after discharge.

Methods

Data source

This research was performed on a large, free, and public database, namely, Medical Information Mart for Intensive Care (MIMIC)-IV (22, 23), which comprised comprehensive clinical information on hospital stays for patients admitted to a tertiary academic medical center in Boston. The latest version, MIMIC-IV 2.0, was updated in June 2022 and contains comprehensive information about patients. The structured query language (SQL) was used for data extraction from the MIMIC-IV database.

Population selection criteria

The inclusion criteria included the following: patients admitted into the ICU for the first time, HbA1c was measured at least once, and there had been no previous diabetes diagnosis. The exclusion criteria were as follows: no height or other measurements and patients under the age of 18 (Figure 1).

Outcomes and covariates

The primary outcome was long-term (1 year) mortality. The secondary outcomes included short-term (30- and 90-day) mortality.

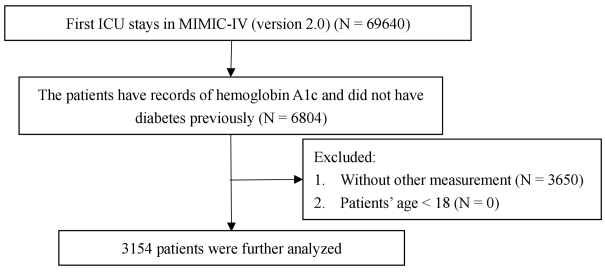

FIGURE 1
Flowchart of study population selection.

The extraction variables included age, gender, body mass index (BMI), admission type, congestive heart failure, renal disease, hypertension, acute myocardial infarction (AMI), creatinine, blood urea nitrogen (BUN), hemoglobin, the Sequential Organ Failure Assessment (SOFA) score, the Simplified Acute Physiology Score (SAPS II), HbA1c, reintubation event, accumulated ventilation time, renal replacement therapy, the heart surgery, the use of some drugs [including aspirin, beta blockers, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), dopamine, dobutamine, norepinephrine, and vasopressin]. In addition, HbA1c measurements were divided into four groups, namely, <5.0 mmol/L, 5.0–5.7 mmol/L, 5.7–6.5 mmol/L, and ≥ 6.5 mmol/L.

Statistical analyses

Categorical data were expressed as percentages and frequencies, while continuous data were expressed as mean SD or median (range) values. Cox proportional hazards regression models were used to calculate the hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs) for 30-day, 90-day, and 1-year mortality. Model I was not adjusted, whereas Model II was adjusted for age, BMI, gender, admission type, congestive heart failure, renal disease, hypertension, acute myocardial infarction, creatinine, BUN, hemoglobin, and the SOFA score. After adjusting with Cox analysis regression using other covariates, restricted cubic splines were used to confirm the linearity between HbA1c and outcome. Using propensity score matching (PSM), the surviving patients were matched with patients of the same size from the deceased. A univariate Cox regression analysis was used to analyze the correlation between HbA1c and outcome. In addition, the XGBoost (24) machine learning algorithm was used to validate the significance of HbA1c on 1-year mortality outcomes. ROC curves were also plotted to confirm the prediction ability of the model, and SHAP figures were plotted to validate the importance of HbA1c on outcomes across all these variables.

R (version 4.1.0) software was used for the statistical analysis. $p < 0.05$ was considered statistically significant.

Results

The study introduced a total of 3,154 patients without diabetes who had HbA1c measurements from the MIMIC-IV database; 581 of those patients were deceased in 365 days, while 2,573 of them survived (Table 1). Patients in the death group were older than those in the survival group, and they also had higher BUN and creatinine measurements, and lower hemoglobin measurements. Moreover, the death group had higher SOFA scores, higher SPASII scores, longer ventilation times, and higher proportion of congestive heart failure, hypertension, renal disease, renal replacement therapy, and reintubation events.

Data are number of subjects (percentage) or mean (standard derivatives); [1] One-way ANOVA test was used to compare the mean ± standard deviance values between deceased and survived participants; [2] Chi-square test was used to compare the percentage between deceased and survived participants.

Restricted cubic splines

Figures 2A–C depict the restricted cubic spline (after adjusting with Cox analysis regression), with Figure 2A revealing the U-shaped association between HbA1c and 1-year mortality, while Figures 2B, C did not reveal the U-shaped association between HbA1c and 30-day and 90-day mortality and only revealed a negative association between mortality and HbA1c in the range of 5% and 6%.

Figures 2D–F show the differences between groups with different ranges of HbA1c for different outcomes, which revealed that the medium ranges of HbA1c (5.0%–5.7% and 5.7%–6.4%) had relatively higher 1-year survival rate than the remaining ranges of HbA1c (less than 5.0% and more than 6.4%). Only the groups with the highest HbA1c (more than 6.4%) had a significantly higher deceased rate for the 30-day and 90-day mortality.

Univariate and multivariate analyses

The Cox model showed that different levels of HbA1c were associated with the occurrence of long-term (1-year) mortality, as well as short-term (30-day and 90-day) mortality when unadjusted and adjusted in Table 2. In the univariate Cox model, those who reported an HbA1c level of less than 5 mmol/L had an HR of 1.65 (95% CI, 1.24–2.19), and those with more than 6.4 mmol/L had an HR of 1.89 (95% CI, 1.42–2.53), compared with the normal range (5.0–5.7 mmol/L). After adjusting for gender, age, admission type, congestive heart failure, renal disease, acute myocardial infarction, creatinine, BUN, hemoglobin, SOFA scores, SAPSII scores, ventilation time, the use of drugs including aspirin, beta blockers, ACEI, ARB, dopamine, dobutamine, norepinephrine, and vasopressin, renal replacement therapy, and heart surgery, compared with the normal range of HbA1c (5.0–5.7 mmol/L), the lower range (less than 5.0 mmol/L) and the higher range (more than 6.4 mmol/L) had HRs of 1.37 (95% CI, 1.02–1.84) and 1.62 (95% CI, 1.20–2.18), respectively. For the 30-day mortality outcome, in the univariate Cox model, those who reported an HbA1c level of less than 5 mmol/L had an HR of 1.58 (95% CI, 1.05–2.38), and those with more than 6.4 mmol/L had an HR of 2.05 (95% CI, 1.39–3.03), while in the adjusted model, the higher level of HbA1c (more than 6.5%) had an HR of 1.81 (95% CI, 1.21–2.71). For the 90-day mortality outcome, in the univariate Cox model, those who reported an HbA1c level of less than 5 mmol/L had an HR of 1.58 (95% CI, 1.13–2.22), and those with more than 6.4 mmol/L had an HR of 1.87 (95% CI, 1.33–2.62), and in the adjusted model, the higher range of HbA1c (more than 6.5%) had an HR of 1.62 (95% CI, 1.14–2.29).

Propensity score matching

After PSM, the whole sample size included 581 deceased patients and 581 survived patients (Supplementary Table 1). The

TABLE 1 Characteristics of the study population (*N* = 3,154).

	Survival	Dead	*p*
N	2573	581	
Age [1] (mean (SD))	65.70 (14.81)	73.80 (15.19)	<0.001
Blood urea nitrogen [1] (mean (SD))	28.20 (19.51)	44.89 (32.46)	<0.001
Creatinine [1] (mean (SD))	1.39 (1.36)	1.93 (1.60)	<0.001
Hemoglobin [1] (mean (SD))	9.54 (2.45)	8.92 (2.45)	<0.001
SOFA score [1] (mean (SD))	4.39 (3.12)	6.16 (3.76)	<0.001
SAPSII score [1] (mean (SD))	32.22 (11.59)	41.82 (12.52)	<0.001
Ventilation time [1] (mean (SD))	875.93 (2,701.94)	2,594.65 (5,554.92)	<0.001
Gender = Male [2] (%)	1,666 (64.7)	293 (50.4)	<0.001
Congestive heart failure[2] (%)	677 (26.3)	203 (34.9)	<0.001
Renal disease[2] (%)	311 (12.1)	146 (25.1)	<0.001
Hemoglobin A1c ranges[2] (%)			<0.001
<5.0%	176 (6.8)	60 (10.3)	
5.0% ≤ HbA1c < 5.7%	1,228 (47.7)	236 (40.6)	
5.7% ≤ HbA1c < 6.5%	1,022 (39.7)	227 (39.1)	
6.5% ≤ HbA1c	147 (5.7)	58 (10.0)	
Hypertension[2] (%)	1,732 (67.3)	429 (73.8)	0.003
Acute myocardial infarction[2] (%)	536 (20.8)	84 (14.5)	0.001
Use of aspirin[2] (%)	2,003 (77.8)	335 (57.7)	<0.001
Use of beta blocker[2] (%)	1,891 (73.5)	369 (63.5)	<0.001
Use of ACEI[2] (%)	881 (34.2)	127 (21.9)	<0.001
Use of ARB[2] (%)	231 (9.0)	34 (5.9)	0.018
Use of dopamine[2] (%)	46 (1.8)	25 (4.3)	<0.001
Use of dobutamine[2] (%)	40 (1.6)	25 (4.3)	<0.001
Use of norepinephrine[2] (%)	356 (13.8)	152 (26.2)	<0.001
Use of vasopressin[2] (%)	122 (4.7)	82 (14.1)	<0.001
Admission type[2] (%)			<0.001
Scheduled surgery	228 (8.9)	14 (2.4)	
Medical admission	1,738 (67.5)	462 (79.5)	
Unscheduled surgery	607 (23.6)	105 (18.1)	
Reintubation event[2] (%)	325 (12.6)	108 (18.6)	<0.001
Renal replacement therapy[2] (%)	63 (2.4)	41 (7.1)	<0.001

mirror histogram in Supplementary Figure 1 described the propensity score distributions in the survived and deceased groups before and after PSM, respectively. In the univariate Cox regression analysis (Supplementary Table 2), only the higher level (more than 6.4%) of HbA1c was significantly associated with 1-year mortality, with an HR of 1.51 (95% CI, 1.13–2.01), which is shown in Figure 3.

XGBoost machine learning algorithm

In this machine learning model, the SHAP figure is shown in Figure 4, which showed that, to some extent, HbA1c was important for the 1-year mortality. The ROCs of the training dataset and testing dataset are plotted in Figure 5, which showed great prediction ability for the 1-year mortality.

Discussion

It has been reported that about one in five ICU survivors would pass away within a year (25) and another study found that the 1-year mortality rate among ICU survivors was approximately 20% (26), which was close to the result in our study. Furthermore, despite the absence of a prior diabetes diagnosis, 46.1% of critically ill patients developed hyperglycemia, with a maximum HbA1c greater than 5.7 mmol/L during their hospitalization. The patients with HbA1c above 6.4% and without diabetes were previously considered to have unknown diabetes, which was associated with various adverse outcomes (18, 27).

Our investigation suggested that HbA1c was associated with 1-year, 30-day, and 90-day mortality among patients without diabetes. The SHAP figure can explain the importance of each variable in this model (28). Through SHAP, we could conclude that HbA1c, to some extent, could predict ICU mortality. According to Lee et al., glycated hemoglobin could predict organ dysfunction in critically ill patients with sepsis and might be a predictor for ICU outcomes (29). Another study found that the risk of death doubled with each increase in HbA1c level, and mortality in the critically ill patients was also affected by the chronic hyperglycemia (30). Furthermore, one study showed that, in critically ill patients without diabetes previously, HbA1c at admission was significantly associated with mortality in ICU (31).

In addition to the ICU research mentioned above, some studies found that among 1,474 non-diabetic patients undergoing cardiac surgery, patients with HbA1c above 6% had a significantly higher 30-day mortality outcome than patients with HbA1c below 6% (32). Furthermore, Halkos et al. discovered a significant reduction in long-term survival per unit increase in HbA1c of 7.0% (33). After multivariable adjustment, a different prospective study discovered that patients with HbA1c above 6.0% had significantly higher 3-year mortality rates than those with HbA1c levels < 6%.

Additionally, previous studies revealed that HbA1c values have a U-shaped relationship with all-cause mortality, showing that both high and low HbA1c values are associated with higher mortality rates in elderly patients with acute VTE (34). Moreover, a U-shaped relationship between HbA1c levels and mortality among diabetics receiving hemodialysis was also discovered in another study (35). In one meta-analysis, participants with diabetes showed a U-shaped association between HbA1c and all-cause mortality while patients without diabetes showed a reversed J-shaped association (36). However, according to the Atherosclerosis Risk in Communities study, glycated hemoglobin was related to all-cause mortality in a J-shaped pattern (37). Higher postprandial glucose levels associated with IGT directly elevate HbA1c, which is directly correlated with

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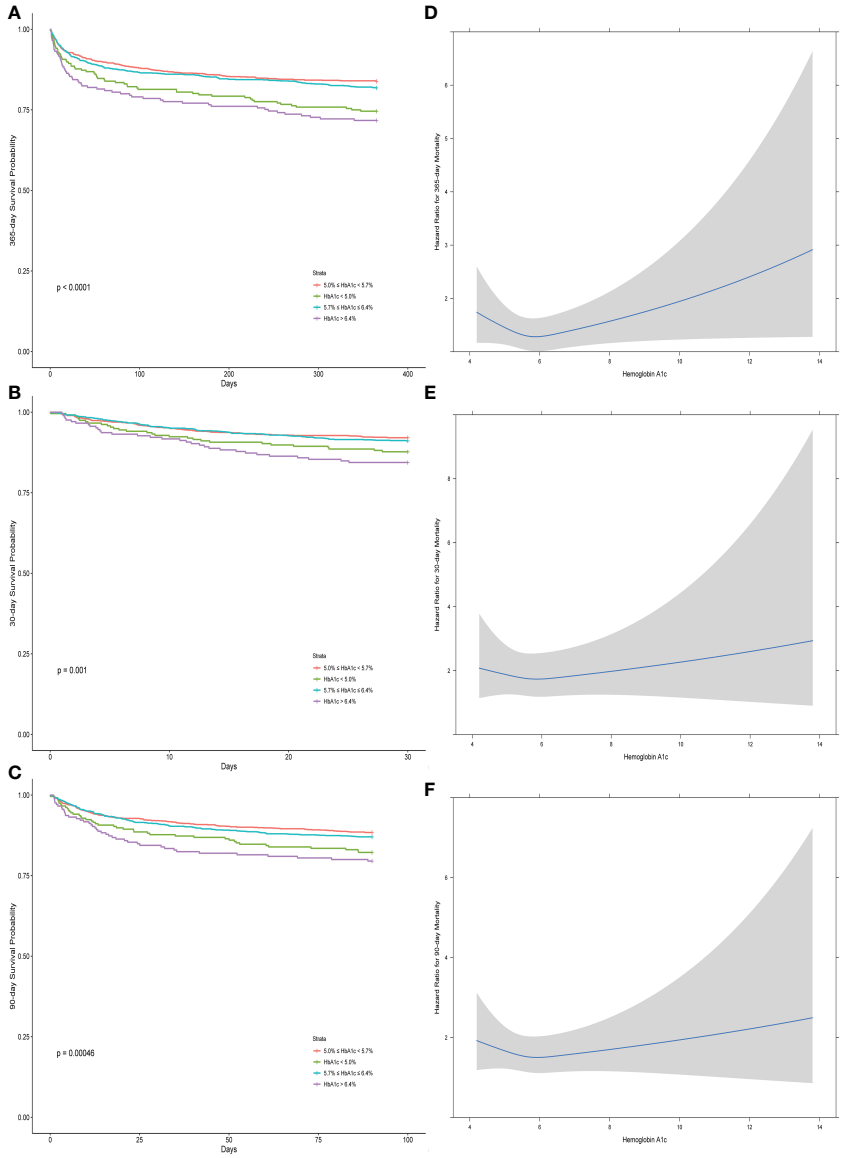

FIGURE 2
The Kaplan–Meier curves **(A–C)** and the restricted cubic splines **(D–F)** between the hemoglobin A1c and different outcomes (30-day, 90-day, and 1-year mortality).

TABLE 2 Univariate and multivariate analysis of the associations between outcomes and different ranges of hemoglobin A1c.

	Group 1 (5.0% ≤ HbA1c < 5.7%)	Group 2 (HbA1c < 5.0%)		Group 3 (5.7% ≤ HbA1c < 6.5%)		Group 4 HbA1c≥6.5%)	
	HR (95% CI)	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Primary outcome							
1-year mortality							
Unadjusted	Reference	1.65 (1.24–2.19)	<0.001	1.13 (0.95–1.36)	0.176	1.89 (1.42–2.53)	<0.001
Adjusted	Reference	1.37 (1.02–1.84)	0.038	1.08 (0.90–1.30)	0.412	1.62 (1.20–2.18)	0.001
Second outcome							
30-day mortality							
Unadjusted	Reference	1.58 (1.05–2.38)	0.027	1.12 (0.86–1.45)	0.392	2.05 (1.39–3.03)	<0.001

(Continued)

TABLE 2 Continued

	Group 1 (5.0% ≤ HbA1c < 5.7%)	Group 2 (HbA1c < 5.0%)		Group 3 (5.7% ≤ HbA1c < 6.5%)		Group 4 HbA1c≥6.5%)	
	HR (95% CI)	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Adjusted	Reference	1.37 (0.89–2.12)	0.150	1.13 (0.87–1.48)	0.364	1.81 (1.21–2.71)	0.004
90-day mortality							
Unadjusted	Reference	1.58 (1.13–2.22)	0.008	1.12 (0.90–1.39)	0.297	1.87 (1.33–2.62)	<0.001
Adjusted	Reference	1.33 (0.93–1.91)	0.113	1.10 (0.88–1.37)	0.416	1.62 (1.14–2.29)	0.007

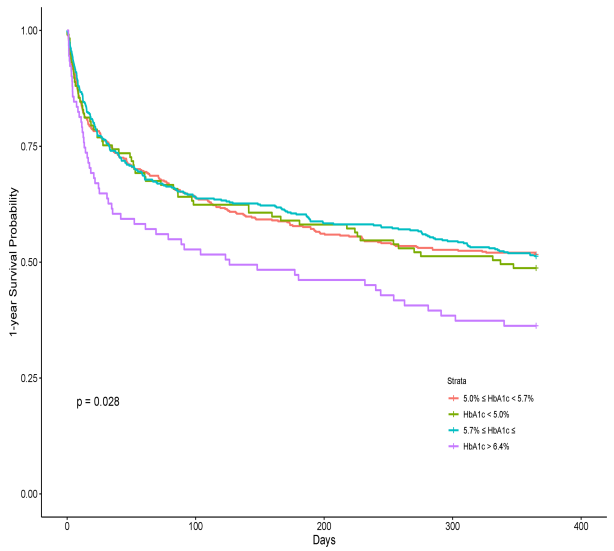

FIGURE 3
The Kaplan–Meier curve between the hemoglobin A1c and 1-year mortality after PSM.

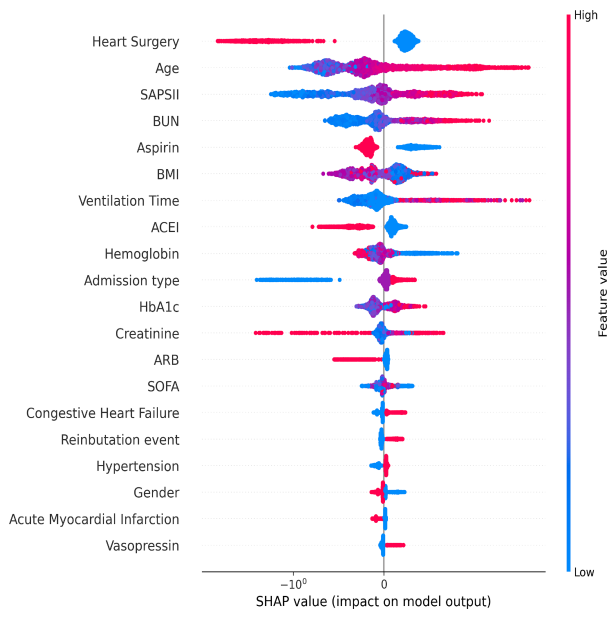

FIGURE 4
The SHAP values of variables in the XGBoost machine learning model.

glycemia (38). Furthermore, the amyotrophic lateral sclerosis mortality risk was twice as high for those with HbA1c levels higher than 6.5% at baseline, compared to those with good HbA1c levels (5.7%) (39). Furthermore, other studies only found a positive association between HbA1c levels and mortality in different population cohorts (40–42), which was somewhat similar to our research result.

Moreover, higher HbA1c levels indicated higher glucose variability (18), which was associated with in-hospital or in-ICU mortality, even in patients with tight glycemic control but high glycemic variability (43). Furthermore, according to Eirini., HbA1c was an independent risk factor for nosocomial infections in critically ill patients (44), a major cause of morbidity and mortality in critically ill patients (45). In addition, there was a review analysis with the conclusion that regardless of the previous diabetic status, HbA1c is a strong predictor of mortality and morbidity (46).

However, there was also a research (47) that revealed that among diabetes patients undergoing cardiac surgery, there was no significant difference between the high-level HbA1c group and the control group, but because this research introduced only 101 patients, this conclusion could not be strongly supported.

The strengths of this study rest on several aspects. Firstly, we chose patients without diabetes from the most recent, complex, and comprehensive MIMIC-IV database (version 2.0). Secondly, we selected a sufficient number of participants for this research, which

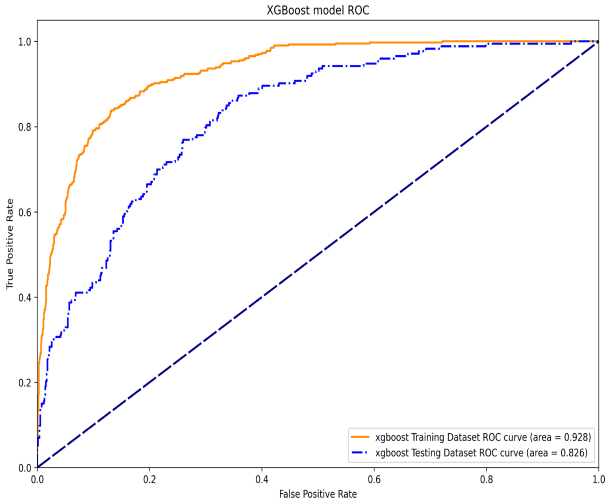

FIGURE 5
The ROC of the training dataset and the testing dataset in the XGBoost machine learning model.

may have made drawing a conclusion safer. Thirdly, we studied the association between 1-year mortality and HbA1c, which was rarely studied.

However, there were also some limitations in this research. First of all, as a single-center retrospective study with a limited sample size, selection bias exists. Secondly, the sample sizes in various groups with different ranges of HbA1c were not balanced, which contributed to the wide range of the CI of the restricted cubic spline's second half. Thirdly, the research lacked prospective studies and mechanism studies, which need to be studied further.

Conclusion

HbA1c (≥6.5%) was associated with harmful effects on the 30-day, 90-day, and 1-year mortality among critically ill patients with undiagnosed diabetes after discharge from the ICU. Measurements on lowering glucose variability should be taken in clinical settings to control hemoglobin A1c.

Data availability statement

The data that support the findings of this study are available from https://physionet.org/content/mimiciv/2.0/ but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of PhysioNet.

Ethics statement

Patient consent was waived because the study was an analysis of a third-party anonymized publicly available database.

Author contributions

WO and YT designed the study. CL and KP organized data. CL and YT analyzed data and wrote the first draft of the manuscript. JT, KP, and LL revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1124342/full#supplementary-material

SUPPLEMENTARY TABLE 1
Characteristics of the study population after PSM (N = 1162).

SUPPLEMENTARY TABLE 2
Univariate Analysis of the associations between outcomes and different ranges of hemoglobin A1c after PSM.

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A systematic review of the safety of tirzepatide-a new dual GLP1 and GIP agonist - is its safety profile acceptable?

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Aims: Tirzepatide is a novel dual glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 receptor agonist (GLP-1 RA). At present, there is no controversy over its effectiveness, but its safety. We conducted a systematic review to assess the safety of tirzepatide.

Methods: We searched PubMed, Embase and Cochrane databases for randomized controlled trials (RCTs) of tirzepatide from databases inception to August 28, 2022 and used the Cochrane Systematic Assessment Manual Risk of Bias Assessment Tool (version 5.1) and modified Jadad scale to assess risk of bias. The systematic review was conducted *via* Revman5.4.

Results: Nine RCTs with a total of 9818 patients were included. The overall safety profile of tirzepatide is similar to GLP-1RAs, except for the hypoglycemia (tirzepatide 15mg, pooled RR=3.83, 95% CI [1.19- 12.30], *P=0.02*) and discontinuation (tirzepatide 10mg, pooled RR=1.75,95%CI[1.16-2.63], *P=0.007* and 15mg, pooled RR=2.03, 95%CI [1.37-3.01], *P=0.0004*). It also showed that the dose escalation could not rise the occurrence rates of total, severe, gastrointestinal adverse events and hypoglycemia (*P>0.05*); Compared with 5mg, tirzepatide 10mg and 15mg were associated with more frequent nausea (*P<0.001*), discontinuation (*P<0.05*) and injection-site reaction (*P<0.01*); The rates of vomiting and diarrhea were dose-dependence at the range of 5-15mg.

Conclusion: The safety profile of tirzepatide is generally acceptable, similar to GLP-1 RAs. It is necessary to pay attention to its specific adverse events (hypoglycemia and discontinuation) at high doses (10mg or higher). Nausea, vomiting, diarrhea, discontinuation and injection-site reaction were dose-dependence among specific dose ranges.As the heterogeneity in different studies by interventions, the results may be with biases and the further confirmation is needed. Meanwhile, more well-designed trials are needed to control the confounding factors and ensure adequate sample size.

KEYWORDS

dual glucose-dependent insulinotropic peptide and glucagon-like peptide-1 receptor agonist, tirzepatide, safety, discontinuation, dose-dependence

1 Introduction

The prevalence of Type 2 diabetes mellitus (T2DM) has reached epidemic proportions and is estimated to afflict over 400 million people worldwide. Moreover, the incidence of diabetes is expected to continue to rise and, in the U.S. alone, is projected to affect nearly one in three people by the year 2050 (1). Its main harm comes from chronic irreversible damages to target organs, including cardiovascular (2), kidney (3), eyes (4), skin and soft tissues (5), etc. However, there is no cure for diabetes so far, but it can be treated and controlled by pharmacological therapy which can delay or possibly to prevent the development of diabetes-related health problems (6). It is suggested that there is a need for the development of a novel and effective treatment agent to combat the rise in T2DM prevalence worldwide.

Tirzepatide (TZP), a novel dual glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) agonist has been approved for the treatment of T2DM in United States on May 13, 2022 (7). TZP targets not only GLP-1 but also GIP receptors and/or glucagon which is intended to address different metabolic pathways for carbohydrate, lipid, and protein metabolism simultaneously (8). In terms of efficacy, almost all randomized controlled trials (RCTs) studies have shown that TZP has outstanding effectiveness in glycaemic control and weight reduction which is significantly better than GLP-1 receptor agonists (GLP-1 RAs). These results are consistent with systematic reviews (9, 10), suggesting that the efficacy of TZP is stable and unparalleled in the treatment of T2DM and obesity. In addition, the molecule including a C20 fatty acid moiety with a half-life of approximately 5 days, allowing for once-weekly subcutaneous injection which can improve the compliance of patients. All these advantages make TZP to be a milestone of anti-diabetic agents. However, the conclusions about safety remain controversial. TZP is currently considered to be as safe as GLP-1 RAs (8–10). But there are still conflicting opinions (11, 12). Meanwhile, there is no special study on safety, which may lead to inaccurate result as the small sample sizes and insufficient outcomes. As the current studies mainly focus on effectiveness, researchers seldom concern about the source of safety heterogeneity, making it difficult to evaluate TZP on a comprehensive basis.

In this manuscript, we provided a systematic review with additional studies and outcomes, and a more detailed analysis was processed. We wonder that, from the current RCTs results, whether TZP has a higher odds of adverse drug event (ADE), than placebo, insulin and especially than GLP-1 RAs; whether there is dose-dependence correlation between ADE of TZP.

2 Materials and methods

2.1 Literature search and data extraction

We performed a systematic search of PubMed, Cochrane Library, and EMBASE databases from the time of databases inception to August 28, 2022. “tirzepatide” and “safety” were used as the Medical Subject Headings (Mesh) and”LY3298176”and”safeties” as the free terms. During the retrieval, Mesh and free terms were combined for the literature search. The literature screening and data extraction were performed independently by two investigators. If there was any argument, it was resolved by a third investigator. Then, we extracted the data including the number of patients in the treatment and control groups, demographics, diseases, intervention methods, concomitant medications and treatment duration.

The outcomes are the odds of total adverse drug event (TADE), serious adverse drug event (SADE), gastrointestinal adverse drug event (GADE), discontinuation by adverse drug event (DADE), hypoglycemia and injection site reaction, etc.

2.2 Literature inclusion and exclusion criteria

The inclusion criteria (1) Randomized controlled trials in any published years and languages; (2) The patients in the treatment groups were given TZP at a maintenance dose of 5, 10 or 15 mg once weekly, and the patients were treated with placebo or other anti-diabetic drugs in the control groups; (3) The main outcomes meet the demand of the research.

The exclusion criteria (1) non-randomized controlled trials; (2) Animal or pharmacokinetic researches, basic studies, systematic reviews, meta-analyses, retrospective studies, case reports, or conference presentations; (3) Abstract-only publications or unpublished studies; (4) Publications missing important information;(5) Duplicate publications.

2.3 Assessment of risk of bias

The quality of the research was assessed according to the Cochrane Systematic Assessment Manual version 5.1 Risk of Bias Assessment Tool and the modified Jadad scale. A study with a modified Jadad scale of more than 3 was considered to be of high-quality and acceptable. The funnel plots were adopted to evaluate the risk of publication bias.

2.4 Statistics analysis

We calculated responder proportions with 95% confidence interval (95% CI) using the suitable model (fixed effects Mantel-Haenszel model or random effects Mantel-Haenszel model) with a double arcsine transformation. The data analysis was performed *via* RevMan 5.4 software. The pooled risk ratio (pooled RR) for safety and 95% CI for the count data measure were calculated. The heterogeneity was measured by Q test and I^2, P >0.10 (Q test) and I^2< 50% among all subgroups suggesting low heterogeneity and fixed effects inverse variance weight model was adopted in the statistical process; $P \leq 0.10$ and $I^2 \geq 50\%$, heterogeneity was large and random effects inverse variance weight model was adopted; whether there was a statistically significant difference between the control and treatment groups depended on the test level ($P=0.05$). When exploring the correlation between ADE and drug dose, we

used the chi-square test to evaluate whether the difference between the does groups was statistically significant, the test level was also *P=0.05*.

3 Results

3.1 Study characteristics

A total of 9 clinical studies were included (13–21)with 9818 cases. The literature selection process is shown in Figure 1. The included studies were published from 2018 to 2022, with treatment duration ranging from 26-72weeks. The treatment groups were all treated with the maintenance dose of TZP (5,10 or 15mg once-weekly), while 5 studies used placebo in control group, 4 with GLP-1RAs and 2 with insulin (2 studies adopted both placebo and GLP-1 RAs). In 3 studies, TZP was used alone, while the other hypoglycemic agents in combination with TZP were applied in 6 studies. The basic characteristics of the included studies are shown in Table 1.

All studies were RCTs, 6 were double-blind and 3 were open-label. We used the Cochrane risk-of-bias tool and modified Jadad scale to assess the risk of bias, as shown in Supplementary Figure 1 and Supplementary Table 1. The quality of all studies was all acceptable.

3.2 Publication bias

From the funnel plots, there was the obvious publication bias in almost all dose groups between TZP and placebo or GLP1-RAs which may have impacts on the stability of the results. But, the assessment may be not accurate enough as the number of studies is less than 10.(Supplementary Figures 2, 3).

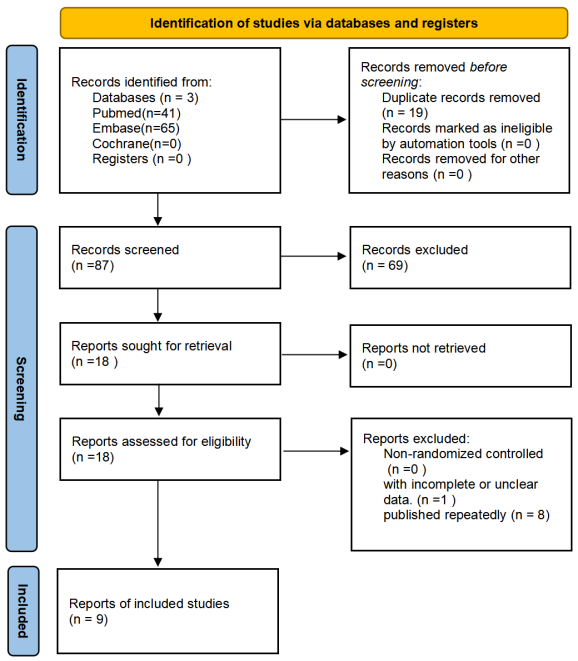

FIGURE 1
Flowchart of the details of the study.

3.3 Meta-analyses

The TADE incidences of 5mg and 15mg TZP were higher than those of placebo, but with no statistically significant differences in each dose group compared to GLP-1 RAs (Figure 2); The odds of SADE were similar between TZP in all groups and GLP-1 RAs (Figure 3); The GADE was more frequent with all TZP doses than placebo, but comparable to GLP-1 RAs (Supplementary Figure 4). Of which, the incidences of nausea, vomiting and diarrhea were higher than placebo in all dose groups but still consistent with GLP-1 RAs (Supplementary Figures 5–7); TZP 15mg was associated with more hypoglycemia than GLP-1 RAs (pooled RR=3.83, 95%CI [1.19-12.30], *P=0.02*) (Supplementary Figure 8); The odds of injection-site reaction were higher in TZP 5mg and 10mg groups than those of placebo, but all dose groups were the same as GLP-1 RAs (Supplementary Figure 9); The risk of discontinuation by ADE was significantly higher in all does groups than placebo. Compared with GLP-1 RAs, more participants receiving TZP 10 mg (pooled RR=1.75, 95%CI [1.16-2.63], *P=0.007*) and 15mg (pooled RR=2.03, 95%CI [1.37-3.01], *P=0.0004*) experienced the discontinuation (Figure 4). TZP had lower odds of hypoglycemia compared to glargine (pooled RR=0.40, 95%CI [0.31-0.51], *P<0.00001*) and degludec (pooled RR=0.21, 95%CI [0.11-0.38], *P<0.00001*) (Supplementary Figure 8), but similar odds of injection-site reaction with insulin; The TADE, GADE and discontinuation were less usual in the insulin groups than TZP.

3.4 Chi-square analysis results of different doses of TZP for adverse drug event

Our study showed that increasing the dose of TZP could not promote the emergence of TADE,SADE,GADE and hypoglycemia (*P>0.05*), suggesting that there may be no dose- dependence; Compared with 5mg, 10mg and 15mg of TZP were also associated with more frequent of nausea (*P<0.001*), discontinuation (*P<0.05*) and injection-site reaction (*P<0.01*), but 10mg and 15mg were equivalent (*P>0.05*).It indicates the obvious dose-dependence in range from 5 to10mg; The incidence of vomiting was 5 mg<10 mg (*P<0.01*), 10 mg<15 mg (*P<0.05*), 5 mg<15 mg (*P<0.001*), illustrating the significant dose-dependence in range of 5-15 mg; For diarrhea, there were no differences between 5mg and 10mg (*P>0.05*), and the same results were observed between 10mg and 15mg (*P>0.05*). But TZP 15mg can lead to more diarrhea than 5mg (*P<0.05*),which revealed that there may be a weak dose-dependence within 5-15mg (Table 2).

3.5 Sensitivity analysis

Considering the possible influence of the blind on the results, open-label studies were excluded (in TZP vs GLP-1 RAs

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TABLE 1 Study-level and participant baseline characteristics of included RCTs.

Study; Clinical Trials;gov registration No.	No. of participants	Study arms	Body weight, kg (mean ± sd)	Age, years (mean ± sd);	Diseases	Background glucose-lowering therapy	Study duration (weeks)
Frias2021 (13) (SURPASS-2) (NCT03987919)	470	TZP 5mg	33.8 ± 6.85	56.3 ± 10.0	Type 2 diabetes	Metformin	40
	469	TZP 10mg	34.3 ± 6.60	57.2 ± 10.5			
	470	TZP 15mg	34.5 ± 7.11	55.9 ± 10.4			
	469	SMG1mg	34.2 ± 7.15	56.9 ± 10.8			
Jastreboff2022 (14) (NCT04184622)	630	TZP 5mg	37.4 ± 6.63	45.6 ± 12.7	Obesity	–	72
	636	TZP 10mg	38.2 ± 7.01	44.7 ± 12.4			
	630	TZP 15mg	38.1 ± 6.69	44.9 ± 12.3			
	643	Placebo	38.2 ± 6.89	44.4 ± 12.5			
Rosenstock2021 (15) (SURPASS-1) (NCT03954834)	121	TZP 5mg	32.2 ± 7.0	54.1 ± 11.9	Type 2 diabetes	–	40
	121	TZP 10mg	32.2 ± 7.6	55.8 ± 10.4			
	121	TZP 15mg	31.5 ± 5.5	52.9 ± 12.3			
	115	Placebo	31.7 ± 6.1	53.6 ± 12.8			
Prato2021 (16) (SURPASS-4) (NCT03730662)	329	TZP 5mg	32.6 ± 6.06	62.9± 8.6	Type 2 diabetes	Mono-therapy with or any combination of metformin, sulfonylurea, or SGLT2 inhibitor	52
	328	TZP 10mg	32.8 ± 5.51	63.7± 8.7			
	338	TZP 15mg	32.5 ± 5.02	63.7± 8.6			
	1000	IG	32.5 ± 5.55	63.8 ± 8.5			
Dahl2022 (17) (SURPASS-5) (NCT04039503)	116	TZP 5mg	33.6 ± 5.9	62 ± 10	Type 2 diabetes	Insulin glargine ± metformin	40
	119	TZP 10mg	33.4 ± 6.2	60± 10			
	120	TZP 15mg	33.4 ± 5.9	61 ± 10			
	120	Placebo	33.2 ± 6.3	60 ± 10			
Heise2022 (18) (NCT03951753)	45	TZP 15mg	31.28 ± 5.01	61.1± 7.1	Type 2 diabetes	Metformin ± another oral hypoglycemic agent	28
	44	SMG1mg	30.82 ± 3.84	63.7 ± 5.9			
	28	Placebo	32.24 ± 3.96	60.4± 7.6			
Frias2018 (19) (NCT03131687)	55	TZP 5mg	32.9 ± 5.7	57·9± 8.2	Type 2 diabetes	± Metformin	26
	51	TZP 10mg	32·6 ± 5.8	56.5 ± 9.9			
	53	TZP 15mg	32.2 ± 6.2	56.0± 7.6			
	54	DLG 1.5mg	32.4 ± 5.4	58.7 ± 7.8			

(Continued)

TABLE 1 Continued

Study; Clinical Trials;gov registration No.	No. of participants	Study arms	Body weight, kg (mean ± sd)	Age, years (mean ± sd);	Diseases	Background glucose-lowering therapy	Study duration (weeks)
	51	Placebo	32.4 ± 6.0	56.6± 8.9			
Inagaki2022 (20) (NCT03861052)	159	TZP 5mg	28.6 ± 5.4	56.8 ± 10.1	Type 2 diabetes	–	52
	158	TZP 10mg	28.0 ± 4.1	56.2± 10.3			
	160	TZP 15mg	28.1± 4.4	56.0± 10.7			
	159	DLG 0.75mg	27.8± 3.7	57.5± 10.2			
Ludvik2021 (21) (SURPASS-3) (NCT038882970)	358	TZP 5mg	33.6± 5.9	57.2± 10.1	Type 2 diabetes	Metformin ± SGLT2 inhibitor	52
	360	TZP 10mg	33.4 ± 6.2	57.4 ± 9.7			
	359	TZP 15mg	33.7 ± 6.1	57.5± 10.2			
	360	ID	33.4± 6.1	57.5± 10.1			

TZP, Tirzepatide; SMG, Semaglutide; DLG, dulaglutide; IG, Insulin glargine; ID, insulin degludec.

subgroups). The results are generally stable, except for "injection-site reaction". The heterogeneity is mainly from Frias (2021), in which the participants were encouraged to change injection sites constantly, whereas the other studies did not mention this method. This may be the source of heterogeneity. Therefore, there is no evidence of the influence by the blind on the results.

In further analyses, the study of Frias (2018) was found to be with high heterogeneity in many subgroup analyses. This may be the initial dose and dose escalation of TZP were different from the others (In this study, initial dose was 5 mg, instead of 2.5 mg, and at the rate of 2 weeks for dose escalation was faster compared to 4 weeks in other studies), which may lead to more injection-site reactions and GADE. Heise (2022) also showed heterogeneity. It may be due to the small sample size which could produce random errors. In addition, the duration of these two trials are shorter which may also lead to heterogeneity.It is because GADE mainly occurs in the first weeks of administration.The short duration may increase the difference between TZP and placebo groups but not GLP1-RAs.

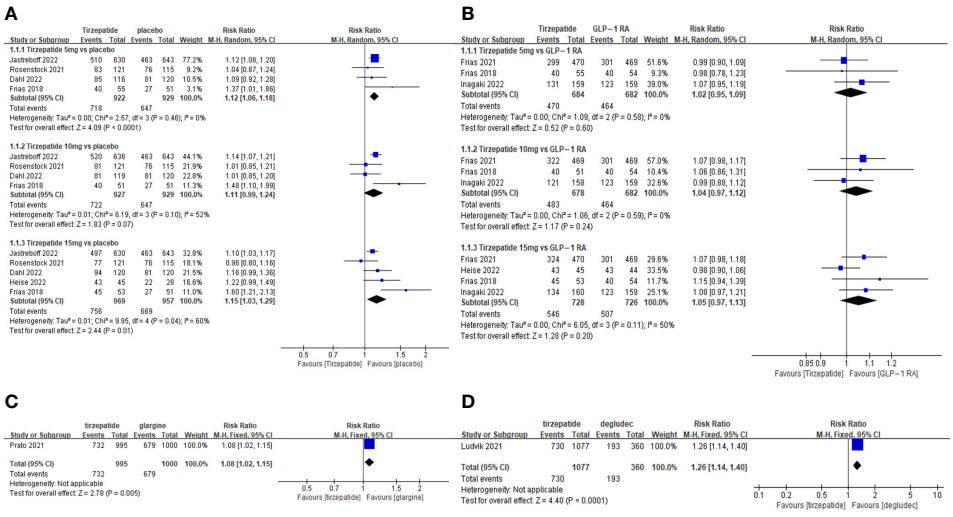

FIGURE 2
Meta-analysis results for tirzepatide of total adverse drug event: **(A)** tirzepatide vs placebo. **(B)** tirzepatide vs GLP-1RAs. **(C)** tirzepatide vs insulin glargine. **(D)** tirzepatide vs insulin degludec.

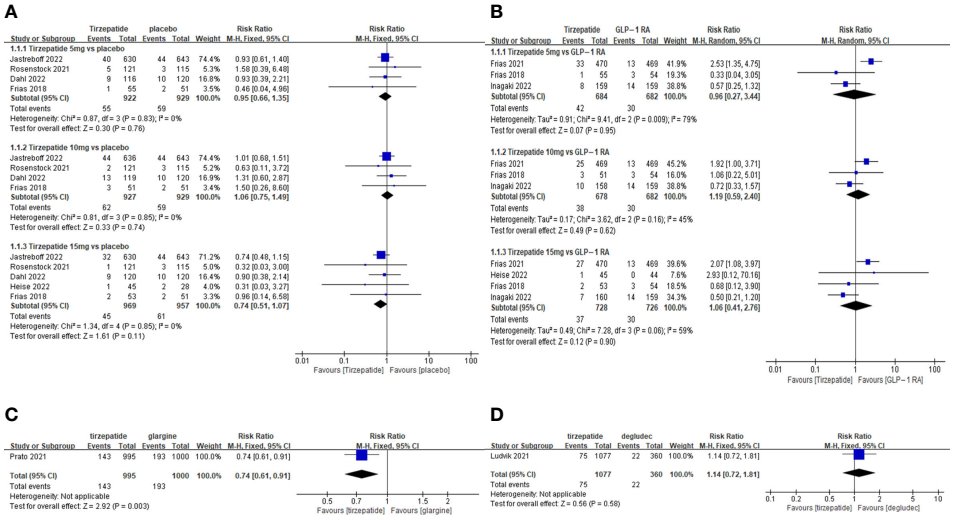

FIGURE 3
Meta-analysis results for tirzepatide of serious adverse drug event: **(A)** tirzepatide vs placebo. **(B)** tirzepatide vs GLP-1RAs. **(C)** tirzepatide vs insulin glargine. **(D)** tirzepatide vs insulin degludec.

Overall, there was high heterogeneity in some outcomes, most of which could be explained by the differences of interventions among studies (clinical heterogeneity). However, due to the lack of sufficient data, it is difficult to evaluate and analyze the degree of heterogeneity caused by these factors.

4 Discussion

In recent years, more has been learned about the safety of GLP-1 RAs, but the safety of TZP, the first dual GLP-1/GIP receptor agonist needs to be further researched as its short history of clinical application. It is currently believed that gastrointestinal events, pancreatitis or elevated serum amylase, cardiac arrhythmias, allergies, injection site reactions, hypoglycemia and acute gallbladder disease could occur during the clinical application of TZP (8).In this systematic review, we have summarized and synthesized the up-to-date RCT results of TZP vs placebo, GLP-1 RAs and basal insulin for ADE evaluation.

The result of this research showed the rates of TADE by TZP with different doses were comparable to those of GLP-1 RAs. The total safety was similar between different dose groups by chi-square tests suggesting that TADE rate was not dose-dependence. This result may not be consistent with some of previous studies (22), possibly due to the inclusion of new research results.

For SADE, the rates of TZP at different doses were similar to placebo, GLP-1 RAs. It seems that the risk of SADE is acceptable. However, the definition of SADE may be not accordant among

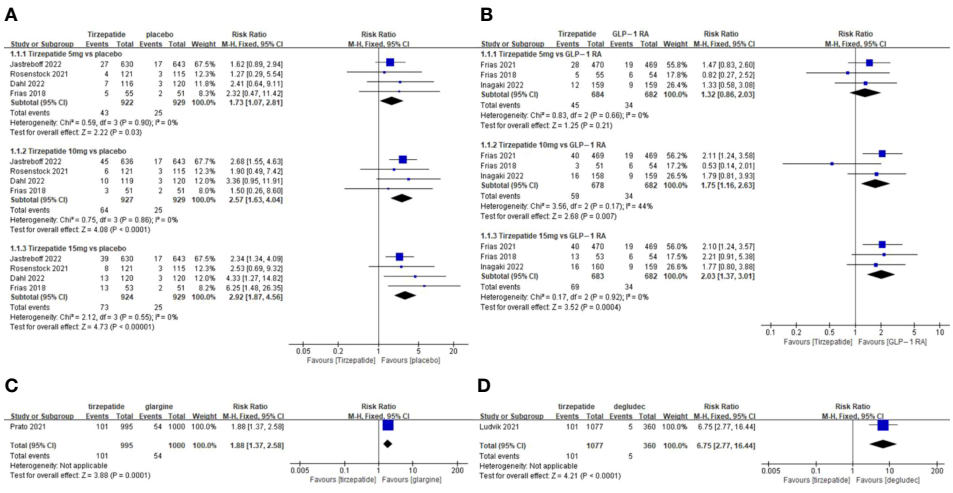

FIGURE 4
Meta-analysis results for tirzepatide of discontinuation by adverse drug event: **(A)** tirzepatide vs placebo. **(B)** tirzepatide vs GLP-1RAs. **(C)** tirzepatide vs insulin glargine. **(D)** tirzepatide vs insulin degludec.

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TABLE 2 Chi-square analysis results of different doses of Tirzepatide for adverse drug event.

Adverse drug event	Comparator arm	No. of studies	Events/total	Events/total	P	Chi^2
Total adverse drug event	5mg vs 10mg	8	1599/2238	1654/2242	0.487	0.483
	10mg vs 15mg	8	1654/2242	1736/2296	0.588	0.293
	5mg vs 15mg	9	1599/2238	1736/2296	0.215	1.539
Serious adverse drug event	5mg vs 10mg	8	173/2238	171/2242	0.905	0.014
	10mg vs 15mg	8	171/2242	146/2296	0.118	2.441
	5mg vs 15mg	9	173/2238	146/2296	0.093	2.829
Gastrointestinal adverse drug event	5mg vs 10mg	3	252/646	292/641	0.130	2.292
	10mg vs 15mg	3	292/641	296/644	0.928	0.008
	5mg vs 15mg	3	252/646	296/644	0.108	2.578
Nausea	5mg vs 10mg	9	376/2238	515/2242	<0.001#	17.894
	10mg vs 15mg	9	515/2242	568/2296	0.273	1.2
	5mg vs 15mg	9	376/2238	568/2296	<0.001#	28.443
Diarrhea	5mg vs 10mg	9	344/2238	395/2242	0.086	2.944
	10mg vs 15mg	9	395/2242	423/2296	0.557	0.346
	5mg vs 15mg	9	344/2238	423/2296	0.021*	5.342
Vomiting	5mg vs 10mg	9	145/2238	197/2242	0.007#	7.261
	10mg vs 15mg	9	197/2242	246/2296	0.047*	3.934
	5mg vs 15mg	9	145/2238	246/2296	<0.001#	21.721
Hypoglycemia	5mg vs 10mg	7	64/2183	58/2191	0.578	0.309
	10mg vs 15mg	7	58/2191	74/2243	0.215	1.537
	5mg vs 15mg	8	64/2183	74/2243	0.496	0.465
Injection-site reaction	5mg vs 10mg	8	46/2238	79/2242	0.004#	8.419
	10mg vs 15mg	8	79/2242	96/2296	0.268	1.226
	5mg vs 15mg	9	46/2238	96/2296	<0.001#	15.862
Discontinuation due to adverse drug event	5mg vs 10mg	8	145/2238	185/2242	0.035*	4.450
	10mg vs 15mg	8	185/2242	204/2251	0.375	0.785
	5mg vs 15mg	8	145/2238	204/2251	0.003#	8.942

*P<0.05, #P<0.01.

studies, because we noticed that "Covid-19 infection" was included in some studies, but was not involved in others (It may be affected by the timing and region of the pandemic of Covid-19). In addition, the different characteristics of patients may also have the influence. In some studies, the patients were older or with more complications which may lead to more SADE and death themselves. These may reduce the differences the of SADE between TZP and controlled agents. Therefore, the final conclusion needs further confirmation.

GLP-1 acts as an inhibitor of gastric and pancreatic motility and maintains postprandial glucose stability. Thus, GADE is the most common, which may not only be one of the reasons for its effect on weight loss *via* reducing appetite but also for the discomfort felt by patients. The odds of nausea, vomiting, and diarrhea by taspoglutide and lixisenatide are more than 80%, and over 50% by exenatide with the obvious dose-dependence (23). The mechanism is considered as the activating the central nervous system (CNS) GLP-1 receptors most likely located in brain stem (area postrema) (23), and gastrointestinal GLP-1 receptors (24). Encouragingly, this study did not show that TZP had a higher risk of GADE compared to GLP-1 RAs, which was similar to some results of the previous study (10). Theoretically, TZP acts on GLP-1 receptor on one hand, and on the other hand when the agent activates GIP receptor, it has no direct effects on gastrointestinal motility and secretory function which does not increase the rate of GADE (8). The result of this study seems to

prove the viewpoint that GIP receptor activation does not cause additional GADE. However, there are still some issues which need to be explored. Previous studies (25–27) showed that reducing refined sugar and fat intake may help to reduce GADE by GLP-1 RAs, but whether these methods are suitable for TZP needs to be confirmed by further research.

The current view is that, consistent with GLP-1 RAs, the higher dose of TZP is associated with more GADE (22). But our result did not seem to support this point. It is notably that the amount of studies included was small (only three) and the final conclusion may not be sufficiently reliable. Although it seem to be contradictory, the derivation may be interesting. Nausea, vomiting and diarrhea were more common in high dose groups although they accounted for the majority of GADE, but were not all the symptoms (13, 15, 19). If there is no dose-dependence of total GADE, it may show the remaining GADE (including abdominal pain, bloating, constipation and decreased appetite, etc) may be with unobvious dose-dependence, which also needs to be further confirmed.

GADE of GLP-1 RAs occurs mainly in the first weeks of treatment and then subsides or stabilizes over time, but severe symptoms can also lead to discontinuation. The discontinuation rate of GLP-1 RAs is currently considered to be 0%-15%, with exenatide slightly lower than semaglutide or dulcolactone (28). An important finding of this study was that discontinuation rates of TZP in all does groups were significantly higher than placebo, with more participants discontinuation at or over 10 mg than GLP-1 RAs, mainly due to intolerable GADE (13, 15–17, 20, 21, 29), which is consistent with the result of the chi-square analysis.The GADE can lead to discontinuation including nausea, diarrhea, vomiting, indigestion, abdominal pain, loss of appetite and constipation.The majority are common in GADE. It may be a disturbing and an alarming signal of TZP safety. Compared to the previous study (10), this study found higher discontinuation of TZP than GLP-1 RAs starting at 10 mg rather than 15 mg, which may affect the suitable dose confirmation of TZP. It might be due to the strengthened effect of GIP on GLP-1 receptors in CNS which can lead to more severe GADE (30). However, it also seems to be difficult to elucidate this mechanism distinctly at this time, as the results of the basal research are not consistent.

Although the overall safety of insulin is better than TZP, however, considering the better efficacy, potential role of cardiovascular protection and convenient administration way, the safety profile may not prevent TZP to replace insulin in T2DM patients. Compared with placebo and GLP-1 RAs, the results of this study showed that TZP did not increase the risk of hypoglycemia. It suggests that TZP, like GLP-1 RAs, might not induce hypoglycemia at appropriate doses. However, more hypoglycemia patients were found in 15 mg TZP group than GLP-1 RAs, and the reason for this needs to be further investigated. Notably, in some studies included, TZP was combined with other anti-diabetic agent usage which may have impacts on the final results. There is still controversy whether TZP itself can cause hypoglycemia. The previous opinion was that TZP alone may not cause hypoglycemia (8). However, some studies believed that the risk still exists (14, 15). Combining with these findings, it suggests that the risk of hypoglycemia by TZP especially at high dose, should not be completely ignored.

This study did not discuss the risk of pancreatitis, tumors, cardiovascular event and hepatobiliary diseases by TZP, as the odds of these ADE were too low to be trusted in clinical trials. These can only be researched by signal mining and retrospective cohort studies in future (31). Meanwhile, due to the insufficient data form the existing trials, some results of this study were affected by small sample sizes thus more confirmation is required. In addition, the higher clinical heterogeneity of some results could lead to instability which also need more well-designed studies.

5 Conclusion

The safety profile of TZP was overall acceptable, similar to GLP-1 RAs. However, TZP 15 mg may be associated with more hypoglycemia than GLP-1 RAs. Meanwhile, it should be noted that more discontinuations were discovered by TZP at 10 mg or over than GLP-1 RAs due to GADE. In addition, TADE, SADE, GADE and hypoglycemia were not dose-dependence; but nausea, vomiting, diarrhea, discontinuation and injection-site reaction were dose-dependence among specific dose ranges. The optimal dose of TZP should be determined by balancing the efficacy and safety. Moreover, some outcomes in this study were with high heterogeneity due to the differences in trial design and they may be with biases and need the further confirmation.Thus, more well-designed trials are needed to control the confounding factors and ensure adequate sample size.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

Conceptualization YW, ZM, MY. Methodology, YW. Software, HW, CX. Validation SZ, YW, ZM, MY. Formal analysis, YW. Investigation, YW, ZM, MY. Resources, HW, SZ. Data Curation, YW, ZM, MY. Writing – original draft preparation, ZM, MY. Writing – review and editing YW, HW, SZ. Visualization, YW, CX. Supervision, YW. Project administration YW, HW. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the

absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1121387/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Assessment of the risk of bias in included studies with cochrane domain-based quality assessment tool.

SUPPLEMENTARY FIGURE 2
Publication bias of safety for tirzepatide vs placebo (Funnel plot). **(A)** total adverse drug event **(B)** serious adverse drug event **(C)** gastrointestinal adverse drug event **(D)** nausea **(E)** vomiting **(F)** diarrhea **(G)** discontinuation by adverse drug event **(H)** hypoglycemia **(I)** injection-site reaction.

SUPPLEMENTARY FIGURE 3
Publication bias of safety for tirzepatide vs GLP-1RAs (Funnel plot). **(A)** total adverse drug event **(B)** serious adverse drug event **(C)** gastrointestinal adverse drug event **(D)** nausea **(E)** vomiting **(F)** diarrhea **(G)** discontinuation by adverse drug event **(H)** hypoglycemia **(I)** injection-site reaction.

SUPPLEMENTARY FIGURE 4
Meta-analysis results for tirzepatide of gastrointestinal adverse drug event: **(A)** tirzepatide vs placebo. **(B)** tirzepatide vs GLP-1RAs.

SUPPLEMENTARY FIGURE 5
Meta-analysis results for tirzepatide of nausea: **(A)** tirzepatide vs placebo. **(B)** tirzepatide vs GLP-1RAs. **(C)** tirzepatide vs insulin Glargine **(D)** tirzepatide vs insulin Degludec.

SUPPLEMENTARY FIGURE 6
Meta-analysis results for tirzepatide of vomiting: **(A)** tirzepatide vs placebo. **(B)** tirzepatide vs GLP-1RAs. **(C)** tirzepatide vs insulin Glargine **(D)** tirzepatide vs insulin Degludec.

SUPPLEMENTARY FIGURE 7
Meta-analysis results for tirzepatide of diarrhea: **(A)** tirzepatide vs placebo. **(B)** tirzepatide vs GLP-1RAs. **(C)** tirzepatide vs insulin Glargine **(D)** tirzepatide vs insulin Degludec.

SUPPLEMENTARY FIGURE 8
Meta-analysis results for tirzepatide of hypoglycemia: **(A)** tirzepatide vs placebo. **(B)** tirzepatide vs GLP-1RAs. **(C)** tirzepatide vs insulin Glargine **(D)** tirzepatide vs insulin Degludec.

SUPPLEMENTARY FIGURE 9
Meta-analysis results for tirzepatide of injection-site reaction: **(A)** tirzepatide vs placebo. **(B)** tirzepatide vs GLP-1RAs. **(C)** tirzepatide vs insulin Glargine **(D)** tirzepatide vs insulin Degludec.

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Patient satisfaction with access, affordability and quality of diabetes care at Mohalla Clinics in Delhi, India

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Introduction: Mohalla Clinics have been set up to provide curative care for minor ailments free of cost within walking distance in the urban slums, thus making primary care more accessible and affordable. Studies evaluating patient satisfaction with treatment of chronic conditions, such as diabetes, in these clinics are lacking.

Methods: A survey of 400 type 2 diabetes patients was conducted, split equally between Mohalla clinics (MC) and Private clinics (PC) in Delhi. Responses were analyzed using STATA17, applying appropriate statistical tests for the data type (Chi-square test, Mann–Whitney *U* test, Wilcoxon signed rank test, or two-sample *t* test).

Results: Satisfaction level was high in both groups with no significant difference between mean satisfaction scores of MC patients and PC patients (Mean 3.79 vs. 3.85 respectively, $p=0.4$). However, MC patients reported a significant improvement in their satisfaction score after switching to MC (Mean 3.79 vs. 3.3 for the previous facility, $p<0.05$). Physician interaction with the patients was the most important factor in influencing the satisfaction score. Proximity to the clinic was the second most important factor for MC patients but was not as important for PC patients. Surprisingly, treatment success was considered an important factor for satisfaction level by <10% MC and <20% PC patients only, pointing to the need for patient education across both the groups. None of the MC patients mentioned free treatment as a contributory factor to high satisfaction, perhaps because most shifted from a government setup to MC. PC patients had more frequent follow-up visits and blood glucose monitoring, and longer consultation duration compared to MC patients, which were offset by access factors, thus not causing much difference to the satisfaction score between the two groups.

Conclusion: Mohalla clinics are making diabetes treatment accessible and affordable for the marginalized population of Delhi, despite not being designed or fully equipped to care for chronic diseases such as diabetes that require multi-specialty care to monitor and manage multiple co-morbidities and long-term complications. Positive perception of physician interaction and convenient location of the clinics are the two major contributors to the high satisfaction patients expressed with diabetes care at these clinics.

KEYWORDS

Mohalla clinic, diabetes, primary care, patient satisfaction, India

1. Introduction

Aam Admi Mohalla Clinics (translated as common man's neighborhood/community clinics) or simply Mohalla Clinics (MC) were launched by the state government of Delhi in 2015 as a flagship scheme to deliver quality primary healthcare closer to communities, especially the underserved ones such as urban slums (1). These clinics have been established primarily to provide basic curative care for common illnesses like fever, diarrhea, skin problems, respiratory problems etc., first aid for injuries and minor wounds, and referral services (2). Patients can simply walk in for a free physician consultation without a prior appointment. Additionally, over 100 medicines included in the essential drugs list are dispensed free of cost to the patients in the clinic itself and over 200 diagnostic tests are also available free of cost through empaneled diagnostic laboratories. Each clinic is staffed with a physician, a nurse, a pharmacist, and a laboratory technician to provide outpatient services (3). Reduced time in commuting to the clinic, less wait time vs. government hospitals, availability of consultation, drugs and diagnostic tests free of cost and under the same roof are the key benefits of MCs which made these clinics quite popular, and the same model is being adopted for provision of primary care services by some other states of India as well (1–5). However, only a limited role of MCs has been envisioned for preventive services, mostly limited to antenatal and postnatal care and nutritional status assessment and counseling. MCs are also not generally meant for specialist consultation, for which a second tier of facilities in the form of Delhi government multispecialty polyclinics are available (2).

A total of 522 MCs are functional as of December 2022, i.e., 1 clinic per 60,000 as against the initial target of setting up 1 per 20,000 population (4). The clinics operate from Monday through Saturday for 6 h every day and have provided over 18 million OPD consultations in the year 2021–2022 (6), which means approximately 117 patients treated per clinic per day. MC doctor, pharmacist and other staff are paid per-patient basis, thus incentivizing treatment of as many patients as possible, even though per patient fee is rather small at INR 40 (roughly USD 0.5 per patient) for the doctor and INR 12 (USD 0.15 per patient) for the pharmacist currently (6). Marginalized groups such as women, older adults, poor and those with education up to primary school, who generally encounter higher barriers in accessing healthcare form a significant proportion of the MC beneficiaries (7). Patient and prescription records are maintained by the MC staff through government provided tablets and clinic software, however there is no published official report or analysis of patient demography or disease types treated at MCs (6). A recently published survey with 356 community participants reported that fever/cough/cold, thyroid, and body ache are the most common medical complaints for younger patients (0–40 years) seeking treatment at MC, and fever/cough/cold and diabetes among beneficiaries older than 40 years of age (8).

Diabetes is one of the leading causes of burden of disease in India and specifically in Delhi, accounting for 3.2% of total disease adjusted life-years (DALYs) in Delhi in 2016, up from 1.3% in 1990 (9). Prevalence of diabetes in Delhi has been reported to be quite high at 18.3% and a diagnosed prevalence of 10.8% (10), thus representing a significant size of patient population of the state seeking treatment. The goals of treatment in diabetes include glycemic control as well as prevention of microvascular complications such as retinopathy, neuropathy and nephropathy, and macrovascular complications such as cardiovascular, cerebrovascular, and peripheral vascular disease. Thus, contrary to the main aim of MCs of curative treatment of acute conditions, treatment of diabetes requires frequent follow-ups, long-term monitoring of the disease and associated complications, and preventative and promotive health services. Despite not having specialists at MCs, diabetes has been reported as one of the major ailments for patients seeking treatment in these clinics (8). There are only a few studies reporting satisfaction with MCs at a community level (7, 8, 11–13), however, in our knowledge no studies have thus far evaluated satisfaction level of patients with diabetes treatment at these community clinics which are not *per se* designed for management of such chronic conditions with long-term sequelae.

2. Methods

The study was conducted using a structured questionnaire-based survey with 400 type 2 diabetes adult patients, split equally between those seeking treatment at MCs and those getting treated at any private clinic (PC) in Delhi. The field survey was conducted between July 2022 and October 2022, using convenience sampling method, with the sample spread out across various zones of Delhi for both MC and PC patients and not concentrated in one or two centers only. The final sample comprised respondents from 19 different MCs and 24 PCs across the city.

2.1. Data collection and study tools

Two separate questionnaires were developed-one for MC patients and the other for PC patients. Both the questionnaires included questions related to demographic details, satisfaction level, factors influencing satisfaction score, access factors and cost of treatment. Additionally, MC patients were specifically asked about their previous facility before shifting to MC, satisfaction score for previous treatment center and facilities available in the MC they currently get treatment from. On the other hand, questions related to awareness about MC and barriers to seeking treatment at MC were unique to PC patients' questionnaire. Before beginning survey data collection, a pilot phase was conducted with 10 patients (not a part of the final analysis), for pre-testing the questionnaires. The final questionnaires were also translated into Hindi (locally spoken language) for convenience of administration to the respondent population. Patients were interviewed in-person while waiting before/exiting after OPD consult at the clinic where they were seeking regular treatment of diabetes. An informed consent was obtained for respondents and anyone who refused to participate was excluded. Each interview took about 15–20 min. The study design was approved by the Ethics Committee of the institute.

2.2. Data analysis

Responses were analyzed using spreadsheets (Microsoft Excel) and statistical analysis performed using STATA17. Appropriate statistical tests of significance were used based on the type of data and objective of the analysis. Chi-square test was used for majority of data points for comparative analyses of MC and PC patients' responses on

close-ended questions. Other tests used were Mann–Whitney *U* test (for Likert scale questions on satisfaction score), Wilcoxon Sign Rank test (for before and after comparison for Mohalla clinic patients), and two sample *t* test (for comparison of cost of treatment).

3. Results

The basic demographic profile of the study population is summarized in Table 1. The survey groups were largely similar in profile in terms of gender and age distribution. However, those getting treated at PCs were more educated (22.5% of MC patients were illiterate vs. none among PC patients, only 10.5% of MC patients were graduate or above vs. 41.5% of PC patients) and had a much higher household income (over two-thirds of MC patients had income less than INR 10,000 ($125) per month while 80% of those treated at PCs had household income over INR 30,000 ($375) per month).

The majority of patients getting treatment at MCs either started their diabetes treatment at MC only (34.5%) or were being treated at a government dispensary (27%) or a government hospital (16%), with only 22.5% seeking treatment at a PC earlier.

3.1. Satisfaction with treatment

Both the groups of patients generally demonstrated a high level of satisfaction with their treatment (Figure 1), with 60% of those being treated at MCs indicating a score of 4 or 5 on a Likert scale of 1 to 5, with 5 being extremely satisfied. The corresponding number for patients undergoing treatment at PCs was 62%. No significant difference in satisfaction score was observed between MC (Mean score 3.79) and PC (Mean score 3.85) patients ($p = 0.4$, Mann–Whitney *U* test). On the other hand, those who had switched to MC from other treatment centers demonstrated significantly better satisfaction score for MC vs. their earlier treatment centers (Mean score 3.3; $p < 0.05$, Wilcoxon sign rank test for before and after comparison).

TABLE 1 Demographic profile of the survey population.

		Mohalla clinics, *n* (%)	Private clinics, *n* (%)
Gender	Male	84 (42%)	105 (47.5%)
	Female	116 (58%)	95 (52.5%)
	Other	0 (0%)	0 (0%)
Age	20–40	77 (38.5%)	84 (42%)
	40–60	89 (44.5%)	94 (47%)
	>60	34 (17%)	22 (11%)
Education	Illiterate	45 (22.5%)	0 (0%)
	Primary school	42 (21%)	13 (6.5%)
	High school	92 (46%)	104 (52%)
	Graduate	21 (10.5%)	80 (40%)
	Postgraduate	0 (0%)	3 (1.5%)
Monthly household income (INR)	<10 K (<$125)	135 (67.5%)	0 (0%)
	10-30 K ($125–$375)	59 (29.5)	38 (19%)
	30-50 K ($375–$625)	6 (3%)	143 (71.5%)
	>50 K > $625	0 (0%)	19 (9.5%)

3.2. Factors affecting satisfaction score

Patients were asked to select the top 3 factors that influenced their satisfaction rating with the treatment. Although there was a significant difference in relative importance of various factors (Chi-square test, $p < 0.05$) between MC and PC patients, there were similarities in some factors chosen by both the groups. Physician interaction (spends adequate time with me, listens to me patiently, explains about the disease and how to take medicines) came up as the most important factor in satisfaction of patients with their treatment with 48.2% of Mohalla patients and 40.8% of PC patients ranking it as one of the top 3 factors influencing their satisfaction levels (Figure 2). Distance to the clinic was the second most important factor for MC patients with about 19% mentioning proximity to the clinic as one of the 3 most important factors for high satisfactions levels. On the other hand, distance to clinic was considered an important factor only by 7% of PC patients (Chi-square test, $p < 0.05$). Surprisingly, treatment success (my diabetes is under control) was considered one of the top 3 factors for satisfaction by only a minority of patients, more among the PC patients 19.7 vs. 8.8% of MC patients. Treatment cost/doctor's fee was another factor showing significant difference as a contributor to satisfaction level between MC and PC patients (Chi-square test, $p < 0.05$). Remarkably, none of the MC patients mentioned free treatment as a contributory factor to high satisfaction, perhaps because most shifted from government setup to MC. On the other hand, 15% of PC patients considered doctor's fee as one of the top 3 factors. There was no significant difference between timing convenience as a factor for satisfaction score (Chi-square test, $p = 0.5$).

3.3. Access factors

Patients were specifically asked about distance to the clinic, ease of getting appointment, waiting time and consultation duration that contribute to convenience and access to treatment (Figure 3). There was a significant difference (Chi-square test, $p < 0.05$) between MC patients and PC patients on the access factors tested, with MC patients having a favorable situation in terms of proximity to the clinic and ability to walk in without prior appointment but PC patients having a better position in terms of frequency of consultations and time spent with the physician. More than 85% of MC patients travelled less than 2 km to reach their treatment facility. Travel distance for PC patients, on the other hand, was longer with about 76% needing to travel 2 kilometers or more. Further, 70% of MC patients and 86.5% of PC patients have a follow-up consultation at least once a month for diabetes treatment, but significantly more PC patients (44.5%) have a follow-up consultation at least once in 15 days vs. only 11.5% MC patients. While all MC consults are on walk-in, 44% of PC patients need to book a prior appointment for meeting their treating physician. Despite prior appointments, PC patients indicated similar wait time for consultation as the MC walk-in patients. However, MC patients indicated shorter consultation duration, with 80% of PC patients spending 10 min or more with their doctor but none of the MC patients get >10 min with their treating physician.

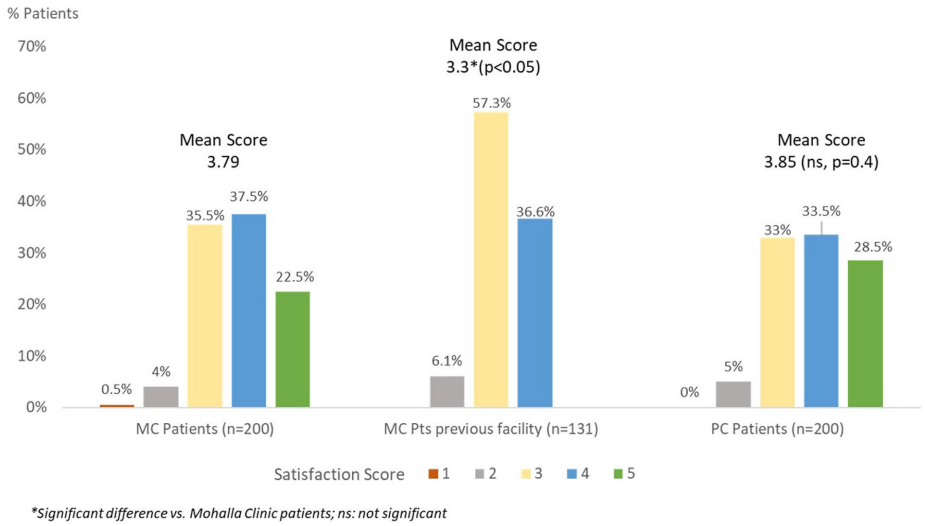


FIGURE 1
Overall satisfaction score of Mohalla clinic and private clinic patients with diabetes treatment.

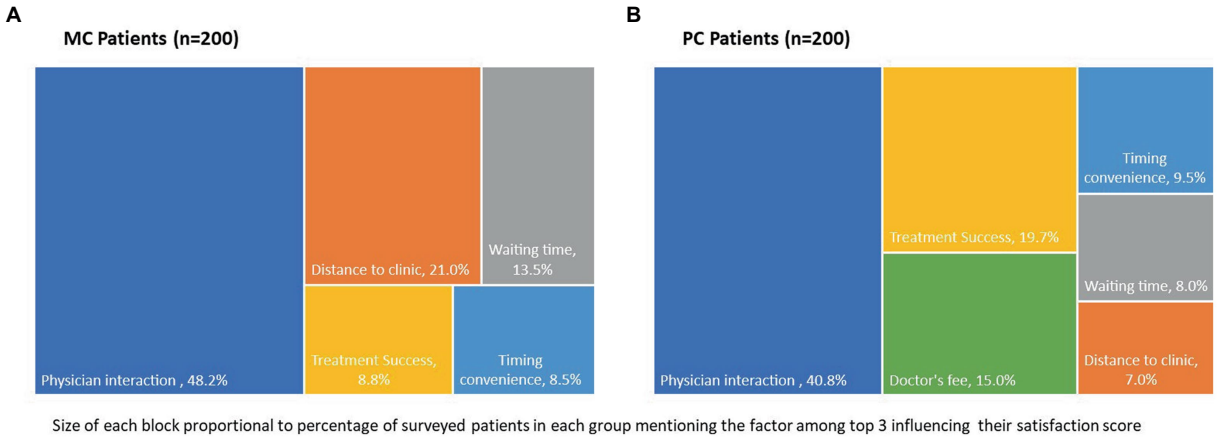


FIGURE 2
Top 3 factors influencing satisfaction with diabetes treatment for **(A)** Mohalla clinic patients and **(B)** private clinic patients.

3.4. Facilities available at Mohalla clinic

Patients at MC are provided with free medicines and diagnostic tests that are prescribed by the MC physician. On being asked about the availability of these services, 25.5% indicated availability of all medicines on every visit while another 8.5% mentioned getting all medicines on repeat visit. 55% of MC respondents indicated that some medicines have to be purchased from the private market. Only 11% indicated not receiving medicines they needed. For diagnostic tests, more than half stated consistent availability of diagnostic tests on every visit and 31% shared the need to get some of the tests from the private market.

3.5. Treatment cost

Total monthly cost of treatment for MC patients were expectedly low (Mean: INR 108.4, SD: 173.3) which was much lower than the cost these patients had to bear (Mean: INR 717.6, SD: 338.5) before shifting to MC and also significantly lower than the PC patients (Mean: INR 3071.5, SD:982.0). The major contributor to the cost in all groups was for medicines. Expectedly, many MC patients (62%) have zero expense, while before moving to MC only 3% had no expense on diabetes treatment (Figure 4).

3.6. Glycemic control

Patients were asked about frequency of getting fasting blood glucose (FBG) checked and their last FBG value. PC patients had more frequent monitoring of FBG in comparison to MC patients (Chi-square test, $p < 0.05$). Based on self-reported most recent FBG level, almost all surveyed patients had levels above the desired level of $\leq$100 mg/dl in both the groups. However, correlation with disease duration, treatment adherence and comorbidities was not made, hence clinical significance of this difference cannot be ascertained.

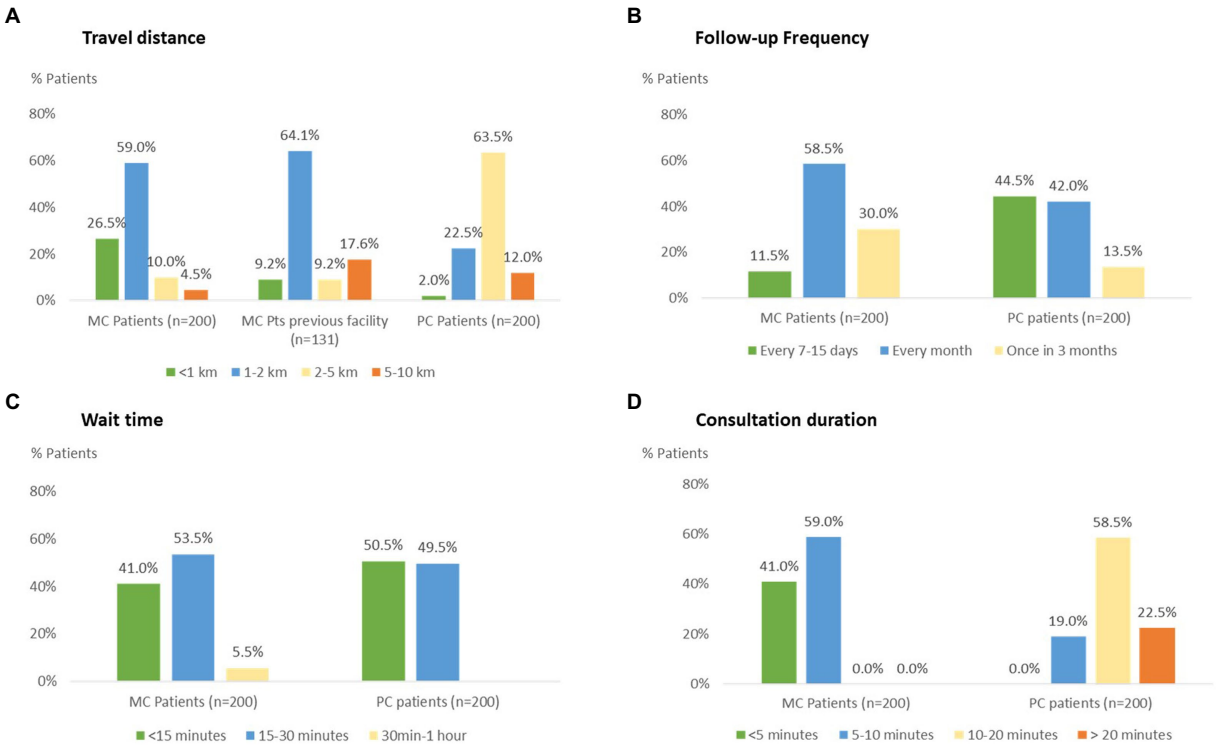
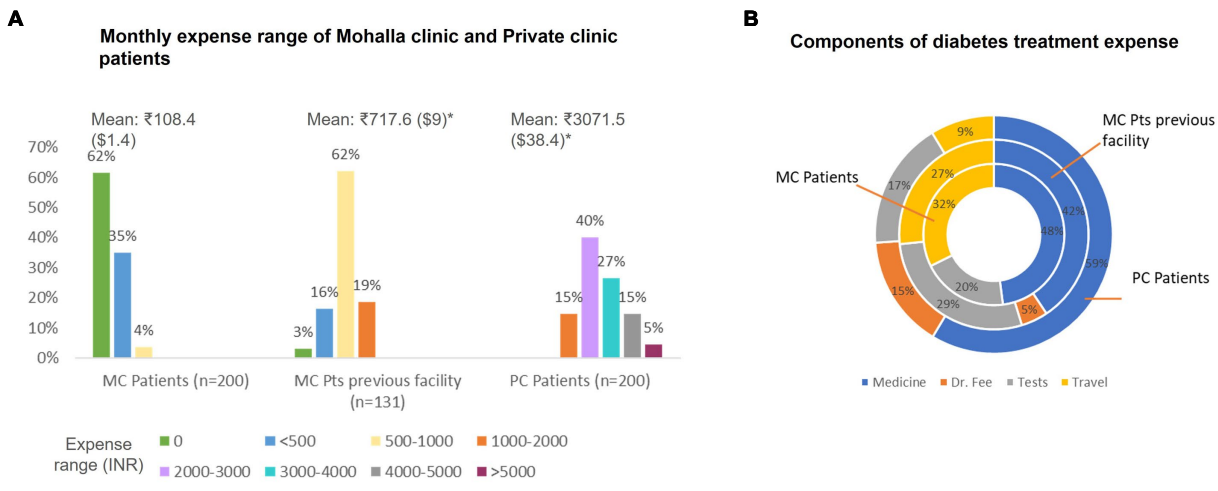


FIGURE 3
Comparison of access factors for Mohalla clinic and private clinic patients: **(A)** travel distance **(B)** follow-up frequency **(C)** wait time for consultation, and **(D)** consultation duration.



**Monthly expense of MC patients significantly lower than that of PC patients as well as expense of MC patients before shifting to MC (paired two tailed test, p<0.05)*

FIGURE 4
Diabetes treatment expense for Mohalla clinic and private clinic patients **(A)** monthly expense range and **(B)** components of treatment expense.

3.7. Barriers to seeking treatment at Mohalla clinic

Private clinic patients were asked about awareness about MCs and why they do not seek treatment at the free, government provided MC facility. All PC patients were aware of MCs, 60% indicated having an MC near their home, but none of the PC patients had ever tried getting treatment for any disease there. A significant percentage carried the bias that quality of care offered there would be sub-par, and some believed that a government run clinic was meant only for the poor (Figure 5).

4. Discussion

Healthcare services in India are provided both by the public sector and private players, with a significant skew towards the private sector.

Private facilities provide healthcare access for about 70% of outpatient visits and 60% of hospital admissions (14), with an even greater bias in urban areas where 79% of outpatient visits are serviced by the private sector (15). However, in the absence of any financial protection or insurance coverage for outpatient treatment costs in India, all such expenses must be borne out-of-pocket by the patients, making private facilities unaffordable for the poor. Public facilities in India are available free of cost but are overburdened and are straddled with deficiencies and inefficiencies of infrastructure. Further, referral pathways have not been established leading to overcrowding of secondary and tertiary health facilities in the public sector as patients can directly go to these hospitals for any disease type/severity. MCs were instituted in Delhi with an aim to decongest hospitals and offer the convenience of treatment access for minor ailments close to the lower socioeconomic neighborhoods. These community clinics have provided over 50 million consultations in the last five years (6), thus easing some of the patient burden on hospitals.

Studies conducted to evaluate the utilization and performance of MCs have reported high rates of satisfaction among the neighborhood community that accesses these clinics. A recent review reported a generally high level of satisfaction (~90%) with the MC services, which were considered either at par with or better than other existing healthcare facilities the patients accessed earlier (7, 8, 11–13). In a recently published community survey, MC users expressed a high level of satisfaction with the MC doctor and gave an average rating of 4.1 out of five (8). In another study, most patients indicated a high intent to return to the MC for seeking care in the future (5). While obvious factors such as proximity to the place of residence, shorter wait time vs. government hospitals, interaction time with the doctor and effectiveness of treatment have been cited as the reasons influencing decision of patients to return to MCs for seeking care, the most important factor suggested in this study is the interaction time with the doctor and other healthcare providers that can play a pivotal role in ensuring success of such initiatives (5).

Our study also pointed to physician interaction as the number one factor influencing satisfaction level of the patients attending MCs as well as those seeking care at private settings with 44.5% of all surveyed patients ranking it as one of the top 3 factors. A doctor who listens patiently and is perceived as providing adequate advice on management of their condition is the critical factor all patients are looking for while evaluating their treatment. MC doctors treat an average of 117 patients every day (6 h) (6) which means roughly 3 min on average per patient. In our survey also, 41% of MC patients said they get less than 5 min with the doctor while 80% of PC patients get more than 10 min with their doctors at each visit. The shorter consultation duration of MC patients appears to have been offset by the proximity of the clinic and the quality of physician interaction, translating into high satisfaction levels. Lower expectations of the marginalized population that these clinics serve may be another reason for ignoring the short consultation duration while indicating satisfaction level. A significant proportion of these patients switched from government dispensaries (another form of primary care set up) or government hospitals to MC and hence they may be used to overcrowding and being rushed through the appointment. In fact, those that shifted from government hospitals showed an improvement in satisfaction score from a mean of 3.4 at earlier hospital to 3.6 at MC and those who were receiving care at a government dispensary earlier showed a significant improvement in satisfaction score from a mean of 3.2 at the dispensary to 3.8 at MC. The major reasons cited for improvement in satisfaction after shifting were proximity to the facility and easy availability of free medicines and diagnostic tests vs. the earlier government facility.

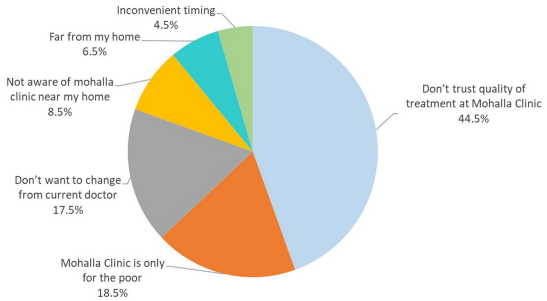


FIGURE 5
Barriers to seeking treatment at Mohalla clinic (Private clinic patients, *n*=200).

A surprising finding in our survey was the lower value both the groups of patients placed on diabetes control (treatment success) relative to other softer aspects like physician interaction or convenience factors like distance and wait time, in choosing the top 3 factors for satisfaction. Less than 10% MC patients and less than 20% PC patients indicated treatment success as a factor influencing their satisfaction score, which may be a reflection of inadequate awareness of the importance of glycemic control. This may also have a correlation with the high self-reported FBG levels at last testing by both the groups. Despite frequent blood glucose monitoring (at least once a month for 80% MC patients and all PC patients) and follow-up appointments (at least once a month for 70% MC patients and over 85% PC patients), self-reported FBG levels were above the normoglycemic range for almost all patients and were also in the very high range for a sizeable survey population. We could not authenticate these self-reported FBG values by comparing them with test reports, nor was our survey designed to correlate these with disease severity, treatment type, lifestyle modification counseling, or treatment adherence related factors. More than one-thirds of MC patients in our survey indicated they started treatment at MC only and have not changed treatment facility in the last 2 years, which means they may not have had a specialist consult even while many of them indicated inadequate glycemic control.

Only a few studies have evaluated patient satisfaction with diabetes care in various healthcare settings in India. A study conducted in urban Puducherry, South India reported high satisfaction levels of about 70% with the health care services received and there was no significant difference in the level of satisfaction between government and private health facility (16). In another study conducted at two centers in the sub-Himalayan region of North India, 70% of patients indicated they were moderately (14%) or highly satisfied (56%) with diabetes treatment, with no significant difference by treatment setting (17). Our survey results indicating high satisfaction level with both the MC (public setting) and the private setting are consistent with the findings in these earlier studies.

Limitations of the study: Our study was designed to understand the satisfaction of chronic disease patients with care at MC, taking diabetes as the representative disease. The finding of high self-reported FBG levels in both the groups was surprising but could not be validated with actual test reports. Further, our study was based on convenience sampling and the sample is not large enough for

generalization of this finding, neither was our survey designed to correlate high self-reported FBG with disease-related, treatment or counseling-related, patient-related or treatment adherence-related factors. Further studies are recommended to specifically assess the success of diabetes treatment in MCs using actual test reports and/or performing the tests as part of the study. This study was also not designed to evaluate the continuum of care and referral practices for diabetes (or other chronic diseases) for specialist consultation, which can be a part of future studies assessing treatment practices and treatment success. Our study focused on patient experiences and perception only, future studies can combine physicians' perspective on treatment practices as well. The study also did not aim to identify differences in patient satisfaction or access factors by location within the city, which could also be a subject of future studies.

5. Conclusion

The marginalized population of Delhi trusts MCs for seeking treatment of not only minor ailments but also for chronic conditions such as diabetes that require long term follow-up, repeated visits, and frequent monitoring. High patient satisfaction with diabetes care at MCs is backed by an overall favorable perception of physician interaction and proximity of the clinics. Better and more consistent availability of medicines, increased consultation time and periodic consultation with specialists is recommended for further improving the quality of care and patient satisfaction at MCs. Of note, the MC essential drugs list includes only three diabetes drugs (glibenclamide, glimepiride and metformin) and only two drugs for hypertension (enalapril and amlodipine, apart from diuretics) (4), which may be widened to enable MCs to comprehensively manage diabetes patients. MCs collect all patient and treatment related data through clinic software, which can be used to analyze the pattern of care for such patients and to check if there are any missed opportunities to refer to specialist care. Based on the analysis, the state government may consider redesigning/strengthening polyclinics and next tier facilities by adding useful features similar to MCs such as location convenience and easy availability of medicines and tests. Consideration also needs to be given for standardizing protocols for chronic conditions such as hypertension and diabetes for initiating treatment and periodic consultation by specialists to ensure patients get adequate care for managing complications, while regular follow-up and continuation of prescription can be done at the MC. The MCs can also play an important role in preventive screening and counseling in the community for high disease burden chronic conditions in India such as hypertension and diabetes, thus fulfilling the more comprehensive role envisaged for a primary health care facility. Further studies are recommended to evaluate the quality of diabetes treatment at MCs including patient counseling for lifestyle modification, referral practices, treatment success in terms of glycemic control and monitoring of long-term complications, which can further guide standardization of diabetes treatment protocols at the MCs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by DPSRU-Biomedical Research Human Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MGS conceptualized and designed the survey and the questionnaire, participated in conduct of the survey, data analysis and manuscript preparation. AG contributed to conduct of the survey, data acquisition, and manuscript preparation. KS assisted in data analysis and statistical analysis. HP guided the study concept, protocol design, questionnaire finalization, and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

AG is employed by Mangrove Creations LLP.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Association of subclass distribution of insulin antibody with glucose control in insulin-treated type 2 diabetes mellitus: a retrospective observational study

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Objective: To examine the distribution and effects of the subclass of insulin antibodies on glucose control and side events in patients with type 2 diabetes treated with premixed insulin analog.

Methods: A total of 516 patients treated with premixed insulin analog were sequentially enrolled from the First Affiliated Hospital of Nanjing Medical University from June 2016 to August 2020. Subclass-specific insulin antibodies (IAs) (IgG1-4, IgA, IgD, IgE, and IgM) were detected in IA-positive patients by electrochemiluminescence. We analyzed glucose control, serum insulin, and insulin-related events between IA-positive and IA-negative groups, as well as among patients with different IA subclasses.

Results: Overall, 98 of 516 subjects (19.0%) were positive for total IAs after premixed insulin analog therapy; of these participants, 92 had subclass IAs, and IgG-IA was the predominant subclass, followed by IgE-IA. IAs were associated with serum total insulin increase and local injection-site reactions but not glycemic control and hypoglycemia. In the subgroup analysis in patients with IA-positive, the IgE-IA and IA subclass numbers were more associated with increased serum total insulin levels. Additionally, IgE-IA might be correlated more strongly with local responses and weakly with hypoglycemia, while IgM-IA might be correlated more strongly with hypoglycemia.

Conclusion: We concluded that IAs or IA subclasses might be associated with unfavorable events in patients receiving premixed insulin analog therapy, which can be used as an adjunctive monitoring indicator in clinical insulin trials.

KEYWORDS

insulin antibody (IA), subclass, type 2 diabetes, glycemic control, retrospective

Introduction

Diabetes has been effectively managed with insulin since it was discovered in the 1920s by Banting and Best (1). However, immunological reactions (especially allergic reactions) to insulin have become increasingly common since that time. In their work, Berson et al. (2, 3) found that most insulin-treated patients had insulin-binding immunoglobulins (Igs), which were later identified as polyclonal immunoglobulin G (IgG). Insulin treatment has progressed through the use of animal insulin, recombinant human insulin, and insulin analogs (1, 4), resulting in a remarkable reduction rather than elimination of insulin antibodies. According to Fineberg et al. (5), insulin antibodies (IAs) were still present in 40–60% of insulin-treated diabetics. Although previous studies showed no significant correlation between IAs and glucose control (6, 7), IA-associated cases leading to severe clinical events, including insulin resistance (8), recurrent diabetic ketoacidosis (DKA) (9), or hypoglycemia (10), have continued to be reported. Recently, these events have garnered a resurgence of attention and have been defined as comprising exogenous insulin antibody syndrome (EIAS) (10, 11). Previous studies on the relationship between IAs and glucose control have mainly focused on total insulin antibodies without considering IA subclasses. Moreover, most of these studies were conducted in the 1980s and 1990s (6, 7) and thus should be updated. Indeed, aside from IgG-IA, several other different subtypes of insulin antibodies have been reported (12–15), including immunoglobulin M (IgM), immunoglobulin A (IgA), and immunoglobulin E (IgE). Studies on patients with type 1 diabetes have indicated that different IA subtypes exhibited various predictive effects in research settings (16, 17). Little evidence exists, however, of analysis regarding the association between IA subclasses and glycemic control in insulin-treated patients with type 2 diabetes (T2D). In most clinical trials involving insulin, hypoglycemia and local injection reactions have been the main adverse effects (18, 19); however, there is a lack of markers suggesting or warning of the occurrence of these unfavorable events. We hypothesized that IA subclasses might have a negative impact on glycemic control in clinical settings. Therefore, our study aimed to demonstrate whether IAs or IA subclasses induced by exogenous insulin affected metabolic control and predicted adverse events in Chinese type 2 diabetic patients receiving insulin treatment.

Methods

Subjects

Between June 2016 and August 2020, we initially collected 612 consecutive patients receiving premixed insulin analogs (lispro mix 50/50). The inclusion criteria were as follows (1): patients with a diagnosis aged ≥18 years (2); patients with type 2 diabetes diagnosed using WHO diagnostic criteria (3); patients negative for glutamic acid decarboxylase antibody, insulinoma-associated protein 2 antibody, zinc transporter 8 antibody, and insulin autoantibody before receiving insulin therapy (4); patients taking combined oral medication—metformin only (0.5 g, thrice times a day)—while not altering the regimen during the first four months of treatment; and (5) patients with well-documented clinical data and laboratory data (insulin antibody results before and after treatment and IA subclasses of IA-positive patients were required). The exclusion criteria were as follows (1): patients who were IA-positive before insulin therapy; and (2) patients with impaired hepatorenal function, acute diabetic complications, history of steroid use, uncontrolled hypertension, moderate to severe anemia, heart disease including decompensated cardiac insufficiency, unstable angina pectoris, myocardial infarction, active proliferative diabetic retinopathy or other unstable retinopathy, as well as drug abuse or alcohol dependence history. Finally, we included 516 patients receiving lispro mix 50/50. Patients were assigned into the following two groups (1): the IA-negative group, including those who were negative for IAs after insulin administration, comprised of 418 patients (207 males and 211 females) with a median age of 56.7 years, ranging from 29 to 75 years; and (2) the IA-positive group, including those who were positive for IAs after insulin administration, comprised of 98 patients (56 males and 42 females) with a median age of 57.7 years, ranging from 38 to 75 years. The flow chart of our study process is illustrated in Supplementary Figure 1.

This project was approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University (2021-SR-075).

Clinical characteristics and biochemical measurements

For all included subjects, detailed demographic profiles, clinical characteristics, and laboratory data before and after insulin administration were retrospectively collected, as well as gender, age, diabetic duration, weight, height, blood pressure, and daily insulin dosage (unit/kg per day) data. Glucose concentrations were measured using hexokinase. Serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDLC), high-density lipoprotein cholesterol (HDLC), blood urea nitrogen (BUN), creatinine (Cr), alanine aminotransferase (ALT), total bilirubin (TBil), and direct bilirubin (DBil) were measured using an automatic biochemical analyzer (Beckman Coulter AU5800). Serum insulin was analyzed using an electrochemiluminescence (ECL) immunoassay (YHLO iFlash3000). Bio-Rad D-100 high-performance liquid chromatography was used to measure hemoglobin glycated (HbA1c).

Assays for IAs and their immunoglobulin subclasses

The total IAs assay in our lab was detected using ECL assay, as described in detail in our previous work (20). The sensitivity of the IAs assay was 82.0%, and the specificity was 98.7%. The cut-off index of positivity for IAs was 0.0042, which was determined to represent the 99th percentile of 142 healthy control subjects. IA

subclasses were analyzed using the same principles as those used for total IAs. Briefly, a mixture of serum samples with sulfo-tag conjugated proteins (Meso Scale Discovery, R91AO-2) in phosphate-buffered solution (PBS) with 5% bovine serum albumin (BSA)was prepared. Overnight incubation at 4°C with secondary antibodies labeled with biotin against IgG1-4, IgA, IgD, IgM, and IgE (Ab 99775; Invitrogen 05-3540; Ab 86252; Ab 99818; Ab 85864; Ab 224182; Ab 99745; Ab 99807) was performed. Meanwhile, a streptavidin-coated (MesoScale Discovery, L15SA-1) plate with blocker buffer (Meso Scale Discovery, R93AA-1) was incubated under the same conditions. The following day, the mixture of serum and antigen was transferred to the streptavidin plate after it had been washed and incubated at room temperature for one hour; the plate shaker was set at low speed. Following another washing of the plate and the adding of a read buffer, the plate was counted on a plate reader. Using positive and negative control serum samples as internal standards, we generated an index to represent the results. A single assay run was conducted on all samples from each individual.

Statistical analysis

Continuous variables are expressed as mean ± SD when data are normally distributed or as median (inter-quartile range) when data are not normally distributed. Categorical variables are reported as the numbers (frequency). Differences in clinical characteristics between the groups were analyzed *via* the Student's *t* test or Mann–Whitney U test for continuous data and the χ^2 test for categorical data. For all tests, p values < 0.05 were considered significant with a two-tailed test. Data analysis was carried out using SPSS v25.0 (IBM Co., Armonk, NY, USA), and graphs were generated using PRISM v9.0.0 (GraphPad Software, Inc., La Jolla, CA) and R software (version 4.1.1).

Results

Baseline clinical and demographic characteristics of the study population

A total of 516 subjects (253 females and 263 males) were included in the final analysis, with a median age of 56.70 years (interquartile range [IQR]: 50.70, 63.60) and a median disease duration of 84.0 months (IQR: 37.75, 132.00) (Table 1). Of these patients, 98 (42 females and 56 males) were positive for total IAs after insulin therapy, whereas 418 (211 females and 207 males) were negative for total IAs. As shown in Table 1, there were no statistical differences in terms of gender, age, diabetic duration, blood pressure, body mass index, blood lipid profile (LDLC, HDLC, TG, TC), hepatorenal function, hemoglobin, erythrocyte, leukocyte, and thrombocyte between the two groups before insulin therapy. Moreover, there were no significant differences in fasting plasma glucose, 2-hour postprandial blood glucose, HbA1c, daily insulin dosage, and fasting insulin. Serum direct bilirubin (3.85 [IQR: 2.69, 5.09] vs. 3.40 [IQR: 2.38, 4.40]; P = 0.016) was slightly elevated in the IA-positive group at baseline compared to the control group.

Insulin-treated T2D patients with IAs predominantly responded to IgG, followed by IgE

Of the 98 study patients who were detected to be positive for total IAs, 92 had subclass IAs as follows: 48 (48.98%) had IgG-IA only; 29 (29.59%) had IgG-IA plus IgE-IA; 7 (7.14%) had IgG-IA plus IgM-IA; 6 (6.12%) had IgG-IA, IgE-IA, and IgM-IA; and 2 (2.04%) had IgG-IA plus IgA-IA, IgE-IA, and IgM-IA (Figure 1A), and thier ECL indexes are shown in Figures 2A, B. However, subclasses of IAs were not measurable in 6 patients, and IgD-IA was absent in all patients. Of the 92 subjects with the IgG-IA subclass, all were detected to have IgG1-IA, and 57 cases had IgG4-IA, whereas IgG2-IA and IgG3-IA were found in 1 and 3 cases, respectively (Figures 1B, 2C).

IAs associated with serum total insulin increase but not glycemic control

Glycemic control, as indicated by the median change from baseline in FPG, 2hPG, and HbA1c, did not differ between the IA-positive group and the placebo group (-3.7 mmol/L [IQR: -5.9, -2.0] vs. -3.1 mmol/L [IQR: -5.4, -1.4], P = 0.10; -7.0 mmol/L [IQR: -9.5, -3.3] vs. -6.9 mmol/L [IQR: -10.3, -3.5], P = 0.46; -1.7% [IQR: -2.7, -0.8] vs. -1.7% [IQR: -2.7, -1.0], P = 0.49; Figures 3A–C). There was also no significant difference in weight change (2.0 kg [IQR: 0.0, 3.4] vs. 2.0 kg [IQR: 0.5, 3.5], P = 0.98; Figure 3D) between the two groups of patients before and after insulin treatment. IA-positive patients received approximately 900 times more serum insulin changes compared with IA-negative patients (45.0 uU/ml [IQR: 25.3, 97.7] vs. 0.05 uU/ml [IQR: -3.4, 3.1], P < 0.0001; Figure 3E), but the increase in daily insulin requirement over our observation period was similar between the IA-positive group and the IA-negative group (0.27 U per kg per day [IQR: 0.13, 0.38] vs. 0.21 U per kg per day [IQR: 0.10, 0.36], P = 0.15; Figure 3F).

IgE-IA and IA subclass numbers associated with increased serum total insulin level

Among patients with different IA subclasses, alterations in FPG, 2hPG, HbA1c, weight, and daily insulin dose were similar (Figures 4A–D, F) before and after insulin treatment. A few minor differences remained—i.e., more decreased FPG (Figure 4A), less decreased 2hPG (Figure 4B), and more increased weight (Figure 4D)—and were observed in patients with all four subtypes (IgG-IA, IgE-IA, IgA-IA, and IgM-IA) compared to those in other groups, albeit not significantly. Patients with IgG-IA and IgE-IA, whether containing other subclasses or not, all had higher serum insulin than those with IgG-IA only or patients with IgG-IA

TABLE 1 Clinical and demographic characteristics of the study population beforeinsulin therapy

Characteristic	Total (n=516)	IA-negative (n=418)	IA-positive (n=98)	P value
Male (%)	263 (50.97%)	207 (49.50%)	56 (57.10%)	0.174
Age at enrollment (years)	56.70 (50.70 - 63.60)	56.70 (50.70- 63.60)	57.70 (49.90 - 63.70)	0.838
Duration of diabetes (months)	84.00 (37.75 - 132.00)	84.00 (36.00 - 132.00)	95.5 (59.00 - 132.00)	0.217
Systolic Blood Pressure (mmHg)	126.66 ± 14.45	126.69 ± 14.42	126.55 ± 14.65	0.932
Diastolic Blood Pressure (mmHg)	79.38 ± 9.43	79.39 ± 9.44	79.34 ± 9.41	0.962
BMI (kg/m^2)	25.66 ± 3.19	25.71 ± 3.23	25.50 ± 3.05	0.561
HbA1c (%)	8.60 (7.90 - 9.80)	8.70 (7.90 - 9.80)	8.50 (7.88 - 9.0)	
HbA1c (mmol/mol)	70.49 (62.84 - 83.61)	71.58 (62.84 - 83.61)	69.40 (62.62 - 82.51)	0.603
Daily insulin dosage (unit per kilogram)	0.29 (0.23 - 0.36)	0.29 (0.22 - 0.35)	0.30 (0.24 - 0.37)	0.143
FPG (mmol/L)	10.71 (8.80 - 13.67)	10.70 (8.64 -13.62)	10.79 (9.29 -13.83)	0.443
2hPG (mmol/L)	17.70 (15.09 - 20.91)	17.44 (15.10 - 20.91)	17.94 (14.97 - 20.90)	0.716
Fasting insulin (uU/mL)*	10.30 (7.00 - 15.05)	9.64 (6.67 - 14.75)	11.00 (7.99 - 16.38)	0.059
Total cholesterol (mmol/L)	4.77 ± 1.16	4.78 ± 1.17	4.71 ± 1.09	0.576
Triglyceride (mmol/L)	1.64 (1.14 - 2.37)	1.68 (1.14 - 2.37)	1.48 (1.13 - 2.46)	0.610
HDLC(mmol/L)	1.150 (0.99 -1.36)	1.15 (0.99 - 1.36)	1.16 (0.99 - 1.37)	0.884
LDLC (mmol/L)	2.77 ± 0.87	2.78 ± 0.88	2.72 ± 0.81	0.571
ALT (U/L)	21.00 (15.00 - 32.00)	21.00 (15.00 - 31.00)	22.00 (14.93 - 32.25)	0.375
AST (U/L)	19.80 (16.00 - 25.00)	19.00 (16.00 - 25.00)	20.20 (16.00 - 27.28)	0.240
TBil (umol/L)	13.10 (10.20 - 16.30)	13.10 (10.19 - 16.10)	13.50 (10.25 - 17.15)	0.407
DBil (umol/L)	3.50 (2.40 - 4.60)	3.40 (2.38 - 4.40)	3.85 (2.69 - 5.09)	0.016
Blood urea nitrogen (mmol/L)	5.29 ± 1.38	5.29 ± 1.41	5.33 ± 1.24	0.785
Serum creatinine (umol/L)	59.24 ± 15.82	58.75 ± 15.63	61.36 ± 16.56	0.142
Hemoglobin (g/L)	143.6 ± 14.43	143.25 ± 14.37	145.04 ± 14.67	0.278
Erythrocyte (10^12/L)	4.77 ± 0.48	4.77 ± 0.48	4.79 ± 0.47	0.679
Leukocyte (10^9/L)	6.61 ± 1.51	6.58 ± 1.56	6.76 ± 1.31	0.283
Thrombocyte (10^9/L)	229.3 ± 56.01	228.66 ± 56.84	232.09 ± 52.52	0.585

Data for continuous variables are expressed as the mean±SD, or as the median (interquartile range). Categorical variables are presented as n (%). BMI, body mass index; FPG, fasting plasma glucose; 2hPG, 2-hour postprandial blood glucose; HbA1c, glycated hemoglobin; LDLC, low-density lipoprotein cholesterol; HDLC, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBil, total bilirubin; DBil, direct bilirubin. * means that data of serum insulin before insulin administration were available in 95 IA-positive patients and 416 IA-negative patients.

and IgM-IA (Figure 4E), showing an increasing trend with the increase of IA subclass numbers.

IAs not associated with hypoglycemia but with injection-site reactions

Regarding insulin-associated adverse events, the injection-site reaction incidence was about four times higher in the IA-positive group than in the IA-negative group (Figure 5; 13.3% vs. 3.3%, P < 0.0001). However, the frequency of hypoglycemia was slightly higher in the IA-positive group than in the control group (Figure 5; 30.6% vs. 25.60%, P = 0.312).

IgE-IA correlated more strongly with local response and IgM-IA correlated more strongly with hypoglycemia

To analyze insulin-associated events among patients with different IA subclasses, we divided them into four subgroups. As Figure 6 shows, patients with IgG-IA and IgE-IA, whether containing other subclasses or not, had the lowest frequency of hypoglycemia (Figure 6A; 16.21%, 6 of 37 cases) and the highest frequency of injection-site reactions (Figure 6B; 24.32%, 9 of 37 patients). However, patients with IgG-IA and IgM-IA had the highest prevalence of hypoglycemia (Figure 6A; 57.14%, 4 of 7 cases), while they did not have injection-site reactions (Figure 6B; 0.0%).

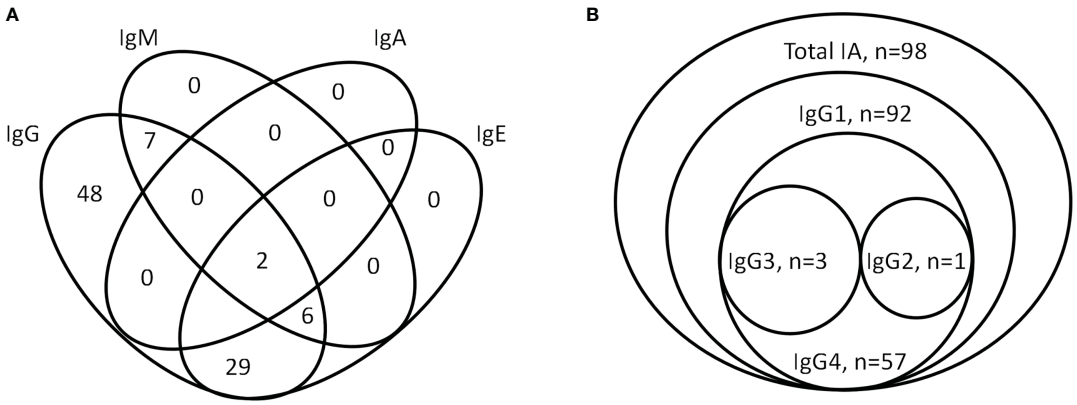

FIGURE 1
Venn diagram showing the number of individuals tested positive for subclass-specific insulin antibodies (IgG, IgA, IgE, and IgM) **(A)** and isotype-specific IgG-IA (IgG1-4) and total IA **(B)**. IA, insulin antibody.

Discussion

This study demonstrated that insulin antibodies were present in approximately 20% of T2D patients treated with premixed insulin analogs for short-term therapy. IgG-IA was the predominant subclass distribution of insulin antibodies (IgG1 was the most dominant isotype of IgG), followed by IgE-IA. However, IA subclasses were not detectable in six patients. One possible explanation is that some non-specific bindings were measurable in detecting the total insulin antibodies, leading to false positives for

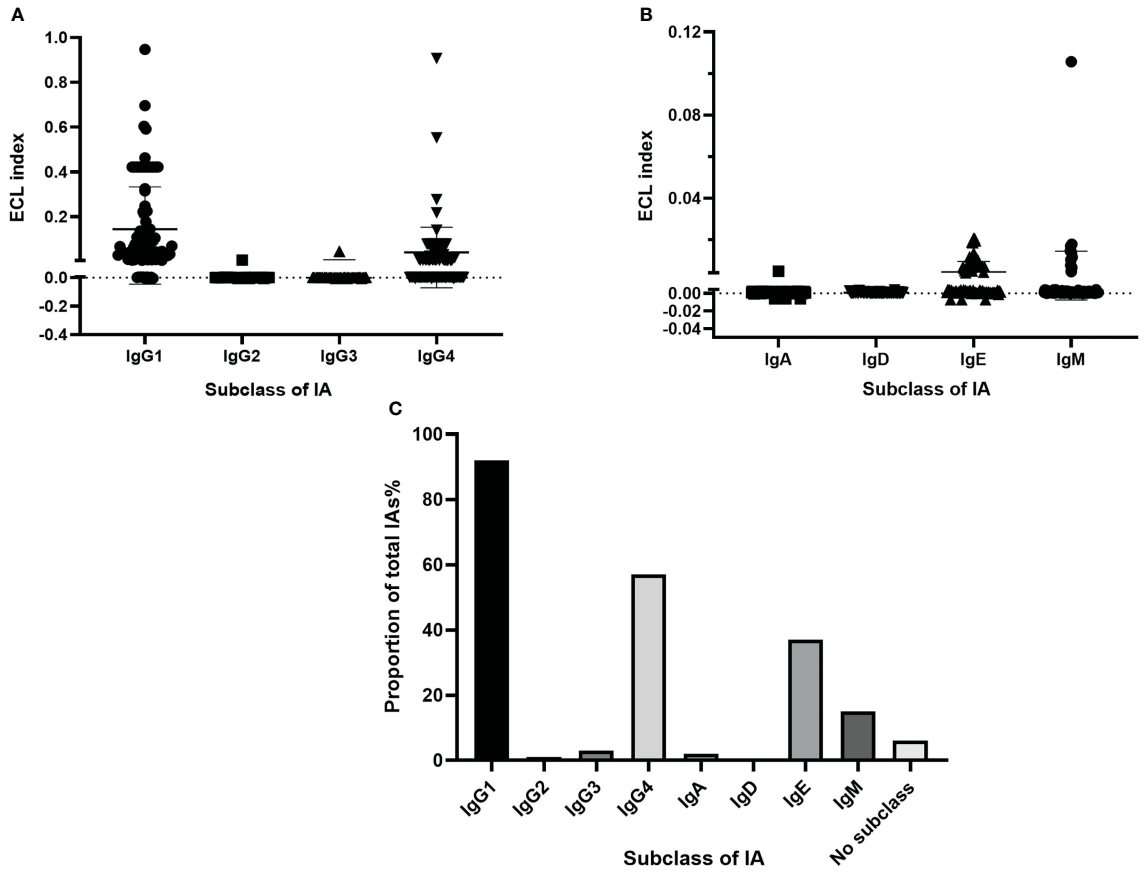

FIGURE 2
Levels (ECL index) of IgG-IA (IgG1-4) **(A)** and IgA-IA, IgD-IA, IgE-IA, IgM-IA **(B)**, and frequencies of positivity of different IA isotypes in patients detected positive for total IAs **(C)**. The solid lines represent median values, and the dotted horizontal lines denote the threshold for positivity. ECL, electrochemiluminescence; IA, insulin antibody.

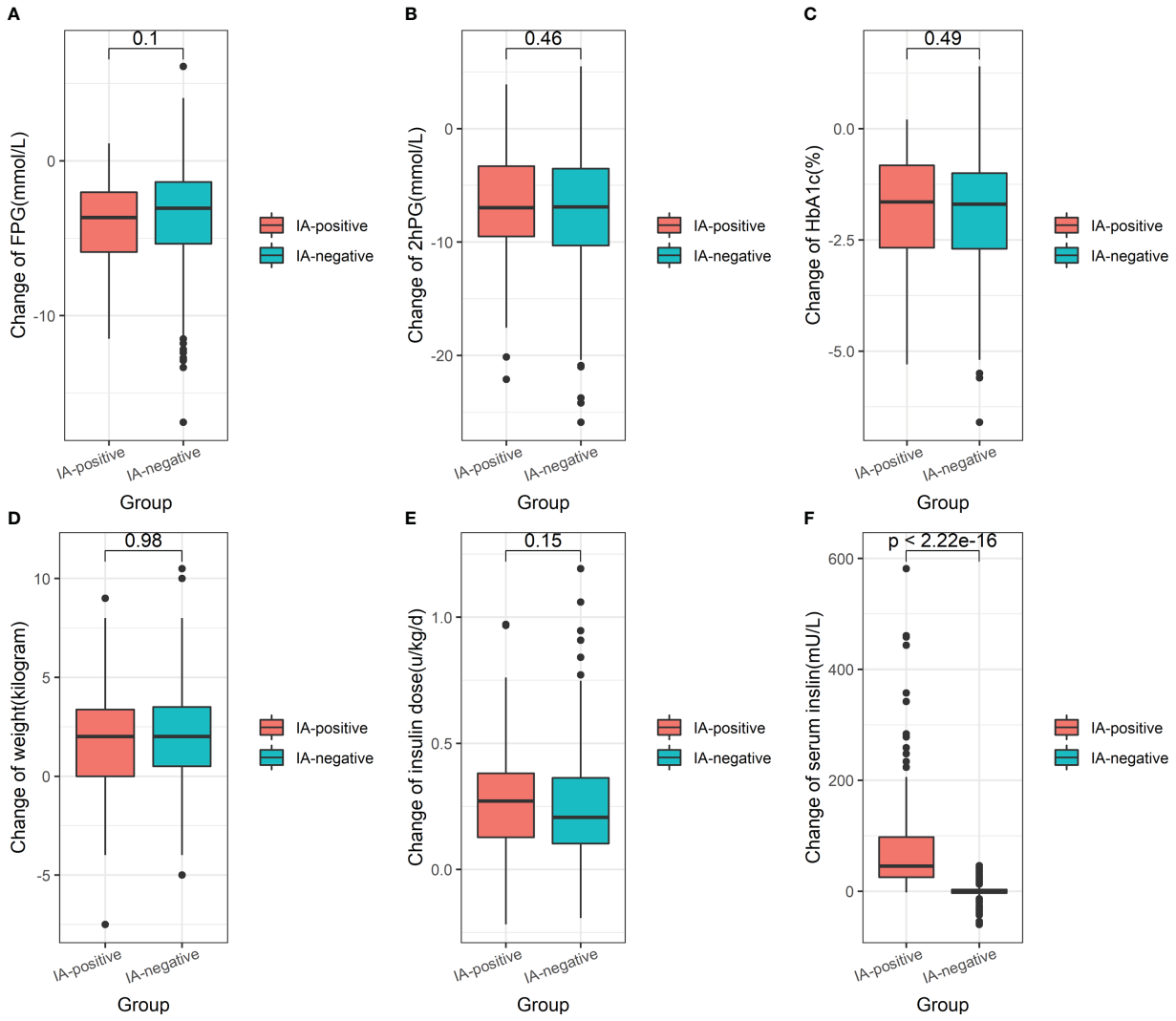

FIGURE 3
The boxplot showing the median change of FPG **(A)**, 2hPG **(B)**, HbA1c **(C)**, weight **(D)**, serum total insulin **(E)**, and daily insulin dose **(F)** from baseline in IA-positive patients and IA-negative patients. FPG, fasting plasma glucose; 2hPG, 2-hour postprandial blood glucose; HbA1c, glycated hemoglobin. Data on serum fasting insulin measured before and post-insulin administration were available in 94 IA-positive and 407 IA-negative patients.

IAs. Another reason was that the relevant total insulin antibodies were genuinely positive, while the low indexes when detecting IA subclasses were reported as negative to ensure accuracy. Taken together, these factors indicate the test's limitations, which comprise a common clinical testing phenomenon. A similar description was reported in the work of Martin Fuchtenbusch et al. (16), in which 2 of 12 patients failed to register any IA subclasses.

According to previous studies, conventional bovine–porcine insulin produces antibodies in > 95% of insulin-treated patients (21, 22). A study (23) examining the immunogenicity of different monocomponent insulins in newly diagnosed patients with type 1 diabetes has shown both human and porcine insulin groups had 24% and 39% of patients with IAs at three months. In another study of > 200 patients without previous exposure to insulin, 44% of patients taking human insulin developed insulin antibodies compared to 60% of those taking porcine insulin at 12 months (5). Accordingly, by using purified and recombinant human insulin preparations, IAs have been markedly reduced but not eliminated. IgG subclass responses to insulin may vary with diabetes type. For example, in T2D patients with high levels of insulin antibody responses, IgG1, IgG3, and IgG4 antibodies have been shown to be elevated, but IgG2 antibodies negligibly absent (24). This is similar to the distribution of IgG subclasses in our study. The frequency of IgG3-IA was lower in our patients. Notably, the IgG1 and IgG4 were the most common subclass responses to both insulin autoantibodies (IAAs) and IAs in patients with type 1 diabetes and insulin-treated prediabetic patients with islet antibody positivity (16). When genetically susceptible young children lack the IgG3-IA, they may be protected from type 1 diabetes (17); conversely, type 1 diabetic patients have been shown to have an elevated IgG3-IA.

Figure 3 illustrates that there was no significant correlation between IAs and glucose control as reflected by FPG, 2hPG, or HbA1c, consistent with most studies from the 1980s and 1990s (6). Similarly, only a marginal effect on glucose control was observed for

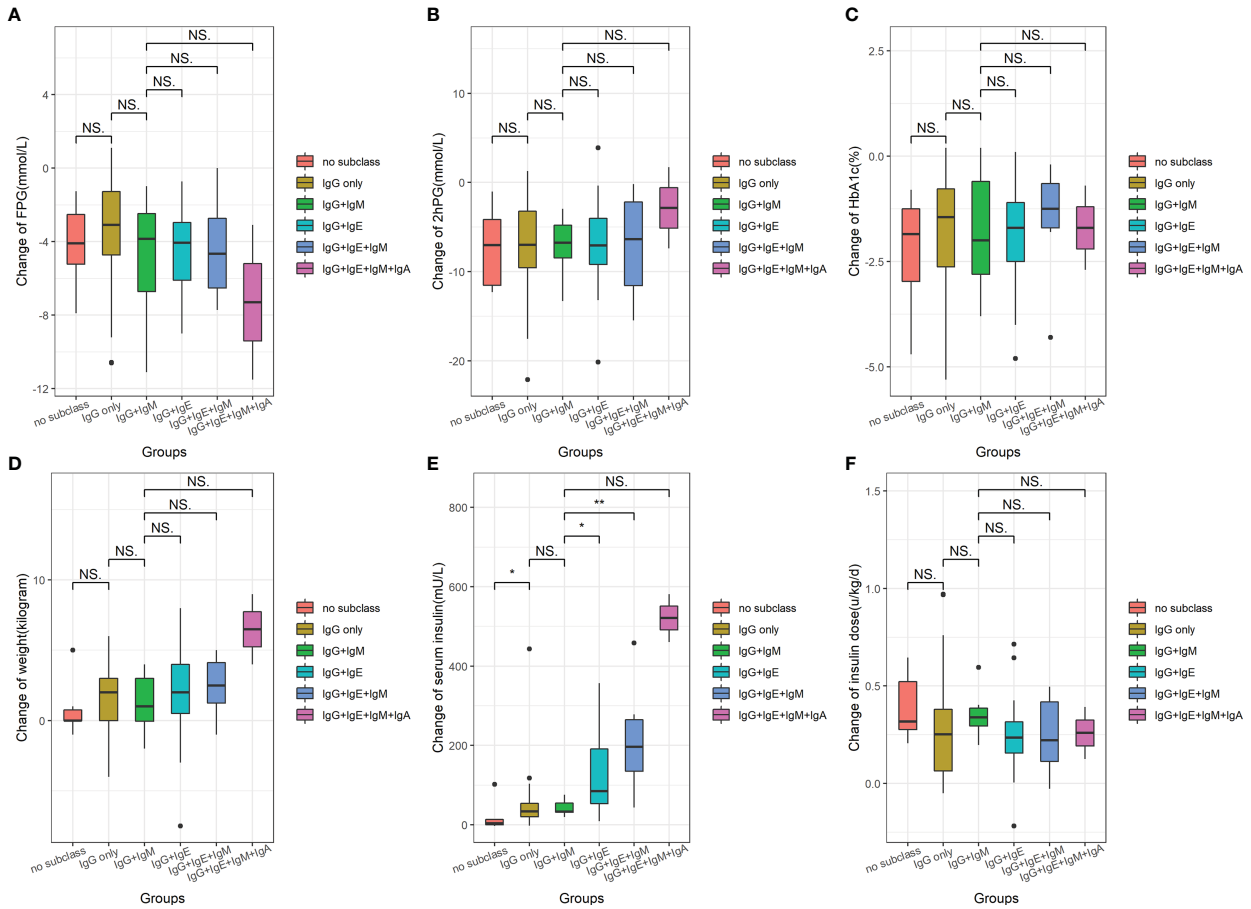

FIGURE 4
The boxplot showing the median change of FPG **(A)**, 2hPG **(B)**, HbA1c **(C)**, weight **(D)**, serum total insulin **(E)**, and daily insulin dose **(F)** from baseline among different IA subclasses groups. NS, no significance; * P<0.05; ** P<0.005. FPG, fasting plasma glucose; 2hPG, 2-hour postprandial blood glucose; HbA1c, glycated hemoglobin. Data of serum fasting insulin measured prior to and post-insulin administration were available in 94 IA-positive patients.

IAs induced by subcutaneous or peritoneal insulin infusions (25). Recently, Philip Home et al. (18) demonstrated no relationship between maximum individual IA titers and changes in HbA1c or insulin dose. However, in our study, more decreased FPG, less decreased 2hPG, and more increased weight were observed in patients with all four subtypes (IgG-IA, IgE-IA, IgA-IA, and IgM-IA), albeit not to the point of statistical significance. The possible explanation for the minor difference provided was that insulin antibodies in these patients with four IA subtypes might bind more tightly (high affinity) to insulin and delay its release, resulting in higher postprandial glucose and less decrease in 2hPG compared with other groups. Meanwhile, delayed hyperinsulinemia has been shown to result in lower fasting glucose and a greater decrease in FPG (26). Unfortunately, due to the limitations of retrospective studies, this remains speculation on our part and warrants confirmation by testing the affinity of different IA subclasses. Regarding insulin dosage requirements, the IA-positive group appeared to require slightly more insulin than the control group, despite there being no statistical difference, suggesting that IAs may not be associated with immune insulin resistance in the short-term, while the long-term effects must be determined by subsequent studies. Although it has been documented that patients with high IA levels may present with a rare syndrome of severe insulin resistance (requiring more than 200 U/d of insulin for at least two days) (9, 27), the underlying causal mechanism remains unclear. Additional prospective treatment trials involving human

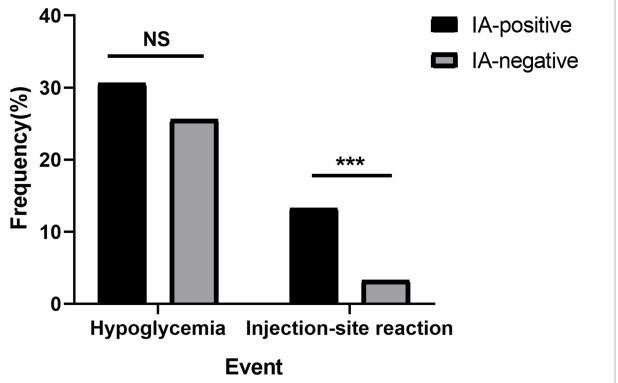

FIGURE 5
Frequency of hypoglycemia or injection-site reactions in the IA-positive group and IA-negative group. NS, no significance; *** P < 0.0001; injection-site reaction, referring to skin itching, local redness and swelling, ecchymosis, subcutaneous nodules, and urticaria.

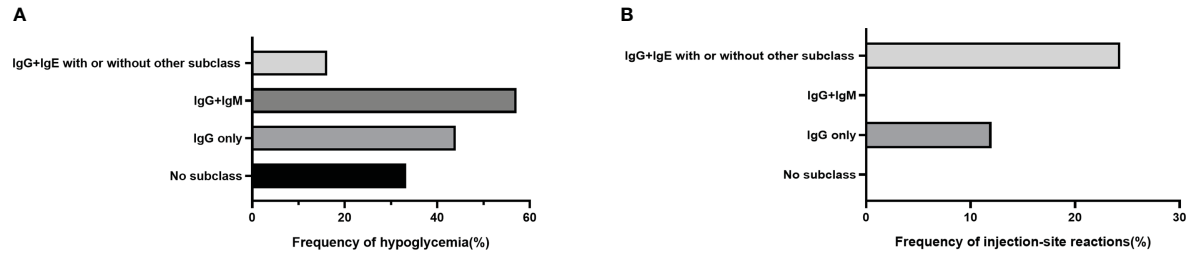

FIGURE 6
Frequency of hypoglycemia or injection-site reactions in the IA-positive group stratified by IA subclasses. **(A)** shows 2 of 6 (33.33%), 18 of 48 (37.50%), 4 of 7 (57.14%), and 6 of 37 (16.21%) patients with no subclass, IgG-IA alone, IgG-IA and IgM-IA, or IgG-IA, IgE-IA whether with or without other subclasses had hypoglycemia respectively. **(B)** shows 4 of 48 (8.30%), and 9 of 37 (24.32%) patients with IgG-IA only, or IgG-IA, IgE-IA, whether with or without other subclasses had injection-site reactions, respectively; other groups had no local responses.

and animal insulin, insulin analogs, and inhaled insulin trials have also shown no significant correlation between IA levels and insulin dose in insulin-naive and insulin-treated patients (5, 7, 28).

Hypoglycemia is a common adverse effect of insulin therapy and an indicator that the safety of insulin requires evaluation. IAs have often been considered relevant to hypoglycemia, especially in EIAS (10). Nevertheless, in our observation, IAs had no relationship with hypoglycemia episodes but instead with increased total serum insulin. Similarly, Fineberg et al. (6) concluded that hypoglycemic events and IA levels were not correlated. Furthermore, sporadic case reports indicated that high levels of IA were associated with clinical hypoglycemia syndromes in a few individuals (29–32); however, none of these studies analyzed IA subtypes. Hypoglycemia in this setting was potentially caused by increased insulin dissociation from the insulin-antibody complex due to low affinity or decreased glucose counter-regulation and prolonged free insulin half-life (5, 33, 34), while other mechanisms warrant further investigation. Whether such low-affinity insulin antibodies are more likely to occur in patients with specific IA isoforms or isoform combinations also deserves further investigation. A previous study (35) on IAAs predicting type 1 diabetes showed that IgM antibodies were of lower affinity than IgG antibodies. Interestingly, in our subgroup analysis, patients with IgG-IA and IgM-IA were more susceptible to hypoglycemia than other subtype combinations; however, patients with IgG-IA and IgE-IA were not susceptible, on the contrary. We speculated that the pentameric structure of IgM-IA might have high capacity and low affinity—that is, it might bind more insulin (high capacity) than other IA subclasses, as well as being more easily dissociated (low affinity). The complex, which combined IgM-IA and insulin, dissociated, then more insulin was released, resulting in a higher incidence of hypoglycemia. The lowest frequency of hypoglycemia in patients with IgG-IA and IgE-IA might be related to the high affinity of IgE-IA; in this case, high-affinity IgE-IA is minimally dissociated, thus leading to less hypoglycemia. Additionally, it has been shown that high-affinity but not low-affinity IgE causes anaphylaxis (36, 37). The detection of IgG-IA, IgM-IA, and IgE-IA in patients with severe hypoglycemia might be suitable to test this hypothesis and provide more insight into the mechanisms involved. In addition, one possible explanation for our study's discrepancy from the previous studies is that the latter mainly enrolled patients with recurrent hypoglycemia or severe hypoglycemia. In contrast, there were few cases of severe hypoglycemia in our present study. Another reason is that, despite elevated insulin levels in our patients with IAs, there may be no abnormal dissociation of insulin as previously described. Notably, hyperinsulinemia has been demonstrated to contribute to diastolic cardiovascular dysfunction and diabetic cardiomyopathy (38, 39). Investigating whether hyperinsulinemia caused by specific IA subtypes is more predictive of this risk or is associated with it will be useful.

In the past, allergy was another frequent adverse effect in patients receiving insulin therapy (40). The prevalence of insulin allergy has decreased since human insulin and its analogs were introduced (41). Such hypersensitivity may result from the insulin molecule itself, as well as from protamine and other components. However, in our study, IA-positive patients suffered a higher rate of injection-site reactions, indicating allergy but unsuitable for all cases, especially in subjects with IgE-IA. Immunoglobulin E is central to type I immediate allergic responses (42). In addition, insulin-specific IgE (type 1) and IgG (type 3) antibodies may mediate local and systemic reactions to insulin administration (6, 43). Additionally, a type IV response can also contribute to insulin hypersensitivity (44). Thus, it is easy to understand why more local reactions occurred in our patients with IgG-IA and IgE-IA. Moreover, IgE-IA has been demonstrated to be present in injection-site reactions by other researchers (45), as has IgG-IA, IgM-IA, and IgA-IA. However, the exact nature of these patients' local responses has yet to be identified, and in-depth evaluations of such patients have not been performed (45).

Finally, it is essential to note that our study has several limitations. First, retrospective data from a single center are subject to the inherent limitations of such investigations. Second, the affinity of IAs or IA subclasses was not routinely performed in clinics. The affinity may indicate the maturity of the immune response (35), and such differences may explain why specific subtypes of IA are more prone to hypoglycemia and other related problems. Third, HLA genotypes have been shown to be associated with IA isotype and affinity (46). However, patients' HLA genotypes were not available in this study, and thus a relationship between IA subclasses and genotypes was not explored. Moreover, the lack of follow-up data prevented us from evaluating the correlation between IA subclasses and long-term clinical outcomes and

seroconversion among different IA subclasses. This deficiency will be explored in future work.

In summary, our findings indicated that IA subclasses might be correlated with adverse effects of premixed insulin analog therapy, despite showing no association with glycemic control. We offer a suggestion for clinical designers and clinicians: If patients exhibit unexplained hypoglycemia or other adverse reactions, IA subtypes should be considered in addition to testing for IAs. In future work, we desire to shed more light on the mechanisms responsible for the maturation of the immune responses to exogenous insulin. Another future study will focus on exploring the relationship between IA subclasses and long-term clinical outcomes.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

This project was approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University (2021-SR-075). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

SC, HC, and YJ contributed equally to the manuscript and shared the first authorship. XZ and MZ contributed to data collection. TY and YG designed the study and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1141414/full#supplementary-material

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U-shaped association between online information exchange and app usage frequency: a large-scale survey of China 's online young and middle-aged people with pre diabetes and diabetes

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Background: China has the world's largest diabetic population, and the cost of caring for all these people every day is substantial. Online information exchange and app usage frequency have been demonstrated to play a significant influence in the management of blood glucose and enhancement of diabetes-related quality of life. However, the association between online information exchange and app usage frequency among actual online populations remains unclear and deserves additional study. Therefore, we evaluated the factors affecting the frequency of app usage in the online glucose management population, with a particular emphasis on the connection between online information exchange and app use frequency, contributing to the expansion of the research of diabetes management models.

Method: This cross-sectional study was conducted by disseminating questionnaires in blood glucose management-related forums and WeChat groups and included 1586 online users concerned about blood glucose management. Information exchange and app usage frequency were considered as independent and dependent variables, respectively. We performed stratified and single factor analysis, multiple equation regression analysis, smooth curve fitting, and threshold effect and saturation effect analysis. R (version 4.1.3, http://www.Rproject.org) and EmpowerStats were used for data analysis.

Result: After adjusting for other covariates, information exchange was independently and positively associated with app use frequency (β = 8.6, 95% CI: 6.5 to 11.2, p < 0.001). Through interaction analysis, the most significant interaction factors influencing the relationship between information exchange and app usage frequency were identified as health insurance status, whether living with parents, glycated hemoglobin status in the previous month, and self-monitoring of blood glucose (SMBG). The association between information exchange and app usage frequency is U-shaped, with information exchange inflection points of 3.0 and 4.2. Information exchange and app usage frequency are negatively correlated when the average information exchange score is less

than 3.0, and for every point increase in the average information exchange score, the likelihood of the app high usage frequency group compared to the app low usage frequency group decreases by 70%. The relationship between information exchange and app usage frequency is strongest when it is greater than or equal to 3.0 and less than or equal to 4.2. The probability of the app high usage frequency group occurring compared to the app low usage frequency group rises 17.3 times for every 1 point increase in the average information exchange score. The probability of the app high usage frequency group occurring in comparison to the app low usage frequency group increased by 1.8 times for every 1 point rise in information exchange when the average information exchange score was higher than 4.2.

Conclusion: Age, body mass index, married, living with parents, hemoglobin level, SMBG, and information exchange were positively connected with app usage frequency in our study of online blood glucose management population. The link between information exchange and app use frequency was significantly U-shaped. The app usage frequency changed the most with the rise in information exchange when the information exchange score was greater than or equal to 3.0 and less than or equal to 4.2. Therefore, we ought to offer effort to concentrate on and increase the health-related behaviors and activities of those in this score interval.

KEYWORDS

diabetes, online, app usage frequency, information, U-shape

1 Introduction

In 2019, the International Diabetes Federation estimated that 9.3% (463 million) people worldwide have diabetes, and that figure is expected to progressively increase, rising by 1.6% (337 million) by 2045. Despite this, approximately 50% of persons with diabetes are unaware of their condition (1). Long-term blood sugar instability will lead to diabetes-related complications (2), and put more of a strain on Medicare's budget for diabetes treatment (3). Fortunately, online interventions found on the Internet play an encouraging role in lowering the risk of diabetes (4). Therefore, the development of simple and effective blood glucose interventions that make it possible for the general population to participate in them is the current focus of diabetes management in China (5–7).

Online information sharing has grown commonplace since the start of the internet era (8). Online information exchange consists mostly of information searching and information sharing. Users can seek information by browsing or posting questions, and they can give information by participating in or initiating forums or by submitting responses to inquiries (8, 9). As far as we know, online users are now also changing from one-way information searching to two-way information sharing (10). Users' perceived social support, blood glucose management, self-care compliance, and quality of life are all enhanced through online information exchange (11, 12). As online information exchange enables a greater number of users to participate, it increases users' pleasure and sense of belonging to online activities, which will lead to a favorable impression of online activities and encourage them to continue to engage in online activities (11, 13, 14).

In recent years, mobile health has increasingly been utilized as an innovative method for diabetes prevention and treatment (15). The number of people who utilize mobile health services is, as we all know, steadily increasing as a result of the popularity of these services and the ease with which they can be accessed (16). It has been proven that mobile health has a substantial effect on both glucose management and weight control (17, 18). In addition, mHealth contributes to an increase in diabetes awareness and medication adherence (19). Mobile health apps are widely used because of their many benefits, which include data storage, instruction, and assistance (20). Surprisingly, it has been demonstrated that information interchange through an app is superior to conventional diabetes management strategies for the self-management of people with diabetes (21, 22). The app's wide audience means it can serve as a platform for cross-border communication and trade, unlocking new possibilities for mobile devices (23).

Results from mobile health interventions are significantly correlated with how often their target population engages in online activities (24), however, it is also influenced by socio-demographic variables such as age, gender, level of education, and health status (25). Furthermore, it is influenced by the app's features, such as information acquisition and resource discovery (26). According to our knowledge, whether participants have health difficulties or the app has numerous virtual or actual incentives, they

are more likely to use it (27). As the frequency of online engagement rises, the behavior of users in day-to-day management may shift (28), while more frequency app usage correlates with better health habits and more normal blood sugar levels (29, 30), without increasing the risk of hypoglycemia (30). Since the app usage frequency is influenced by numerous variables and swings over time (27), it is difficult to maintain a high frequency of use (25, 26). Therefore, it is important for us to find the factors that affect the frequency of app use.

App stickiness refers to the frequency and duration of app usage, and information exchange is a crucial factor in promoting app stickiness, with this link being especially prominent in social and email apps (31–33). According to Chin-Lung et al., social factors like information exchange will positively impact the persistence of app usage by acting directly on users' motivations to keep using the app (32). However, investigations on the frequency of app use indicated that there was no statistically significant difference between the app management group's 10.7 (SD = 9.5) and app information exchange management group's 11.1 (SD = 7.3) app uses per week (p = 0.83) (34). Unfortunately, there are no research on the correlation between online information exchange and app usage frequency, and it is vital to investigate this correlation in order to acquire a deeper understanding of the mechanisms underlying blood glucose management.

Due to the significant relationship between the frequency of app use and daily blood glucose management (30), in order to effectively manage blood glucose, we need to improve the frequency of app use and create interventions to enhance the frequency of app use. According to recent research, apps with real-time information exchange capabilities can better follow online trends (31). According to research by Litchman et al., those who exchange information regularly are more invested in their online activities (35). Shao et al. observed that information exchange increases user satisfaction, which has an effect on the growth of social networks usage (33). However, research into the correlation between information exchange and app usage within digital communities is lacking. We hypothesize that information exchange is a driving force behind regular app use.

The objectives of this study were to determine the relationship between information exchange and app usage frequency, identify factors that affect app usage frequency, and predict daily online behavior of the online glucose management population, particularly app usage frequency, in order to help blood glucose management and find a new online behavioral pathway to effectively assist in blood glucose control.

2 Materials and methods

2.1 Participants

After accepting the researcher's explanation of the purpose and significance of this study and instructions for completing the questionnaire, Chinese online blood glucose management users agreed to accept and complete the electronic questionnaire on their own or with the help of others to become study participants. Participants must meet the following inclusion criteria: First, participants with abnormal blood glucose (including diabetes: fasting blood glucose ≥7.0 mmol/L, 2-hour postprandial blood glucose ≥11.1 mmol/L; prediabetes: fasting blood glucose 6.1-6.9 mmol/L, 2-hour postprandial blood glucose <7.8 mmol/L; impaired fasting blood glucose: fasting blood glucose 5.6-6.1 mmol/L, 2-hour postprandial blood glucose <7.8 mmol/L; abnormal glucose tolerance: fasting blood glucose <7.0mmol/L, 2 hours postprandial blood glucose 7.8-10.9mmol/L), and had been exchanging information online and using the blood glucose management app. Second, participants with normal blood glucose who have interacted with people with abnormal blood glucose, participated in online information exchange activities, and use a blood glucose management app, and who wish to receive assistance and support in this area. Third, participants capable of completing the questionnaire on their own or with the aid of others. Fourth, there was no history of mental illness or cognitive impairment among the participants.

2.2 Data collection

Based on previously conducted studies, we created a thorough questionnaire for this cross-sectional study that included pertinent inquiries about information exchange and app usage frequency (36–38). We evaluated the features of online behavior and the elements that influence app usage frequency and analyzed data on online blood glucose management population characteristics, information exchange behavior, and app usage frequency. We demonstrated reliability through preliminary trials.

In this study, the individuals responsible for administering the questionnaires received professional training. Patients with diabetes who met the inclusion criteria were sent electronic questionnaires *via* the platform "Questionnaire Star" from March 15 to May 15, 2022. These questionnaires were posted in blood glucose management posting bars (such as the Sweet Home Bar, Blood Sugar Bar, High Blood Sugar Bar, Diabetes Bar, etc.), QQ groups (Diabetes Exchange Group), and blood glucose management WeChat groups (Glucose Friends Support Exchange Group). Based on the following exclusion criteria: (1) any two or more questionnaires with the same network IP address; (2) questionnaires with highly repetitive answers; (3) questionnaires that took less than 120s to complete; (4) questionnaires with illogical or contradictory content or omissions in the responses, 336 questionnaires were excluded and 1586 questionnaires were ultimately included. 82.9% of tests were valid.

The questionnaire included demographic data (age, gender, height, weight, education, residence, marital status), social support status (family size, whether living with parents), blood glucose related information (glycosylated hemoglobin status, SMBG), active information searching frequency (before joining the online group, after joining the online group), online information exchange scale (5 items; valid and reliable, Cronbach's α= 0.85>0.80 and KMO= 0.85>0.800, respectively) and app usage frequency scale (5 items; valid and reliable, Cronbach's α= 0.79>0.70 and KMO= 0.79>0.70, respectively),

The assessment of the frequency of active searching for online health-related information was based on two items on a 5-point Likert scale: 1 = "never" (0 searches per day), 2 = "occasionally" (1-2 searches per day), 3 = "generally" (3-5 searches per day), 4 = "sometimes" (6-10 searches per day), and 5 = "often" (more than 10 searches per day) with the following content: (1) before joining the online group; (2) after joining the online group. Online information exchange was assessed based on a 5-point Likert scale (1 = "strongly disagree", 2 = "disagree", 3 = "neutral", 4 = "agree", and 5 = "strongly agree") of 5 items, which are as follows: (1) The exchange of knowledge and experience related to the group or forum is very convincing to me; (2) The members of the group or forum exchange knowledge and experience frequently, seriously, and enthusiastically; (3) I can ask relevant questions and answer them for more informative answers to my inquiries; (4) I can exchange more symptoms, conditions, or ideas with members of the group or forum; (5) When I feel confused or uncomfortable, members of the group or forum will provide me with useful online help or information. The evaluation of app usage frequency was based on 5 items on a 6-point Likert scale (1 = "never", 2 = "less than once a month", 3 = "once a month", 4 = "several times a month", 5 = "several times a week", 6 = "every day") with the following content: (1) WeChat (including WeChat groups), (2) Weibo, (3) QQ (including QQ groups), (4) Jitterbug (5) others. The above-mentioned scales with good reliability were used for patient self-assessment. The scores of the scales were determined by calculating the mean of the scale items. The flow chart of the study is shown in Figure 1.

2.3 Ethics approval and consent to participate

All participants provided verbal informed consent to inclusion before they participated in the study, and the protocol was approved by the Ethics Committee of the Medical College of Shantou University (Code: SUMC-2021–064).

2.4 Statistical analysis

Continuous variables are expressed as mean ± standard deviation and for categorical variables as number of cases (N, N%), ratio (OR) and 95% confidence interval (CI), respectively. For app usage frequency, we first convert it into a dichotomous variable based on the median, with less than 50% being the app low usage frequency group and greater than or equal to 50% being the app high usage frequency group. Information exchange was transformed into a trichotomous variable according to the trichotomous method, where the low information exchange group scored ≤ 3.8, the medium information exchange group scored > 3.8 and ≤ 4.2, and the high information exchange group scored > 4.2. Using single-factor and multi-factor binary logistic regression models, in order to further analyze the relationship between online information exchange and app usage frequency, we used three different models, model 1: no adjustment for variables; model 2: adjustment for sex and age; model 3: adjustment for sex, age, BMI, education, medical insurance, marital status, residence, family size, whether living with parents, glycated hemoglobin, and SMBG. In the sensitivity analysis, we also use the trichotomous variables of information exchange obtained by the trichotomous method, and calculate the trend to observe the possibility of nonlinearity. A weighted generalized additive model and a smooth curve fitting were performed to address nonlinearity between information exchange and app usage frequency. When nonlinearity was identified, the critical inflection point was determined using a recursive method. Then, a two-piecewise linear regression model was conducted on both sides of the inflection point. Interaction tests were conducted to assess whether patient characteristics influenced the association between online information exchange and app usage frequency. In addition to further study of the data, we subsequently performed subgroup

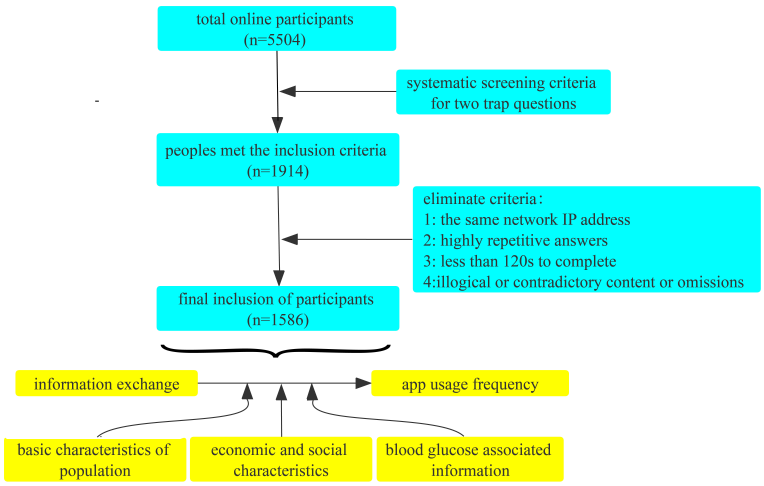

FIGURE 1
Research flow chart.

analyses. The statistical analyses were conducted using package R (version 4.1.3, http://www.Rproject.org) and EmpowerStats software (http://www.empowerstats.com).

3 Results

3.1 Descriptive statistical analysis of participants

Participants' ages ranged from 29 to 90, with a median of 54 years old, and there were 1,586 blood glucose managers analyzed in this study. Among the entire population, 1,321 individuals (83.3% of the total) were <40 years old, whereas 265 individuals (16.7% of the total) were ≥40 years old. Of the total number of participants, 679 were female (42.8%) and 907 were male (57.2%). More than half of the participants have a normal BMI, and there are 1358 participants with a BMI between 18.5 and 24 who make up 66.90% of the total. The average score for information exchange was 4.1 ± 0.6. There were no statistically significant differences in the proportions of gender, education, BMI, medical insurance, residence, or family size between groups with high and low app usage frequency. In contrast, there were statistical differences in age, marital status, whether living with parents, glycosylated hemoglobin, SMBG and information exchange scores between groups with high and low app usage frequency (Table 1). From the IP address of the respondents, the respondents came from 32 provinces, autonomous regions and municipalities directly under the Central Government of China, of which Hebei, Beijing and Tianjin ranked the top three, which can be seen in Figure 2. Due to the wide geographical distribution of participants, there are many participants in each region, so the results of this study are representative of all regions in China. Figure 3 maps the number of respondents in each region.

Figure 4A demonstrates while the information exchange has the best score for information credibility (4.12 points), while it has the lowest score for user sincerity and passion (4.01 points). Overall, participants rated the online information exchange highly on average. WeChat has the greatest usage frequency score, accounting for 4.94 points in Figure 4B, followed by Tiktok, QQ, and Weibo. Other app is utilized less frequently than the above four online information exchange applications, with only 4.14 points. Figures 4C, D show that most people used active search for online health-related information both before and after joining the online group. Prior to joining the online group, participants scored 3.481

TABLE 1 Baseline characteristics of eligible participants, including their online app usage frequency classifications.

App usage frequency	Low	High	Total	P-value
N	673	913	1586	
Information exchange	3.8 ± 0.6	4.3 ± 0.4	4.1 ± 0.6	<0.001
Sex				0.928
male	384 (57.1%)	523 (57.3%)	907 (57.2%)	
female	289 (42.9%)	390 (42.7%)	679 (42.8%)	
Age				0.017
<40	578 (85.9%)	743 (81.4%)	1321 (83.3%)	
≥40	95 (14.1%)	170 (18.6%)	265 (16.7%)	
BMI				0.706
<18.5	86 (13.4%)	102 (11.4%)	188 (12.2%)	
>=18.5, <24	430 (66.9%)	609 (68.1%)	1039 (67.6%)	
>=24, <28	108 (16.8%)	157 (17.6%)	265 (17.2%)	
>=28	19 (3.0%)	26 (2.9%)	45 (2.9%)	
Education				0.247
junior high school and below	36 (5.3%)	36 (3.9%)	72 (4.5%)	
high school/technical secondary school/technical school	105 (15.6%)	163 (17.9%)	268 (16.9%)	
junior college	171 (25.4%)	200 (21.9%)	371 (23.4%)	
undergraduate	325 (48.3%)	465 (50.9%)	790 (49.8%)	
postgraduate and above	36 (5.3%)	49 (5.4%)	85 (5.4%)	
Medical security				0.826
urban employees' medical insurance	242 (36.0%)	330 (36.1%)	572 (36.1%)	

(Continued)

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TABLE 1 Continued

App usage frequency	Low	High	Total	P-value
urban residents' medical insurance	179 (26.6%)	226 (24.8%)	405 (25.5%)	
new rural cooperative medical insurance	169 (25.1%)	235 (25.7%)	404 (25.5%)	
others	83 (12.3%)	122 (13.4%)	205 (12.9%)	
Marital status				<0.001
single or unmarried	238 (35.4%)	228 (25.0%)	466 (29.4%)	
married	416 (61.8%)	667 (73.1%)	1083 (68.3%)	
divorced or widowed	19 (2.8%)	18 (2.0%)	37 (2.3%)	
Residence				0.176
Urban	391 (58.1%)	559 (61.2%)	950 (59.9%)	
Suburban	154 (22.9%)	215 (23.5%)	369 (23.3%)	
Township	95 (14.1%)	110 (12.0%)	205 (12.9%)	
Countryside	33 (4.9%)	29 (3.2%)	62 (3.9%)	
Family size				0.092
≤3 persons	348 (51.7%)	511 (56.0%)	859 (54.2%)	
≥4 persons	325 (48.3%)	402 (44.0%)	727 (45.8%)	
Cohabitation with parents				<0.001
Yes	511 (75.9%)	761 (83.4%)	1272 (80.2%)	
No	162 (24.1%)	152 (16.6%)	314 (19.8%)	
Glycosylated hemoglobin				<0.001
≤6.4%	373 (55.4%)	529 (57.9%)	902 (56.9%)	
≥6.5%	203 (30.2%)	329 (36.0%)	532 (33.5%)	
Not tested	97 (14.4%)	55 (6.0%)	152 (9.6%)	
SMBG				<0.001
Not tested or occasionally tested	500 (74.3%)	516 (56.5%)	1016 (64.1%)	
Daily testing	173 (25.7%)	397 (43.5%)	570 (35.9%)	
Information exchange tertile				<0.001
Low	373 (55.4%)	150 (16.4%)	523 (33.0%)	
Medium	167 (24.8%)	259 (28.4%)	426 (26.9%)	
High	133 (19.8%)	504 (55.2%)	637 (40.2%)	

Divide into two groups according to the median of app usage frequency, less than 50% is the low usage frequency group of app and more than or equal to 50% is an app high usage frequency group; Information exchange was transformed into a trichotomous variable according to the trichotomous method, where the low information exchange group scored ≤ 3.8, the medium information exchange group scored > 3.8 and ≤ 4.2, and the high information exchange group scored > 4.2.

(SD=1.127) for online search, while after joining the online group, they scored 3.745 (SD=0.876), an increase in score compared to before joining that was statistically significant (p<0.001).

3.2 The relationship between online information exchange and app usage frequency

App use frequency increased significantly with information exchange score (p<0.05). The frequency of application use was higher among participants who were at least 40 years old, married, cohabited with their parents, had an elevated hemoglobin level in the previous month, and had regular daily blood glucose testing (p<0.05). However, the frequency of app use was not affected by gender, BMI, education, medical insurance, residence, or family size (Table 2).

In this study, we developed three models to determine if information exchange has an independent effect on app usage frequency. Adjusted and unadjusted data are used to examine the relationship between information exchange and app usage frequency in Table 3. Using the information exchange score as a

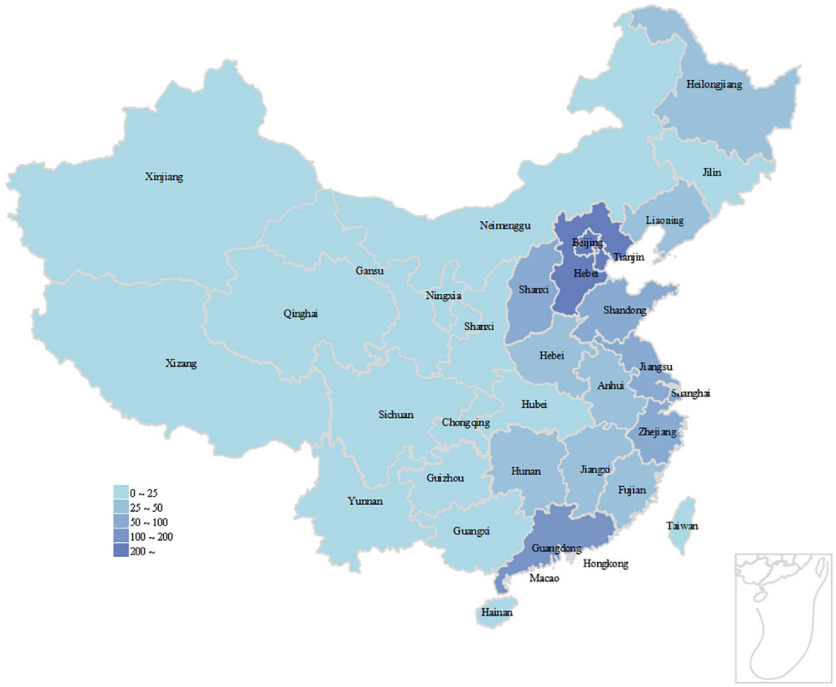

FIGURE 2
Distribution of participants' region of residence.

continuous and categorical variable, the relationship between information exchange and app usage frequency was confirmed. Model 1 and 2 found a positive correlation between information exchange and app usage frequency when information exchange was modeled as a continuous variable (OR=8.2, 95% CI: 6.4 to 10.5, p<0.001; OR=8.3, 95% CI: 6.4 to 10.6, p<0.001, respectively). In model 3, we also found a similar correlation (OR=8.6, 95% CI: 6.5 to 11.2, p<0.001). Using the trichotomous approach, information exchange was positively associated with app usage frequency in Model 1 (OR = 9.4, 95% CI: 7.2 to 12.3, p<0.001). Similarly, we found no significant difference between model 2 and 3 compared with model 1 (OR=9.5, 95% CI: 7.2 to 12.5, p<0.001; OR=9.6, 95% CI: 7.2 to 12.9, p<0.001 respectively). In the sensitivity analysis, we also calculated p-values for trends to observe the likelihood of non-linearity based on information exchange trichotomous variables derived from the trichotomous method, and we observed comparable trends (p<0.01).

As information exchange is a continuous variable, it is necessary to examine the nonlinear connection between online information exchange and app usage frequency. Using a generalized additive model and a smoothed curve fit, we then sought to establish a connection between online information exchange and app usage frequency. The relationship between information exchange and app use frequency was nonlinear, as shown in Figure 5. (after adjusting for sex; age; BMI; education; medical insurance; marital status; residence; family size; cohabitation with parents; glycosylated

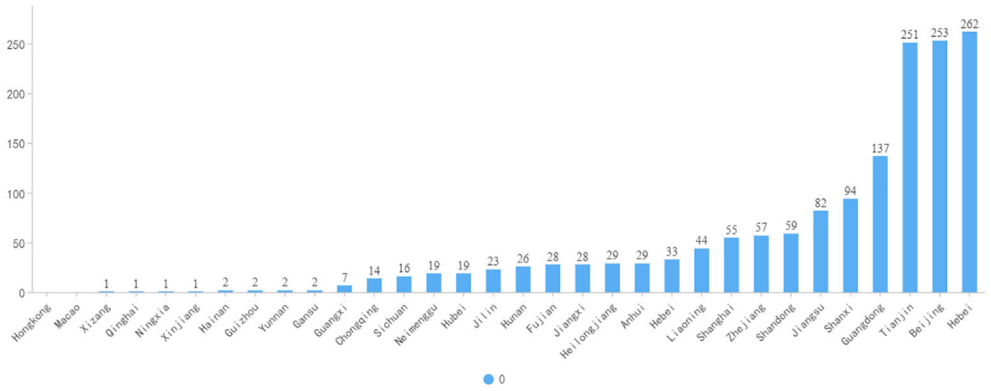

FIGURE 3
Participants' provinces of residence.

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TABLE 2 Univariate analysis for app usage frequency.

	Statistics	App usage frequency
Sex		
male	907 (57.2%)	1.0
female	679 (42.8%)	1.0 (0.8, 1.2) 0.928
Age		
<40	1321 (83.3%)	1.0
≥40	265 (16.7%)	1.4 (1.1, 1.8) 0.018
BMI		
<18.5	188 (12.2%)	1.0
>=18.5, <24	1039 (67.6%)	1.2 (0.9, 1.6) 0.266
>=24, <28	265 (17.2%)	1.2 (0.8, 1.8) 0.291
>=28	45 (2.9%)	1.2 (0.6, 2.2) 0.670
Education		
junior high school and below	72 (4.5%)	1.0
high school/technical secondary school/technical school	268 (16.9%)	1.6 (0.9, 2.6) 0.099
junior college	371 (23.4%)	1.2 (0.7, 1.9) 0.543
undergraduate	790 (49.8%)	1.4 (0.9, 2.3) 0.146
postgraduate and above	85 (5.4%)	1.4 (0.7, 2.6) 0.338
Medical insurance		
urban employees' medical insurance	572 (36.1%)	1.0
urban residents' medical insurance	405 (25.5%)	0.9 (0.7, 1.2) 0.557
new rural cooperative medical insurance	404 (25.5%)	1.0 (0.8, 1.3) 0.882
others	205 (12.9%)	1.1 (0.8, 1.5) 0.650
Marital status		
single or unmarried	466 (29.4%)	1.0
married	1083 (68.3%)	1.7 (1.3, 2.1) <0.001
divorced or widowed	37 (2.3%)	1.0 (0.5, 1.9) 0.974
Residence		
Urban	950 (59.9%)	1.0
Suburban	369 (23.3%)	1.0 (0.8, 1.2) 0.849
Township	205 (12.9%)	0.8 (0.6, 1.1) 0.173
Countryside	62 (3.9%)	0.6 (0.4, 1.0) 0.064
Family size		
≤3 persons	859 (54.2%)	1.0
≥4 persons	727 (45.8%)	0.8 (0.7, 1.0) 0.092
Cohabitation with parents		
yes	1272 (80.2%)	1.0
no	314 (19.8%)	0.6 (0.5, 0.8) <0.001
Glycosylated hemoglobin		

(Continued)

TABLE 2 Continued

	Statistics	App usage frequency
Not tested	152 (9.6%)	1.0
≤6.4%	902 (56.9%)	2.5 (1.8, 3.6) <0.001
≥6.5%	532 (33.5%)	2.9 (2.0, 4.2) <0.001
SMBG		
Not tested or occasionally tested	1016 (64.1%)	1.0
Daily testing	570 (35.9%)	2.2 (1.8, 2.8) <0.001
Information exchange	4.1 ± 0.6	8.2 (6.4, 10.5) <0.001
Information exchange tertile		
Low	523 (33.0%)	1.0
Medium	426 (26.9%)	3.9 (2.9, 5.1) <0.001
High	637 (40.2%)	9.4 (7.2, 12.3) <0.001

Divide into two groups according to the median of app usage frequency, less than 50% is the low usage frequency group of app and more than or equal to 50% is an app high usage frequency group; Information exchange was transformed into a trichotomous variable according to the trichotomous method, where the low information exchange group scored ≤ 3.8, the medium information exchange group scored > 3.8 and ≤ 4.2, and the high information exchange group scored > 4.2.

hemoglobin and SMBG), and a descending and ascending curve can be seen from left to right. In Table 4, we employ a two-segment regression model in which the inflection points are 3.0 and 4.2. When the information exchange score was below 3.0, there was a negative correlation between information exchange and app usage frequency, with each 1-unit increase in information exchange reducing the probability of occurrence by 70% in the high app usage frequency group compared to the low app usage frequency group, but without a statistically significant difference in outcome (OR=0.3, 95% CI:0.1 to 1.7, p=0.169). When the information exchange score was between 3.0 and 4.2, there was a positive correlation between information exchange and app usage frequency, with a 17.3-fold increase in the probability of occurrence of the high app usage group for every 1-unit increase in the information exchange score (OR=18.3, 95% CI:11.0 to 30.4, p<0.001). For every 1 unit increase in the information exchange score, the app high usage frequency group increased by 1.8 times relative to the app low usage frequency group when the information exchange score was higher than 4.2 (OR=2.8, 95% CI:1.1 to 6.9, p<0.05).

3.3 A subgroup analysis of the relationship between online information exchange and app usage frequency

Subgroup analyses were performed to investigate the connection between information exchange and app usage frequency. Besides, we also evaluated were the interrelationships among the subgroup's various strata. As shown in Table 5, there is a positive and statistically significant correlation between online information exchange and app usage frequency across all study subgroups. There were no statistically significant differences by gender (p=0.7329), age (p=0.1363), BMI (p=0.9791), education (p=0.5576), marital status (p=0.2765), residence (p=0.6802), or family size (p=0.8488) after the interaction. In contrast,

TABLE 3 Multiple regression equation analysis for the relationship between online information exchange and app usage frequency.

Outcome	Model 1	Model 2	Model 3
	β (95%CI) P-value	β (95%CI) P-value	β (95%CI) P-value
Information exchange	8.2 (6.4, 10.5) <0.001	8.3 (6.4, 10.6) <0.001	8.6 (6.5, 11.2) <0.001
Information exchange tertiles			
Low	1.0	1.0	1.0
Medium	3.9 (2.9, 5.1) <0.001	3.9 (3.0, 5.1) <0.001	4.0 (2.9, 5.3) <0.001
High	9.4 (7.2, 12.3) <0.001	9.5 (7.2, 12.5) <0.001	9.6 (7.2, 12.9) <0.001
Information exchange tertile trend	3.1 (2.7, 3.5) <0.001	3.1 (2.7, 3.5) <0.001	3.1 (2.7, 3.6) <0.001

Non-adjusted model adjust for: None.
Adjust I model adjust for: sex; age.
Adjust II model adjust for: sex; age; BMI; education; medical insurance; marital status; residence; family size; cohabitation with parents; glycosylated hemoglobin; SMBG.

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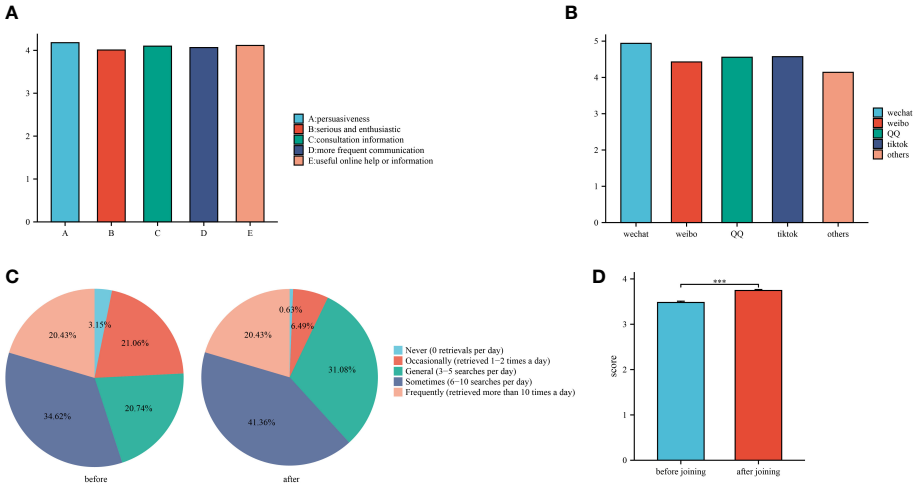

FIGURE 4
(A): Scores for different online information exchange behavior; **(B)**: Score for different app usage frequency. **(C)**: Proportion of active search frequency before and after joining an online group. **(D)**: Average score of active search frequency before and after joining an online group.

interaction tests were significant in different strata of the subgroups of medical insurance(p=0.0190), cohabitation with parents (p=0.0048), glycosylated hemoglobin (p=0.0076) and SMBG (p=0.0269).

4 Discussion

There is growing evidence that online information exchange and regular app use play an important role in blood glucose control and daily blood glucose management, and these factors have become the focus of online users for blood glucose management (30, 39–41). We can see a statistically significant difference in app usage frequency in accordance with the extent of online information exchange, and this is the first large-scale study to our knowledge to explore the connection between information exchange and app usage among Chinese internet users.

4.1 Basic demographic characteristics

Among the survey population of respondents to the various clinical stages of blood glucose management included in this study, the ratio of males to females is roughly 1:1, and the population tends

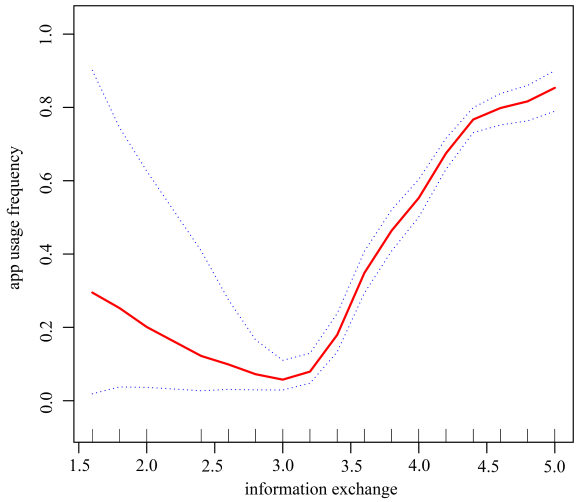

FIGURE 5
Analysis of the relationship between information exchange and app usage frequency in the online blood glucose management population by smoothing curve fitting The red solid bars indicate a smooth curve fit between information exchange and app usage frequency in the blood glucose management population, and the blue dashed strip indicates the 95% confidence interval of the fit between information exchange and app usage frequency. Adjusting variables: sex; age; BMI; education; medical insurance; marital status; residence; family size; cohabitation with parents; glycosylated hemoglobin; SMBG Note:Divide into two groups according to the median of app usage frequency, less than 50% is the low usage frequency group of app and more than or equal to 50% is an app high usage frequency group.

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TABLE 4 Independent correlation between online information exchange and app usage frequency analysis by multiple segmented linear regression.

Outcome:	App usage frequency		
	β	(95%CI)	P-value
Model I			
A straight-line effect	8.6	(6.5, 11.2)	<0.001
Model II			
Fold points (K1, K2)	3, 4.2		
< K1 segment effect 1	0.3	(0.1, 1.7)	0.169
K1-K2 segment effect 2	18.3	(11.0, 30.4)	<0.001
>K2 segment effect 3	2.8	(1.1, 6.9)	0.028
Log likelihood ratio tests	<0.001		

Adjusting variables: sex; age; BMI; education; medical insurance; marital status; residence; family size; cohabitation with parents; glycosylated hemoglobin; SMBG.

to be younger, with the number of people aged 40 years accounting for approximately 83.3% of the total. In addition, of the total number of participants, 55.2% have some college education. Similar findings were found in a Chinese online health community survey (42), proving that our sample is a good representation of the whole.

The fact that young people and student groups make up the bulk of China's internet users may be related to the fact that they lack the knowledge to support their health management and the confidence to refute dubious and untrue opinions, so they prefer to communicate with people who have had similar experiences *via*the internet and rely on online health information to aid them in managing their health (43).

We observe that married people and cohabitation with their parents make up a significant portion of the high app usage frequency group, accounting for 73.1% and 83.3% respectively of the total. Evidence suggests this may have its origins in the advantages of family behavioral therapy, in which members of the family can actively communicate and take responsibility for one another in learning about and applying the latest developments in blood glucose management *via*the use of apps (44, 45). Brew-Sam et al., on the other hand, suggest that parental encouragement does not always lead to more app usage. Users may turn to third parties, such as blood glucose management apps, for help when they encounter unhelpful family behaviors. On the other hand, the need for self-care apps may be reduced if families are very involved in their loved ones' day-to-day management (44). Those in our study population who were married or still living at home with their parents were more likely to use the app, which may have something to do with the relative stability of these households and their members' willingness to lend a helping hand without being intrusive.

We were surprised to find that SMBG, as well as having a test for glycated hemoglobin, was also positively correlated with app usage frequency. Previous research has shown that patients who are

TABLE 5 (A)subgroup analysis of the correlation between online information exchange and app usage frequency.

	N	App usage frequency	P for interaction
Sex			0.7329
Male	878	8.2 (5.8, 11.6) <0.0001	
Female	659	9.0 (6.0, 13.7) <0.0001	
Age			0.1363
<40	1277	9.3 (7.0, 12.6) <0.0001	
≥40	260	5.5 (3.0, 10.2) <0.0001	
BMI			0.9791
<18.5	188	7.7 (3.9, 15.1) <0.0001	
>=18.5, <24	1039	8.5 (6.2, 11.8) <0.0001	
>=24, <28	265	9.3 (4.8, 17.8) <0.0001	
>=28	45	9.9 (2.1, 47.0) 0.0041	
Education			0.5576
Junior high school and below	67	6.5 (2.0, 20.9) 0.0017	
High school/technical secondary school/technical school	261	6.9 (3.9, 12.2) <0.0001	
Junior college	359	10.1 (5.8, 17.5) <0.0001	
Undergraduate	766	8.1 (5.5, 11.9) <0.0001	
Postgraduate and above	84	20.2 (5.4, 75.5) <0.0001	
Medical security			0.0190

(Continued)

TABLE 5 Continued

	N	App usage frequency	P for interaction
Urban employees' medical insurance	563	5.8 (3.8, 8.9) <0.0001	
Urban residents' medical insurance	387	8.4 (5.1, 13.9) <0.0001	
New rural cooperative medical insurance	391	17.7 (9.8, 31.8) <0.0001	
Others	196	6.9 (3.4, 14.3) <0.0001	
Marital status			0.2765
Single or unmarried	448	9.1 (5.7, 14.6) <0.0001	
Married	1056	7.9 (5.7, 11.0) <0.0001	
Divorced or widowed	33	40.6 (3.8, 428.5) 0.0021	
Residence			0.6802
Urban	929	8.2 (5.8, 11.5) <0.0001	
Suburban	355	9.2 (5.3, 15.8) <0.0001	
Township	197	11.2 (5.4, 23.2) <0.0001	
Countryside	56	4.8 (1.5, 15.9) 0.0101	
Family size			0.8488
≤3 persons	829	8.4 (5.8, 12.0) <0.0001	
≥4 persons	708	8.8 (6.0, 13.0) <0.0001	
Cohabitation with parents			0.0048
Yes	1236	10.5 (7.7, 14.3) <0.0001	
No	301	4.2 (2.5, 7.2) <0.0001	
Glycosylated hemoglobin			0.0076
Not tested	145	4.1 (2.0, 8.3) 0.0001	
≤6.4%	877	7.3 (5.2, 10.3) <0.0001	
>6.5%	515	15.1 (9.1, 25.2) <0.0001	
SMBG			0.0269
Not tested or occasionally tested	984	7.0 (5.2, 9.6) <0.0001	
Daily testing	553	13.5 (8.1, 22.4) <0.0001	

successful at sticking to SMBG have higher levels of health-related knowledge, self-efficacy, and education (46), while all these factors work in favor of encouraging self-management of blood glucose (47, 48).

4.2 High score in online information exchange

This study's research of the online population reveals that the online population scored high on information exchange, with scores of 4 or above, with the highest ratings for information trustworthiness and usefulness being 4.18 and 4.11, respectively. Although there is a wealth of information available on various online information exchange platforms, its veracity varies widely, and misinformation and falsehoods that have not been verified are widely disseminated. This leaves users feeling overwhelmed when trying to decide which platform is best for them to access information and communicate with others (49). As a result, managers of online platforms should increase their oversight of online information exchange platforms and monitor misleading information in real time, and the general public should train itself to be more critical in its information selection and identification rather than blindly following the herd. Currently, some online platforms include not only specialist institutions, but also people in similar situations who assist one another. Meanwhile, credibility of the data is ensured by the platform's ongoing efforts to improve its management and introduce a system for reporting false information (50).

The frequency of active searches by online users increased after joining online groups relative to before joining online groups (before joining: 3.481 ± 1.127 *vs*after joining: 3.745 ± 0.876, $p<0.001$), which is because, once a user joins a group, they are more likely to be affected by the group's members, to become more

interested in health information, or to experience a decline in their own health (51). However, a cross-sectional study conducted by Kalantzi et al. at the Athens Diabetes Clinic found that users actively sought information in the beginning, but less so as time progressed and basic knowledge was learned (52). This contradicts our findings, possibly because our participants included may have been diagnosed with abnormal blood glucose or aware of blood glucose related issues for a relatively short period of time, and the participants were still in the knowledge building phase of a continuous active search. At present, the most frequently searched questions online remain relate to the pathophysiology, complications, diet, and prevention of diabetes, and the most reliable internet resources continue to come from medical specialists (52, 53). In order to create mobile health apps that help users better regulate their blood glucose levels in accordance with clinical standards, healthcare professionals and app developers should work together to better understand user preferences.

4.3 The relationship between information exchange and app usage frequency

Presently, China's blood glucose management app primarily provides information on disease treatment, health status, self-care, psychosocial, laboratory test results, and information released by healthcare professionals. Online information learning and exchange among blood glucose management users has become an important method of blood glucose management (54). This online exchange is also gradually shifting from a purely receptive to a two-way exchange of information, which enables patients to gain emotional and social support from interacting with users who share their experiences, leading them to derive more benefit from, and become more enthusiastic about, participating in online activities (34). According to a study conducted by Jin et al., health information shared by doctors or other self-glucose managers in China's largest online health community had an initiating and spreading effect, leading to the sugar users receiving the information becoming the dominant ones sharing the information and integrating themselves into the online internet (11). Increasing adherence to blood glucose management is aided by both faith in the Internet and the information exchange online, according to the results of another Chinese online poll (55). Previous research has found a link between health literacy and effective blood glucose self-management (11, 56). In a randomized controlled trial of diabetes patients lasting 6 months, Zhang et al. found a statistically significant drop in HbA1c of 2.03% in the app-interactive management group compared to 1.37% in the app-managed group, but no statistically significant difference in blood glucose self-monitoring (57), which means that information exchange is a potential factor in improving glycemic status and is a starting point for future research into glycemic control.

The blood glucose self-management potential of mobile health apps is substantial. In spite of the fact that mobile health apps simplify blood glucose self-management, not all users are successful in maintaining their routines. Therefore, it is particularly important to examine the factors that influence the frequency of app use. A meta-analysis that included 99 articles suggested that users are more likely to regularly check their mobile health app if it is tailored to their specific needs, provides alerts in the form of personalized push messages, is easy to navigate and operates smoothly technically, and offers personal support in addition to digital interventions (58). Other research indicate that online information exchange is a significant factor of app usage frequency, with this association being especially prominent in some social applications and email apps (31–33). It is worth emphasizing that information with visual characteristics boosts the user's reliance on the app, which means that apps with graphic and video-based information have an edge in enticing people to use them (59). This is in line with our findings, with picture or video-based WeChat, Tiktok and QQ ranking in the top three for frequency of use, with scores of 4.97, 4.57 and 4.56 respectively. In Chin-Lung et al., it is suggested that social impacts, such as information exchange, have a beneficial effect on the frequency of app use (32). However, a different perspective has been proposed. A 6-month single-center prospective randomized controlled trial in China found that people who used the app for blood glucose self-management (with Welltang installed for self-management) used it 10.7 ± 9.5 times per week, while those who used the app for interactive management (with Welltang installed for self-management + information exchange interaction) used it 11.1 ± 7.3 times per week, and the difference was not statistically significant (p=0.83) (34). Unfortunately, there are no research on the association between online information exchange and app usage frequency, and it is necessary to analyze and quantify this relationship in order to obtain additional insight into the mechanisms of blood glucose management.

In our study, online information exchange scores were correlated with app use frequency across subgroups of age, gender, BMI, education, medical insurance, marital status, residence, family size, cohabitation with parents, glycated hemoglobin, and SMBG. The interaction test was significant ($p<0.05$) in the different strata of the subgroups with medical insurance derived from the interactivity calibration, with the online information exchange of the urban employees' medical insurance population contributing least to app use frequency at 5.8 (3.8, 8.9) and the highest contribution among the new rural cooperative medical insurance population at 17.7 (9.8, 31.8). This is partly related to the low accessibility of health services in rural areas and the relative lack of medical knowledge (60, 61). Most of those who participate in the new rural cooperative medical care are from relatively backward rural areas, and their limited medical level and lack of medical knowledge lead them to look for ways to help with health management through the Internet, so when they engage in online information exchange, they are more motivated to learn how to use the Internet, so those who participate in the new rural cooperative medical care show a stronger promotion of app usage frequency by those who participate in the new rural cooperative medical care. An interaction test revealed a statistically significant difference ($p<0.05$) in the effect of online information exchange on the frequency of app use between those who lived with their parents and those who did not, which is directly related to the beneficial effect of family support in diabetes treatment. Family support can help users manage their health by reducing stress, changing their

emotional states, enhancing self-efficacy, and encouraging negative health behavior modification, which is consistent with the increased frequency of app usage among individuals cohabiting with parents in our study (62).

Our research is the first to find a statistically significant U-shaped correlation between information exchange and app usage frequency among Chinese internet users and this correlation was unaffected by other potential dangers. The relationship between information exchange and app usage frequency is strongest when the average information exchange score is in the range of 3.0-4.2, with each increase in the average information exchange score increasing the high app usage frequency by 18.3 times, while when the average information exchange score is >4.2, each increase in the average information exchange score increases the high app usage frequency by 2.8 times. Once patients have a sense of belonging in the process of exchanging information, their online satisfaction rises, they begin to trust the app, and they are more likely to utilize it (63). However, we have also found that as users move from satisfied to very satisfied with the act of information exchange, the frequency of app use slows down, which may have to do with the fact that the more information is exchanged, the more users are able to identify mismatches between the app and their needs, and thus the app takes up less and less time in their lives (64). This may also explain why the more information people exchange, the less often they utilize apps.

4.4 Limitations

Although this is the first study to investigate the relationship between information exchange and app usage frequency, it does have some limitations. At first, our cross-sectional design precludes us from drawing any firm conclusions about the cause and effect of information exchange and app usage frequency; Secondly, some confounding factors that were not included may have had an impact on this study. Third, we have not yet validated the potential processes of information exchange and app usage frequency, which will be the focus of future research.

5 Conclusions

This study showed that among the online blood glucose management population, participants' age, BMI, marital status, cohabitation with parents, glycosylated hemoglobin, SMBG, and information exchange scores were positively associated with app usage frequency, with online information exchange being independently associated with app usage frequency. We found a curvilinear relationship between online information exchange and app usage frequency. Online information exchange appears to be a risk factor for high app usage when the online information exchange score is <3.0, but can contribute to an increase in app usage when the information exchange score is ≥3.0, and this increase decreases when the score is >4.2. Therefore, online information exchange may be a simple and accurate way of predicting the frequency of app use for the online blood glucose management population.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by The Ethics Committee of Shantou University Medical College. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional req uirements.

Author contributions

HG undertook the data analysis, complete statistical tables and result analysis, wrote the research process, literature reviews, and interpreted the results. YBX, CL, JS, YCX, YZ performed the design of the questionnaire and data collection, GF was in charge of the conception, undertook the design of the study framework, participated the data collection, complete the whole of statistical tables, takes responsibility for the integrity of the data and the accuracy of the data, revised the entire manuscript, and interpreted the conclusion. GF ultimately modified the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Trends of the burden of type 2 diabetes mellitus attributable to high body mass index from 1990 to 2019 in China

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Background: Overweight and obesity are well-known risk factors for developing type 2 diabetes (T2DM). However, details on the evolution of the T2DM burden attributed to China's high body mass index (BMI) in China have not been thoroughly studied. This study aimed to investigate the temporal trends of the T2DM burden attributable to a high BMI in China from 1990 to 2019 and to evaluate the independent effects of age, period, and cohort on the burden of T2DM attributed to a high BMI.

Methods: Data on T2DM burden attributable to a high BMI from 1990 to 2019 were obtained from the Global Burden of Disease Study 2019. Deaths, disability-adjusted life years (DALYs), age-standardized mortality rate (ASMR), and age-standardized DALY rate (ASDR) of T2DM attributable to a high BMI were estimated by age and sex. The joinpoint regression model was performed to calculate the annual percentage change (APC) and the average annual percentage change (AAPC) in the burden of T2DM attributed to a high BMI. The age–period–cohort analysis was applied to estimate the independent effects of age, period, and cohort on the temporal trends of mortality and the DALY rate.

Results: In 2019, deaths and DALYs from T2DM attributable to a high BMI in China were 47.53 thousand and 3.74 million, respectively, five times higher than in 1990. Among those under 60 years of age, men had higher deaths and DALYs than women, while the gender differences reversed in those over 60 years of age. Furthermore, the ASMR and ASDR in 2019 were 2.39 per 100,000 (95%UI 1.12–3.90) and 181.54 per 100,000 (95%UI 93.71–286.33), respectively, representing a 91% and 126% increase since 1990. In China, women previously had a higher ASMR and ASDR than men, while the differences in the ASMR and ASDR between the sexes were reversed in recent years. From 1990 to 2019, the ASMR in women increased before 2004 and then decreased from 2004 to 2015, and increased again after, with an overall AAPC value of 1.6%. In contrast, the ASMR in men continued to increase, with an overall AAPC value of 3.2%. The ASDR continued to increase in men and women, with AAPCs of 2.2% and 3.5%, respectively. The age effect showed that the relative risk of mortality increased with age in both men and women, except for the 75–84 age group. The impact of the age on the DALY rate revealed a trend of first rising and then decreasing, peaking at 65–69

years. The effect of the period on the burden of T2DM attributable to a high BMI increased from 1990 to 2019. The cohort effect generally showed a downward trend.

Conclusion: The burden of T2DM attributed to a high BMI in China increased substantially from 1990 to 2019, particularly in men. Therefore, there is an urgent need for gender- and age-based public health guidelines on prevention strategies, early diagnosis, and effective management of T2DM, overweight, and obesity in China.

KEYWORDS

type 2 diabetes, high body mass index, China's burden of disease, mortality, disability adjusted life year, trends

Introduction

Diabetes mellitus (DM) is a serious public health problem that significantly influences the lives and well-being of individuals, families, and societies around the world. It is listed as one of the five non-communicable diseases prioritized in the United Nations and WHO Action Plan to address non-communicable diseases (1, 2). The incidence of DM is increasing and has become the eighth leading cause of disease burden in 2019 (3). The International Diabetes Federation Diabetes Atlas study revealed that 536.6 million people aged 20–79 years have diabetes in 2021, with a projected increase to 783.2 million by 2045 (4). Furthermore, DM was responsible for 6.7 million deaths worldwide in 2021, which equates to one diabetes death every 5 s (5). The rising prevalence of DM imposed a significant burden on healthcare systems. In 2021, health expenditures attributable to diabetes were estimated to be 966 billion USD (4).

Among type 1 DM, type 2 DM (T2DM), and gestational DM, T2DM accounts for 90%. According to the most recent study using Global Burden of Disease (GBD) data in 2019, the prevalence, deaths, and disability-adjusted life years (DALYs) of T2DM were 437.9 million, 1,472.9 thousand, and 66.3 million worldwide, respectively (6). With a fifth of the world's population, China had a rapidly growing prevalence of T2DM and had the highest prevalence, death, and DALY of T2DM in 2019 (6). According to the latest edition of the International Diabetes Federation Diabetes Atlas, there were 140 million adults living with diabetes in China by 2021, accounting for a quarter of the global total, putting tremendous pressure on the public health system (5).

Among numerous metabolic and behavioral risk factors, such as smoking, diet, and physical inactivity, overweight and obesity are the leading contributors to the development and progression of T2DM, as they lead to insulin resistance and beta cell dysfunction (5, 7, 8). Obesity can make it difficult for patients with T2DM to control their weight and blood sugar levels; worsen insulin resistance; aggravate structural and cognitive brain injury; and increase the risk of chronic complications such as diabetic nephropathy, cardiovascular disease, and cerebrovascular disease (9–12). Obesity is also a risk factor for other types of conditions, such as cancers (13). Despite this, the number of obese people continues to rise worldwide. As China's economy has grown rapidly in the last 30 years, the Chinese lifestyle has changed considerably, with more sedentary behavior and a higher-fat and -energy diet (14). This change has also led to more people being overweight and obese in China.

A recent report on the Nutrition and Chronic Diseases of Chinese Residents (2020) found that the prevalence of diabetes was greater (50%) among adults 18 years or older, a notable increase from 42% in 2015 in the previous 5 years. In addition, according to China's survey results in 2010, 2013, and 2015–2017, the prevalence of diabetes was 14.3%, 14.7%, and 13.8% among individuals with a BMI range of 25–30 kg/m^2 and 19.6%, 19.6%, and 20.1% among those with a BMI greater than 30 kg/m^2, respectively. More striking is that approximately 65% of Chinese people with diabetes are overweight (15, 16). Therefore, evaluating the current landscape and the possible trajectories of the burden of T2DM attributable to a high BMI is crucial for preventing and treating T2DM and overweight in the future.

Although previous studies have reported the latest global spatiotemporal patterns of T2DM burden attributable to high BMI (17), this overall pattern may not properly represent the disease burden in China. Jiang et al. examined the association between the burden of diabetes and high BMI exposure in China, utilizing GBD 2016. Still, this study did not separately address the burden of type 1 DM, T2DM, and gestational DM attributable to a high BMI (18). Comprehensive research has not evaluated long-term trends of T2DM burden attributable to a high BMI in China, specifically concerning the effects of age and sex. Additionally, no study has decomposed the impact of chronological age, period, and birth cohort on the trend of T2DM burden attributable to a high BMI in China. Therefore, this study aimed to investigate the current landscape of the burden of T2DM due to a high BMI and to assess the long-term trend of T2DM burden due to a high BMI in China from 1990 to 2019 using joinpoint regression and age–period–cohort analysis.

Materials and methods

Data sources

Data on the burden of T2DM attributable to a high BMI in China were extracted from the GBD 2019 using the Global Health Data Exchange GBD Results Tool (http://ghdx.healthdata.org/gbd-results-tool). The GBD collaborators conducted a comprehensive and systematic evaluation of prevalence and years lived with disabilities for 369 diseases and injuries, mortality for 286 causes, and comparative risks for 87 risk factors by age and sex, covering the period 1 January 1990 and 31 December 2019 in 204 countries and territories, 21 regions, and seven superregions. The general approach to GBD 2019 has been reported in previous studies (3, 19). The epidemiological burden and trend of T2DM attributable to a high BMI in China from 1990 to 2019 was assessed by extracting annual deaths, DALYs, and their corresponding 95% uncertainty intervals (UI)s and age-standardized rates stratified by age (5-year age groups of patients aged 20–94 and ≥95) and sex (both genders, male and female).

Definitions of type 2 diabetes mellitus and high body mass index

The reference case for T2DM is fasting plasma glucose (FPG) greater than 126 mg/dl (7 mmol/L) or reporting being on drug or insulin treatment for T2DM. Because other measures of blood sugar (glycated hemoglobin A1c, oral glucose tolerance test, and postprandial glucose test), which are inconsistent with the reference case definition, were also accepted to define diabetes in GBD 2019, alternative case definitions used as data inputs were considered and adjusted before beginning the modeling process. The sequelae of T2DM include diabetic neuropathy, foot ulcer, amputation, and retinopathy (moderate and severe vision loss and blindness due to diabetic retinopathy). They are identified by codes E11-E11.1 and E11.3-E11.9 according to version 10 of the International Classification of Diseases (ICD). A high BMI was defined as BMI ≥ 25 kg/m^2 for adults (age 20+ years).

Estimation of type 2 diabetes mellitus burden attributed to high body mass index

Previous studies have extensively described fundamental modeling strategies for the GBD 2019 and specific methods to assess the burden of T2DM due to a high BMI (3, 6, 17). Here, we provide a brief overview of approaches to estimate the burden of T2DM due to a high BMI. The first step was data seeking. A comprehensive systematic review of diabetes prevalence, incidence, and mortality was performed in GBD 2019. The epidemiology of diabetes was systematically searched by using the Global Health Data Exchange for multicountry survey programs, national surveys, and longitudinal studies. Meanwhile, additional data that other research groups used to report on the global burden of diabetes, microdata from unpublished national studies, and publications not captured in the PubMed were obtained by enlisting the help of other leaders in the field. Eventually, 1,289 original data sources covering 171 countries were used in the diabetes modeling in GBD 2019. The sequelae of diabetes included diabetic neuropathy and foot ulcers, and amputation due to diabetes was also performed a comprehensive systematic review in GBD 2019. The second step was data inputs, which included estimates of diabetes in a representative population, estimates of mean FPG in a representative population, and individual-level FPG data from surveys and insurance claims in the US and Taiwan. In particular, only 20% of the research provided the type of diabetes estimates, and some studies reporting T2DM separately did not detail the diagnostic criteria. Therefore, the estimates of T2DM were determined by deducting the estimates of type 1 DM from the total estimates of diabetes for age, sex, and location from 1990 to 2019. In addition, the prevalence of obesity per location was used as a covariate. The third step was data processing. To solve the problem of inconsistent sampling and measurement to ensure that data are comparable between different data sources and between high fasting glucose modeling efforts, several data processing steps were performed, including small sample size, mean FPG processing, age splitting, and bias adjustments. The fourth step was modeling. The processed and standardized data were modeled using DisMod MR-2.1, a Bayesian meta-regression tool, to produce the prevalence and age-standardized prevalence rates of diabetes and the sequelae of diabetes for age, sex, geographic location, and year. The proportions extracted from the diabetes sequelae models were multiplied by the parent diabetes model to ensure that all estimates were in the same population space.

The death of a vital registration resource in ICD 10 was used to model T2DM mortality. Data processing for deaths caused by T2DM has also been previously described (3, 6). The Cause of Death Ensemble model was used to estimate T2DM death rates. Years of life lost (YLLs) from T2DM were calculated by multiplying death estimates from T2DM with the corresponding standard life expectancy at the age of death. The prevalence of T2DM sequela multiplied by its disability weight (Supplementary Table S1) was used to calculate years lived with disability (YLDs) (3). DALYs for T2DM were calculated as the sum of YLDs and YLLs.

A comprehensive systematic review of the prevalence of a high BMI was also performed. Finally, 2,022 original data sources from 190 countries with a high BMI were entered into the models. The proportion of T2DM burden attributable to a high BMI was estimated using a comparative risk assessment framework, which has been previously described (19). The proportional population attributable fraction (PAF) was used to quantify the contribution of a high BMI to the subsequent occurrence of the T2DM burden.

Statistical analysis

To assess the magnitude and temporal trend of the burden of T2DM attributable to a high BMI from 1990 to 2019, we employed joinpoint regression analysis, also known as change-point regression, to calculate the annual percentage change (APC), the

average annual percentage change (AAPC), and the corresponding 95% confidence intervals (95% CI). As introduced by Kim et al., the joinpoint regression model can be defined as follows (20):

$$\log(y|x) = c + \beta Year + \delta 1(x - \tau 1)^{+} + \cdots + \delta_k(x - \tau_k)^{+} \quad (1)$$

$$APCi = (e^{bi} - 1) \times 100\,\% \quad (2)$$

$$AAPC = (e^{\frac{\sum b_i w_i}{\sum w_i}} - 1) \times 100\,\% \quad (3)$$

where y is the rate indicators, c is the constant, *Year* is the observation year, β is the coefficient of year, τ_k is the unknown turning time point (joint point) that needed to be identified, b_i is the estimation of β on the i-th identified trend, and w_i is the length of the i-th identified trend.

If the lower limit of the 95%CI for the related APC/AAPC estimation was higher than zero, there is an increasing trend in the burden of T2DM associated with an increased BMI. Inversely, if the upper limit of the 95% CI for the associated APC/AAPC calculation is less than zero, it suggests a declining trend. If the 95% CI of APC/AAPC includes 0, the trend for the burden of T2DM due to a high BMI is stable.

The age-period-cohort model analysis was used to estimate the impact of age, period, and cohort effects on temporal trends in the mortality and DALY rates of the T2DM attributable to a high BMI. The age-period-cohort model considers age, period, and cohort factors and is commonly used to analyze trends in chronic disease morbidity and mortality and predict future disease burden changes. In the age-period-cohort model, the period effect refers to influence of human factors on the mortality and DALY rates of T2DM due to a high BMI, such as advances in disease diagnosis technology, screening, and early detection; changes in disease definition and registration; and improvements in treatment. All these human factors can affect the mortality and DALY rates of T2DM attributable to high BMI in different periods and produce a period effect. The effect of age is one of the most important determinants of differences in the epidemiology of T2DM, which is the impact of changes in incidence with age. The cohort effect refers to changes in T2DM mortality and DALY rates attributed to a high BMI due to different levels of exposure to risk factors among different generations. Mortality and DALY data for T2DM attributable to a high BMI, population, and period are divided into continuous 5-year intervals from the 20–24 age group to the 90–94 age group and the 95 plus age group, and from 1990–1994 to 2015–2019, respectively. Consecutive 5-year cohorts were defined from 1895–1899 to 1995–1999. The age-period-cohort model is as follows:

$$R = c + \alpha_i \text{Age} + \beta_j \text{Period} + \gamma_k \text{Cohort} + \epsilon$$

where R is the mortality and the DALY rate for the age i age group during the j period; c is the intercept term of mortality and the DALY rate; α_i, β_j, and γ_k are the coefficients of the effects of age, period, and cohort at all levels, respectively; and ϵ is the residual. The exponential values of the coefficients represent the relative risk (RR) of death and DALYs attributed to T2DM for a given age, period, and birth cohort compared to the reference groups.

The Joinpoint Regression Program v4.9.1.0 (April 2022) developed by the US National Cancer Institute Surveillance Research Program was used for the joinpoint regression analysis. Analysis and plot drawing of the age-period-cohort model were performed by R (version 4.2.2) and Stata (version 16.0, StataCorp LP, TX, UA). A two-sided *P*-value less than 0.05 was considered statistically significant.

Results

Description analysis of type 2 diabetes mellitus deaths and disability-adjusted life years attributable to a high body mass index in China

T2DM deaths and DALYs attributable to a high BMI increased five times in China from 10.51 thousand in 1990 to 47.53 thousand in 2019 and from 0.77 million to 3.74 million in 2019, respectively (Table 1). In 2019, the estimated ASMR and ASDR of T2DM associated with a high BMI were 2.39 per 100,000 (95%UI 1.12–3.90) and 181.54 per 100,000 (95%UI 93.71–286.33), respectively (Table 1). Between 1990 and 2019, the ASMR and ASDR increased by 91% (95%UI 33%–284%) and 126% (95%UI 60%–365%), respectively (Table 1). The ASMR and ASDR of T2DM in 2019 were attributed to a high BMI in proportions of 26.01% (95%UI 12.92%–40.78%) and 38.06% (95%UI 21.09%–55.09%) respectively, as shown in Table 1. From 1990 to 2019, the age-standardized proportion of deaths and DALYs from T2DM due to a high BMI had more than doubled (Table 1). Figure 1 shows the trends in deaths and DALYs due to T2DM related to a high BMI in both sexes and all ages from 1990 to 2019. There was a consistent increase in all age group numbers and rates of deaths and DALYs for T2DM related to a high BMI in both men and women over the past 30 years. Furthermore, women consistently had higher death numbers and mortality rates from T2DM attributable to a high BMI than men, but the disparity gradually decreased. The number of DALYs was significantly higher for women than for men before 2008; the opposite was true after that year. The gender gap in all-age DALY rates showed similar trends to the gender gap in the number of DALYs between 1990 and 2019. However, before 2015, the ASMR of T2DM attributable to a high BMI was higher in women than in men, and before 2010, the ASDR of T2DM attributable to a high BMI was also higher in women than in men. After these years, the ASMR and ASDR were lower in women than in men (Supplementary Figures S1A, B).

The number and rate of deaths and DALYs from T2DM related to high BMI by gender and age group in 2019 are shown in Figure 2. In both men and women, the number of T2DM deaths attributable to a high BMI increased with age and peaked in the 70–74 age group; the trend began to decline after this age. The number of T2DM DALYs attributable to a high BMI reached the highest level in the 50–54 age group in men and in the 65–69 age group in women; then, a declining trend was observed in the older age group. The number of deaths and DALYs from T2DM attributable to a high BMI in 2019 for men and women varied substantially by age

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TABLE 1 Deaths and disability-adjusted life years (DALYs) in 1990 and 2019 for type 2 diabetes mellitus (T2DM) attributable to a high body mass index (BMI) in China.

Measure	Year					
	1990			2019		
	Total	Male	Female	Total	Male	Female
Death						
Age-standardized PAF%, (95%UI)	14.45 (4.05, 29.53)	12.30 (2.97, 26.8)	16.01 (4.79, 31.75)	26.01 (12.92, 40.78)	23.65 (11.18, 38.04)	27.69 (14.12, 42.85)
Cases, No.×10^3 (95% UI)	10.51 (2.94, 21.51)	4.03 (1.00, 8.84)	6.49 (1.91, 13.15)	47.53 (22.51, 76.63)	22.83 (10.50, 38.35)	24.70 (11.55, 40.50)
Mortality per 100,000, No. (95% UI)	0.89 (0.25, 1.82)	0.66 (0.16, 1.45)	1.13 (0.33, 2.29)	3.34 (1.58, 5.39)	3.15 (1.45, 5.29)	3.54 (1.66, 5.81)
Age-standardized rate per 100,000, No. (95% UI)	1.25 (0.34, 2.60)	1.00 (0.24, 2.25)	1.49 (0.43, 3.04)	2.39 (1.12, 3.90)	2.47 (1.10, 4.18)	2.35 (1.09, 3.90)
DALYs						
Age-standardized PAF%, (95%UI)	18.40 (5.25, 36.63)	16.88 (4.39, 34.78)	19.77 (6.07, 38.26)	38.06 (21.09, 55.09)	37.30 (20.49, 54.08)	38.48 (21.28, 55.83)
Cases, No.×10^6(95% UI)	0.77 (0.21, 1.61)	0.35 (0.09, 0.77)	0.42 (0.12, 0.84)	3.74 (1.91, 5.90)	1.97 (1.00, 3.11)	1.77 (0.93, 2.82)
All-age rate per 100,000, No. (95% UI)	65.20 (17.72, 135.96)	58.12 (14.47, 126.18)	72.74 (21.08, 147.10)	262.77 (134.56, 415.08)	271.31 (138.08, 428.90)	253.90 (133.12, 404.05)
Age-standardized rate per 100,000, No. (95% UI)	80.21 (21.50, 167.40)	71.48 (17.59, 156.52)	88.84 (25.71, 178.47)	181.54 (93.71, 286.33)	195.12 (99.28, 309.13)	167.70 (86.86, 268.54)

DALYs, disability-adjusted life years; PAF, proportional population attributable fraction; UI, uncertainty interval.

group. Men had a much higher number of deaths and DALYs than women before the age group of 60–64, while women experienced a significantly higher number of deaths and DALYs than men beyond that age group. Furthermore, except for death rates occurring in adults 75–84 years of age, death rates from T2DM attributable to high a BMI increased with age, starting in the age group of 50–54 for both sexes. Both men and women showed a peak in the T2DM DALY rate attributable to a high BMI in the age group of 70–74 years, followed by a decline in the rate until the age group of 80–84 years for men and the oldest age group for women. There was a substantial difference between men and women in the rate of T2DM mortality and DALY associated with a high BMI.

Temporal trends for the burden of type 2 diabetes mellitus attributable to high body mass index in China by gender

The trends of the ASMR and ASDR for T2DM attributable to a high BMI across all ages from 1990 to 2019 in China are shown in Figure 3 and Table 2. The ASMR and ASDR increased from 1990 to 2019, with AAPC values of 2.3% (95% CI 2.1%–2.5%) and 2.8% (95%CI 2.7%–3.0%), respectively. Interestingly, men had higher AAPCs than women for both the ASMR and ASDR. The ASMR of T2DM attributable to a high BMI in men continuously increased from 1990 to 2019, with three significant increase segments ($APC_{1990-1995}$ = 1.9%, $APC_{2005-2004}$ = 5.5%, and $APC_{2007-2019}$ = 2.6%) and one stage of slight increase ($APC_{2004-2007}$ = 0.8).

However, the trend for the ASMR in women differs from that of men. Women showed an upward trend during 1990–2004 (APC = 1.1%), 1996–2004 (APC = 5.5%), and 2015–2019 (APC = 2.2%), while a decrease was observed between 2004 and 2008 (APC = −1.9%) and between 2008 and 2015 (APC= −0.5%). Additionally, the joinpoint analysis of the ASDR for T2DM attributable to a high BMI in men exhibited a continuously increasing trend from 1990 to 2019, with the highest increase between 2001 and 2004 (APC = 8.4%). For women, the ASDR trend for T2DM attributable to a high BMI during 1990–2019 indicated that the six segments showed an upward trend, except for a slight decrease from 2014 to 2019 (APC = 0). The highest increase in ASDR in women was observed between 2001 and 2004, with an APC of 8.0%.

Variation in temporal trends for the burden of type 2 diabetes mellitus attributable to high body mass index in the age, period, and cohort in China by gender

The age-specific change trend for the mortality rate of T2DM attributable to a high BMI during the observation period is shown in Figures 4A, B. The mortality rate of T2DM attributable to a high BMI increased significantly, decreased, and then rose again with age in women from 1990 to 2019. For men, the mortality rate of T2DM attributable to a high BMI showed a decreasing trend with an increase in age among those aged over 90–94 years during 1990–2019, while the mortality rate of other age groups showed a similar

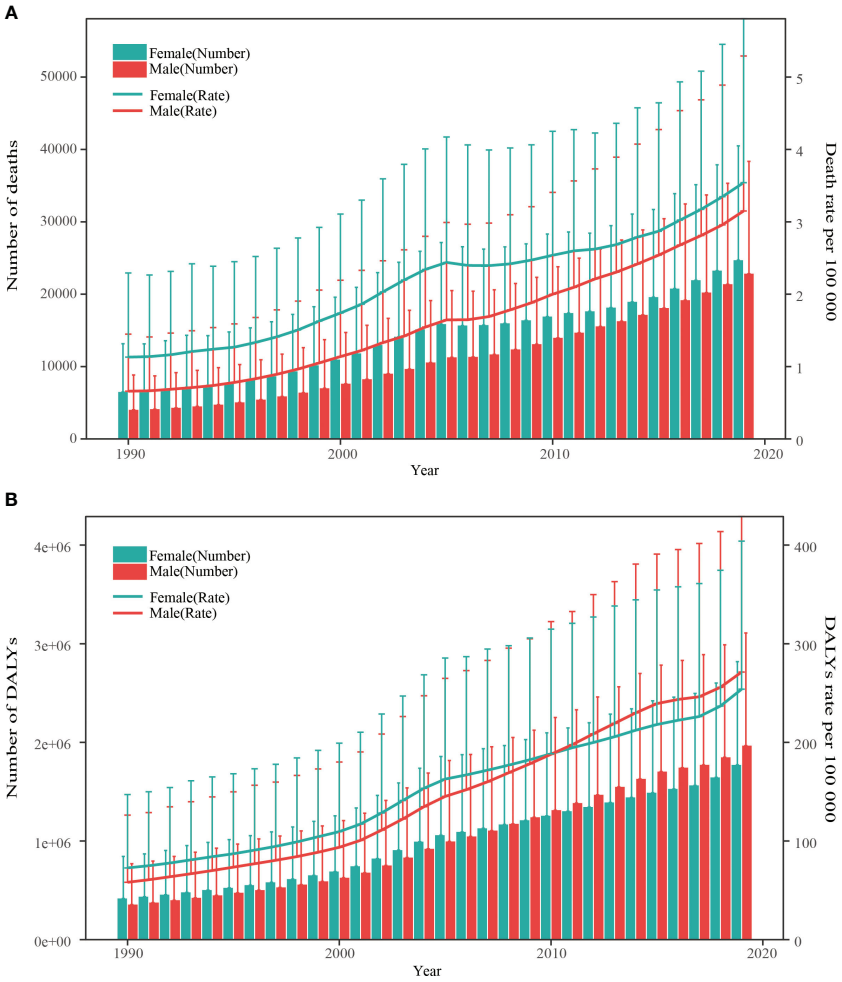

FIGURE 1
Trends in the all-age number and rate of deaths and DALYs of type 2 diabetes mellitus attribute to high body mass index by sex from 1990 to 2019. **(A)** Death number and rate. **(B)** DALYs number and rate. DALYs, disability-adjusted life years. The vertical lines represent the upper limit of the 95% uncertainty interval.

trend for women. A rapidly increasing trend was observed between each observation point of mortality from T2DM attributable to a high BMI among those younger than 75–79 years, a decreasing trend was observed for those 75–79 years to 80–84 years, and an increasing trend was observed for those over 80–84 years. The DALY rates of T2DM attributable to a high BMI in both men and women increased and then decreased with age across all periods, with a peak in the 65–74 age group followed by a rapid decline before the age group of 80–84 and then a slower decline (Supplementary Figures S2A, B).

The period effects of the mortality rate of T2DM attributable to a high BMI are presented in Figures 4C, D. Two types of changes were observed in both women and men in different age groups over time. The first type showed little change in the T2DM mortality rate attributable to a high BMI among people aged 20–64 in women and people aged 20–54 in men during the observation period, indicating a minimal period effect. The second type showed a significant increase in the period effect of the mortality rate of T2DM attributable to a high BMI for women aged over 60–64 years and men aged over 50–54 years between 1990 and 2019. The DALY rates of T2DM attributed to a high BMI in the 20–49 age group for women and in the 20–29 age group for men did not show a significant trend between 1990 and 2019 (Supplementary Figures S2C, D). Although the DALY rates of T2DM attributable to a high BMI in other age groups for both women and men showed an overall upward trend, the 70–74 age group had the most significant increase. Furthermore, the rise in DALY rates women with T2DM attributable to a high BMI leveled off after 2005 and showed a downward trend in the 60–69 age group.

The effects of the cohort on the mortality rate of T2DM attributable to high BMI increases are displayed in Figures 4E, F. An analysis of birth cohorts revealed that women aged over 60–64 and men aged over 50–54 had a lower mortality rate of T2DM due to a high BMI in the early period compared to the later period. However, for age groups younger than the 60–64 female group and 50–54 male group, the mortality rate of T2DM attributable to a high BMI was less influenced by the birth cohort. Supplementary Figures S2E, F indicated that the DALY rates of T2DM attributable to a high BMI in both men and women were lower in the early period than in the later period. However, the trend in DALY rates for T2DM

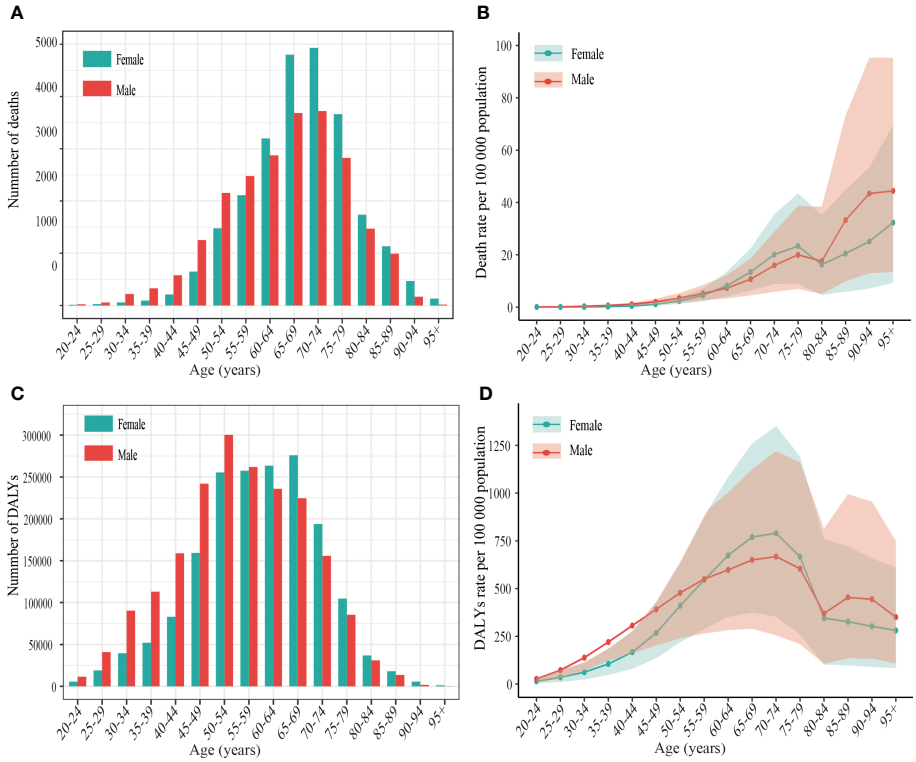

FIGURE 2
The number and rate of deaths and DALYs of type 2 diabetes mellitus attributable to high body mass index per 100 000 population by age and sex in China, 2019. **(A)** Age-specific number of deaths. **(B)** Age-specific rate of deaths. **(C)** Age-specific number of DALYs **(D)** Age-specific rate of DALYs. DALYs disability-adjusted life years.

attributable to a high BMI with the passage of the birth cohort varied slightly between women and men. In men, DALY rates increased with birth cohorts, but the effect of birth cohorts on DALY rates was insignificant in the 20–25 age group. For women, DALY rates in the 20–44 and 75–95 age groups increased with birth cohorts, but the increase tended to plateau in the 20–44 age group. However, the DALY rates of T2DM attributable to a high BMI in the 50–74 age group first increased and then decreased with the passage of the birth cohorts.

The age–period–cohort model results for the burden of type 2 diabetes mellitus attributable to high body mass index in China by sex

Figure 5 and Supplementary Table S2 demonstrate that the age, period, and cohort effects impacted the mortality and DALY rates of T2DM attributed to a high BMI for both sexes. After controlling for period and cohort factors, the effect of age on the mortality rates of T2DM attributable to a high BMI showed that the relative risk of mortality generally increased with age for both men and women, except for the 75–84 age group. Calculating the effect coefficients for different age groups revealed that the highest relative risk of death from T2DM attributed to a high BMI occurred in women over 95 years and in men 90–94 years of age, with RRs of 4.43 and 4.89, respectively. Additionally, the age group of 20–24 years had the lowest relative risk of death from T2DM associated with a high BMI, with women experiencing an RR of 0.06 and men experiencing an RR of 0.04. Furthermore, women and men over 50–54 years of age were at risk of T2DM mortality attributable to a high BMI, with an RR > 1. The effect of age on DALY rates showed bell-shaped curves with advancing age in both men and women (Figure 5B; Supplementary Table S2). An increase with age was observed in the age group under 65–69, followed by a gradual decline. The highest relative risk associated with age for DALY rates was found in the 65–69-year-old group, with an RR of 3.05 (95%CI 3.04–3.07) in women and 2.25 (95%CI 2.22–2.28) in men. The relative risk of T2DM DALY rates attributed to a high BMI was greater than 1 for the age group of 40–79 in men and 45–79 in women.

There was an upward trend in the period effect of T2DM mortality attributed to a high BMI for both women and men between 1990 and 2019 (Figure 5C; Supplementary Table S2). The age-period-cohort model analysis for the period effect further showed that the RR for men increased more rapidly from 0.47 in the 1990–1994 year group to 2.01 in the 2015–2019 year group than the RR for women from 0.60 in the 1990–1994 year group to 1.55 in the 2015–2019 year group. Women had a relatively higher risk of T2DM mortality attributed to a high BMI than men between 1990 and 2004, while men had a higher risk after the 2000–2004 year group. The 2005–2019 year groups were risk groups with an RR > 1

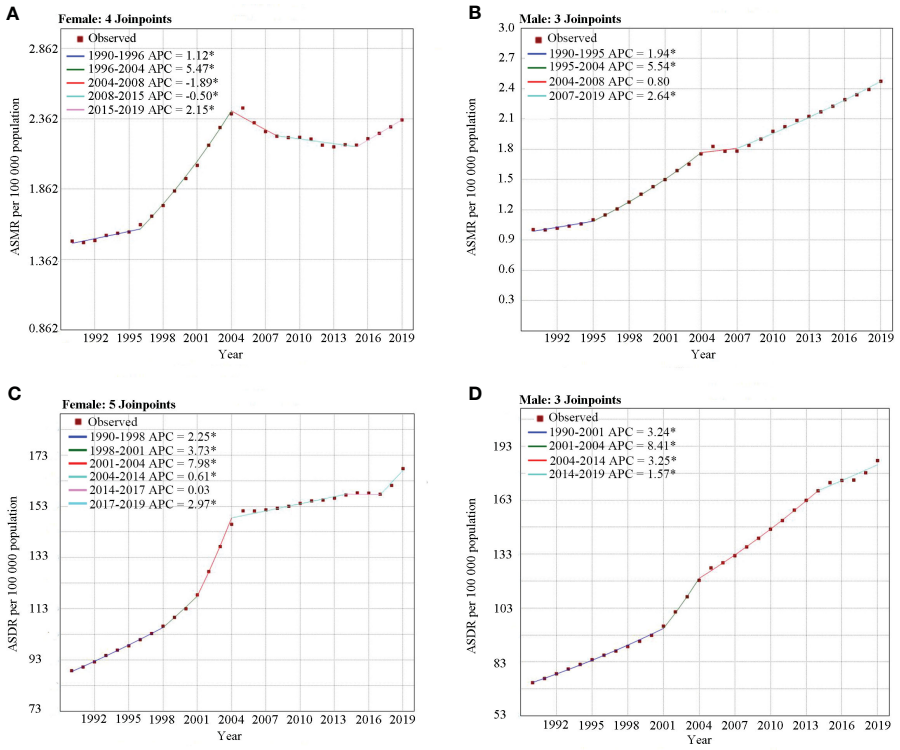

FIGURE 3
Joinpoint analysis of the ASMR and ASDR for type 2 diabetes mellitus attributable to high body mass index in China from 1990 to 2019 by sex. **(A)** ASMR for females. **(B)** ASMR for males. **(C)** ASDR for females. **(D)** ASDR for males. APC, annual percentage change; ASMR, age-standardized mortality; ASDR, age-standardized disability-adjusted life years rate. *Indicates that the APC is significantly different from zero at the alpha = 0.05 level.

in mortality in both sexes. Similar trends were observed in the period effect of the T2DM DALY rates attributable to a high BMI (Figure 5D; Supplementary Table S1).

The cohort effect showed an overall reduction in the risk of T2DM mortality attributed to a high BMI from earlier to later birth cohorts (Figure 5E; Supplementary Table S2). However, the birth cohort groups of 1895–1954 in women and 1895–1949 in men had a higher relative mortality risk with an RR > 1. In contrast, the effects of the cohort on DALY rates showed a horizontal J-shaped flip, with a decreasing trend in both women and men born before 1974 and an increasing trend in those born after that (Figure 5F; Supplementary Table S2). In addition, during the birth cohort of 1900–1964, the cohort RR of DALY rates associated with T2DM attributable to a high BMI was slightly lower in men than in women.

Comparisons of the difference between the burden of type 2 diabetes mellitus attributable to high body mass index in China and in worldwide

Figure S3 shows the trends in the ASMR and ASDR for T2DM attributable to a high BMI for both sexes combined in China and globally from 1990 to 2019. During this period, the ASMR and ASDR were higher worldwide compared to China, and the difference lasted for 30 years. Furthermore, the ASMR and ASDR for T2DM attributable to a high BMI in China and the world increased from 1990 to 2019.

Discussion

T2DM is one of the most serious and widespread chronic metabolic diseases that can cause life-threatening complications and disability. It can also result in significant financial costs and reduce life expectancy. In China, the number of people with diabetes has a dramatic increase from 98.4 million in 2013 to 140.9 million in 2020 (21). Obesity is a significant risk factor for T2DM, with over two-thirds of T2DM patients being overweight or obese. The prevalence of overweight and obesity in adults aged 18 years or older increased substantially from 16.4% and 3.6% in 1992 to 34.3% and 16.4% in 2015–2019 in China, respectively (22, 23). This study evaluated the temporal trends of the T2DM burden attributable to a high BMI in China from 1990 to 2019 using joinpoint regression and age-period-cohort analysis. We found that the absolute numbers of death and DALYs in T2DM attributable to a high BMI in China were 47.53 thousand and 3.74 million in 2019, respectively, approximately five times higher than that in 1990. However, the total population only increased by 24.34% from 1990 to 2019 in China, indicating a faster growth rate of the burden of T2DM attributable to a high BMI than that of the country's population growth, which accurately represented a continuously

TABLE 2 The data from joinpoint regression analysis on the age-standardized mortality rate and age-standardized DALY rate of T2DM attributable to a high BMI in both sexes, males and females, from 1990 to 2019 in China.

ASMR			ASDR		
Period	APC,% (95% CI)	AAPC,% (95% CI)	Period	APC,% (95% CI)	AAPC,% (95% CI)
Overall					
1990–1996	1.7 (1.3, 2.0)	2.3 (2.1, 2.5)	1990–2000	2.7 (2.6, 2.7)	2.8 (2.7, 3.0)
1996–2004	5.6 (5.3, 5.8)		2000–2004	7.3 (6.9, 7.8)	
2004–2007	-1.1 (-2.6, 0.4)		2004–2014	2.0 (1.9, 2.1)	
2007–2015	0.9 (0.7, 1.1)		2014–2017	0.6 (-0.1, 1.3)	
2015–2019	2.4 (2.0, 2.9)		2017–2019	2.8 (2.1, 3.4)	
Female					
1990–1996	1.1(0.7, 1.6)	1.6 (1.4, 1.8)	1990–1998	2.2 (2.1, 2.3)	2.2 (2.1, 2.4)
1996–2004	5.5 (5.2, 5.8)		1998–2001	3.7 (2.9, 4.6)	
2004–2008	-1.9 (-2.8, -0.9)		2001–2004	8.0 (7.2, 8.8)	
2008–2015	-0.5 (-0.8, -0.2)		2004–2014	0.6 (0.5, 0.7)	
2015–2019	2.2 (1.6, 2.7)		2014–2017	0.0 (-0.7, 0.6)	
			2017–2019	3.0 (2.3, 3.6)	
Male					
1990–1995	1.9 (1.3, 2.5)	3.2 (3.0, 3.5)	1990–2001	3.2 (3.1, 3.4)	3.5 (3.2, 3.7)
1995–2004	5.5 (5.3, 5.8)		2001–2004	8.4 (5.9, 11.0)	
2004–2007	0.8 (-1.2, 2.9)		2004–2014	3.2 (3.1, 3.4)	
2007–2019	2.6 (2.5, 2.7)		2014–2019	1.6 (1.2, 2.0)	

AAPC, average annual percent change presented for full period; APC, annual percent change; ASMR, age-standardized mortality rate; ASDR, age-standardized disability-adjusted life years rate; CI, confidence interval.

increasing burden of T2DM attributable to a high BMI. Furthermore, a roughly one-fold increase in age-standardized mortality and DALY rates of T2DM attributed to a high BMI. The trends in the ASMR and ASDR of T2DM attributed to a high BMI in China from 1990 to 2019 were similar to the worldwide. The significant increase in the burden of T2DM due to a high BMI can be attributed to multiple factors, such as changes in dietary patterns, aging and population growth, urbanization, and environmental pollution (24).

The burden of T2DM caused by a high BMI increased significantly for both men and women between 1990 and 2019 but grew more for men. In addition, the burden of T2DM attributable to a high BMI used to be consistently higher for women than men, but the difference has decreased or even changed in recent years. The factors that led to this phenomenon are not fully understood. The patterns of changes in the prevalence rates of overweight and obesity among men and women were similar to the changes in the burden of T2DM attributed to a high BMI between sexes. In China, men previously had a lower prevalence of overweight and obesity than women, but this gap has shrunk or even been reversed in recent years. According to the 2002 China National Nutrition Surveys (CNNSs), the prevalence of overweight and obesity among adults was 30.3% in women and 29.6% in men. However, in the 2010–2012 CNNSs, the prevalence values were 41.6% in women and 42.4% in men, consistent with the trend of increasing disparity between women and men in the prevalence of obesity observed in the China Chronic Disease and Risk Factor Surveillance (25–27). Other possible explanations could be gender differences in treatment and control rates for T2DM. Although the rate of T2DM treatment in Chinese men doubled in 2017 compared to the previous year, the control rate did not increase significantly. Both rates have improved in women since 2010 (28). Furthermore, bariatric surgery has been rising in China in recent years. However, the proportion of obese patients undergoing bariatric surgery is highly uneven in China, with women accounting for 75.1% and men only 24.9% (29). Such sex differences in bariatric surgery may explain the sex disparity in the burden of T2DM attributable to a high BMI. These findings highlight the need for targeted policies and interventions that address the growing trend and gender disparities in the burden of T2DM attributed to a high BMI in China. Such measures should be implemented properly and appropriately based on gender-specific considerations.

The number of deaths and DALYs of T2DM attributable to a high BMI was primarily concentrated in age groups 50–89 and 40–79, respectively, consistent with other studies (17). Our analysis also

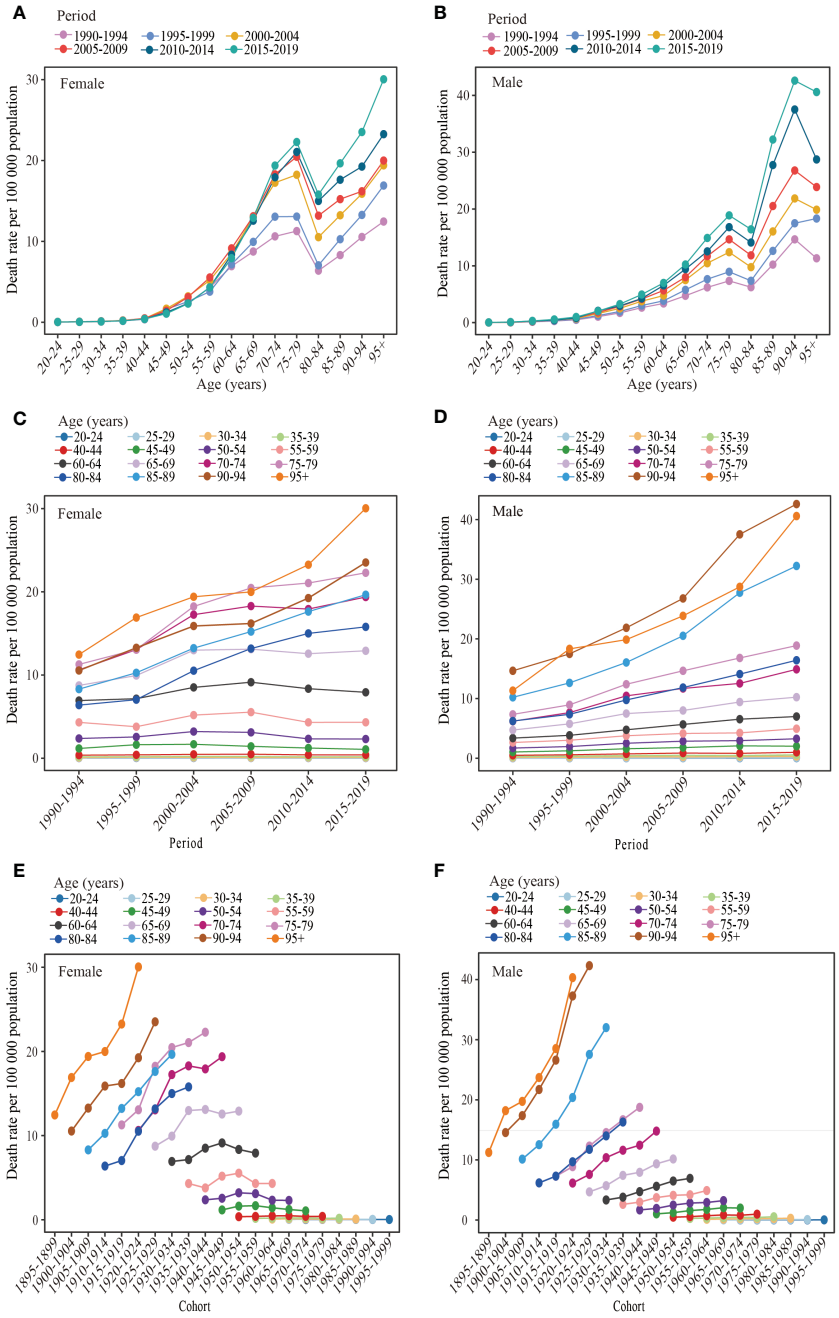

FIGURE 4
Long-term trends of the mortality rate of type 2 diabetes mellitus attributable to high body mass index in China during 1990-2019 by age, period, and cohort. **(A, B)** Age-specific trends of the mortality rate of type 2 diabetes mellitus attributable to high body mass index for females and males, **(C, D)** Period-based trends of the mortality rate of type 2 diabetes mellitus attributable to high body mass index for females and males. **(E, F)** Cohort-based trends of the mortality rate type 2 diabetes mellitus attributable attributable to high body mass index for females and males.

shows that the number of deaths and DALYs of T2DM due to a high BMI was higher in men than in women in age groups<60 years, while it is lower in men than in women in age groups ≥60 years, consistent with global data (17). Unhealthy lifestyle behaviors, such as smoking and alcohol consumption, are more prevalent among men than women in China, which is an essential factor contributing to the higher burden of T2DM due to a high BMI among men than among women in age groups<60 years. The age-dependent sex difference in the burden of T2DM due to a high BMI could be attributed to the combined effect of age-dependent sex differences in T2DM and obesity. According to The China Diabetes Atlas, there was no significant difference in the prevalence of T2DM between different genders before 2002, with rates high and low in both men and women (28). However, since the 21st century, the prevalence of T2DM in Chinese men has been higher than in women. Another study has shown that the sex disparity in the incidence of T2DM fluctuates throughout life, and women experience a higher prevalence than men during youth. In contrast, men have higher

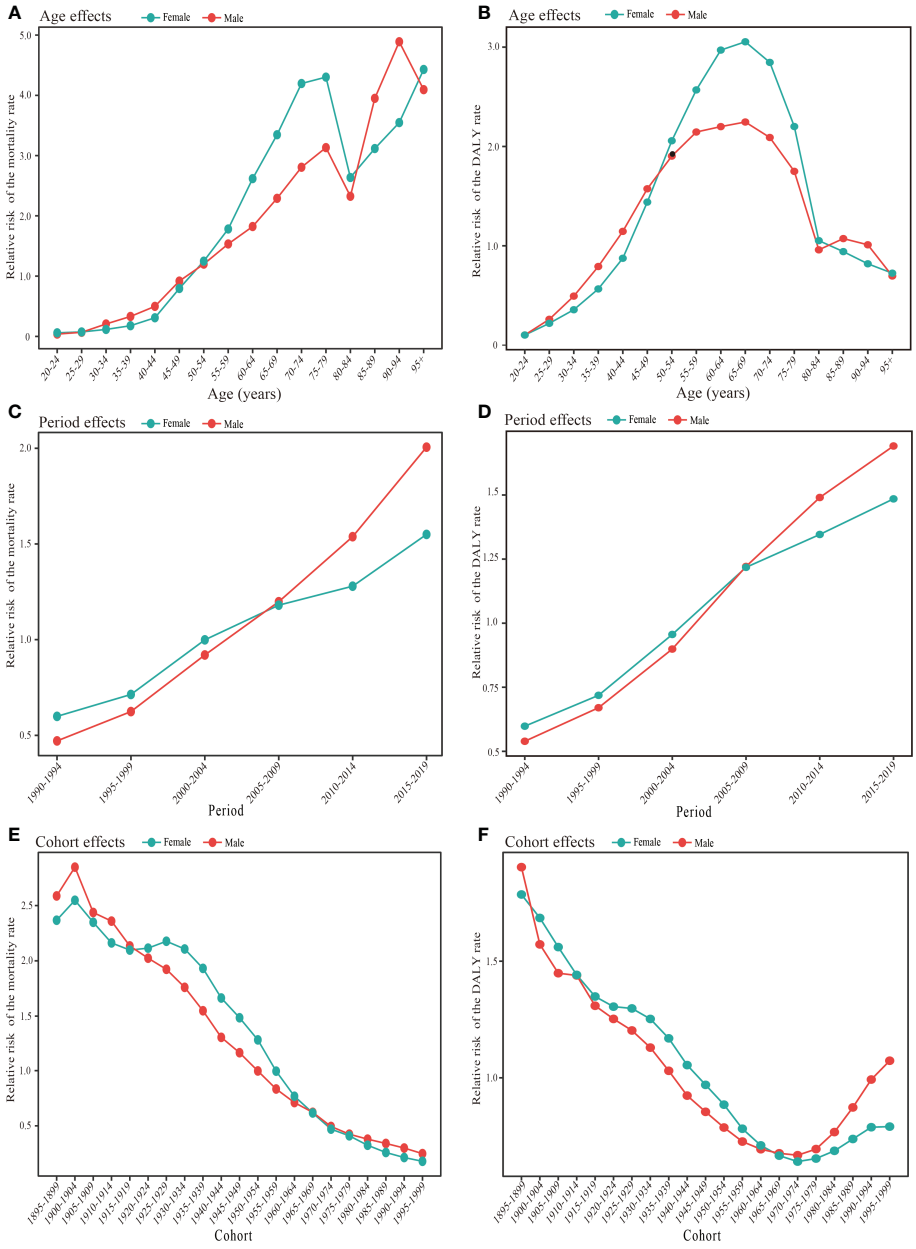

FIGURE 5
Age, period, and cohort effects on type 2 diabetes mellitus burden attributable to high body mass index in China during 1990-2019 by gender. **(A)** Age effects for types 2 diabetes mellitus mortality rate in females and males. **(B)** Age effects for type 2 diabetes mellitus DALY rate in females and males. **(C)** Period effects for type 2 diabetes mellitus mortality rate in females and males. **(D)** Period effect for type 2 diabetes mellitus DALY rate in females and males. **(E)** Cohort effect for type 2 diabetes mellitus mortality rate in females and males. **(F)** Cohort effects for type 2 diabetes mellitus DALY rate in females and males. DALY, disability-adjusted life year.

rates of T2DM than women in midlife, and the incidence of T2DM is roughly equivalent between the sexes in later life (30). Insulin clamp studies also show that men have stronger resistance to insulin than women in late puberty and as adults (31). In addition, women who develop T2DM typically have a higher BMI than men. The average age-adjusted BMI of women at the time of diagnosis is 1.8 kg/m^2 higher than men's (32, 33). As a result, women can have insulin resistance and metabolic dysfunction for a long time before they are diagnosed with T2DM, putting women with diabetes at increased cardiovascular risk than men with diabetes (34). Combined with the fact that women have a longer life expectancy than men, this could explain why the burden of T2DM attributable to a high BMI is higher in older women than in older men (35).

This study used joinpoint regression analysis to evaluate changes in the ASMR and ASDR of T2DM attributable to a high BMI in China. From 2004 to 2016, the trend for the ASMR and ASDR for women decreased slightly and increased slightly, respectively. However, both the ASMR and ASDR in men showed a substantial increase from 2004 to 2016, which may have been driven by rapid social, economic, and environmental transitions

and cultural factors. First, China has made significant efforts to prevent and control obesity since 2003, issuing various policies, recommendations, and guidelines (36–38). Meanwhile, China has made significant efforts and advances in diabetes prevention, risk factor management, self-management education and support, and the integration of medical care modalities. However, there is always an opportunity for improvement, especially in rural areas with limited healthcare services and education access. Second, the perception of the female body image in Chinese society has favored a lean body type in recent years.

In contrast, a larger body size for men is often viewed as a symbol of strength and masculinity, influenced by long-standing social and cultural norms. In addition, as China continues to modernize, physical activity and labor intensity decrease in urban and rural areas and significantly more for women than for men (39). However, unlike the trend in most countries, the prevalence of physical inactivity in China in recent years has been significantly higher for men at 16% than for women at 12% (40). One possible reason for the lower rate of physical inactivity among women compared to men in China is the popularity of "square dancing," a type of fitness activity that has gained widespread popularity among middle-aged and elderly women in recent years. Square dancing is an excellent way to get women to exercise, especially in rural areas, where finding other exercise methods can be challenging. Additionally, square dancing has been shown to have social and mental health benefits, which can further contribute to its popularity among women (41, 42).

Using the age-period-cohort method, we further examined the effects of age, period, and cohort factors on the mortality and DALY rates of T2DM attributable to a high BMI in China. Age effects are typically defined as modifications caused by changes in physical, psychological, and social status resulting from biological age changes. As expected, the effect of age on T2DM mortality due to a high BMI generally increases with age. The 10th edition of the International Diabetes Federation Diabetes Atlas showed that age was a significant and independent risk factor for T2DM, with a similar trend expected to continue until 2045 (5). In addition, the prognosis is also adversely affected by the fact that T2DM complications such as cardiovascular disease, neuropathy, retinopathy, and nephropathy are more prevalent and worse in older patients. However, the effect of age on the DALY rate exhibited bell-shaped curves as the age increased. This may be related to the lower prevalence of overweight and obesity among older adults in China. Previous epidemiological evidence has shown that obesity and overweight generally increased with age, but later in adulthood, there was a slight decrease (43).

The period effect refers to the risk of morbidity or mortality caused by changes in natural conditions or social environments during a specific period. This is reflected mainly in the differences in disease risk caused by the changes in medical levels, diagnosis and treatment technology, health knowledge, and economic and cultural factors. Similarly, the effect of the period on mortality and DALY rates of T2DM attributable to a high BMI showed an increasing trend throughout the period, indicating that the period effect played a significant role in the growing trend of the burden of T2DM attributable to a high BMI. Over 40 years of reform and opening, China's economy, science and technology, overall quality of life, and medical services have all improved significantly. With the improvement of the living standard of Chinese residents, the dietary structure has changed. However, these have also brought new health threats to the Chinese people, mainly including environmental pollution and destruction, changes in lifestyle, the intrusion of large amounts of synthetic chemicals into human life, and mental health problems.

China is changing from a high-carb, high-fiber, and low-fat diet to a high-fat, high-energy, and low-carb diet (44). The daily dietary energy supply per capita in China has also increased significantly, from 2,100 to 2,400 kcal in the early 1980s to 3,000–3,100 kcal in the early 2010s, which is the direct cause of the increase in the overweight and obese population (44). In addition, unhealthy processed foods are rising and becoming more accessible and affordable (45). These trends have increased non-communicable diseases such as diabetes, hypertension, and cardiovascular disease. In the last 40 years, with the improvement in the consumption level and the lifestyle transformation, the sports time of Chinese residents decreased rapidly and the sedentary time gradually increased. With the pace of urbanization, modernization, and informatization, people are spending more and more times sitting in front of their desks. The proliferation of private cars, computers, smartphones, and other devices that make people's lives more convenient has dramatically reduced the amount of daily physical activity. Cable TV, internet entertainment, fast video streaming, mobile games, e-sports, and other forms of entertainment also increase the time people spend sitting. Furthermore, due to the improvement of medical treatment and the popularization of diabetes screening in China in recent years, an increasing number of diabetes cases are being screened at an early stage, which is one of the reasons for the growing burden of T2DM attributable to a high BMI.

The cohort effect represents the impacts specific to one's age group, those born in the same calendar year due to exposure to the same cultural, environmental, and social changes. Our findings showed that the RR of the cohort effect on T2DM mortality and DALY rates attributable to a high BMI have decreased, suggesting that earlier birth cohorts have a higher burden of T2DM attributable to a high BMI than later birth cohorts. This phenomenon may be related to better education and increased health awareness in the later cohorts than in the earlier cohorts. Furthermore, a previous study indicated that the group with unfortunate experiences in early life would have poorer physical health in adulthood (46). Studies have shown that pregnant women who experience nutritional deprivation during pregnancy are particularly vulnerable and that the physical health of their children is often shaped throughout their lives by their mothers' experiences of starvation during pregnancy. As adults, these children have relatively high levels of triglycerides and low-density lipoprotein cholesterol and are at greater risk of developing diseases such as obesity and diabetes (47).

According to these findings, multiple policy changes and interventions are needed to reverse the increasing trend of the burden of T2DM attributable to a high BMI in China. Fortunately, some initiatives are already in place, such as the National Basic Public Health Service Program and the Healthy China 2030 Plan, which aims to improve the prevention and management of T2DM and obesity. However, more efforts are needed to ensure its effective implementation and sustainability. Therefore, it is crucial to engage all stakeholders, including governments, civil society organizations,

and the private sector, in the implementation process and to establish monitoring and evaluation mechanisms to track progress and identify areas for improvement. First, we should improve public awareness and education on chronic diseases such as diabetes and obesity, increase public awareness of healthy lifestyles, and encourage people to improve their ability to care for themselves. Second, the food safety administration department should strengthen the supervision of food production, processing, sales, and other links and establish a food safety system. Third, the education and health departments must jointly strengthen nutrition education; promote a balanced diet; reduce the intake of foods high in sugar, fat, and salt, and increase the intake of vegetables and fruits. Fourth, the healthcare sector should establish a health management mechanism, regular physical examinations, and health counseling for high-risk groups. At the same time, a chronic disease management system should be installed. Collaboration between doctors, nurses, nutritionists, and other healthcare professionals allows for comprehensive treatment and management of patients. Fifth, researchers should strengthen basic and applied research on diseases such as T2DM and obesity. This will enable a solid a scientific foundation and technical support to prevent and treat these conditions.

This study has the following limitations: (1) it was a secondary analysis of data collected from the GBD 2019 study. Therefore, the general limitations of GBD studies, such as potential bias, are unavoidable, which can lead to some degree of deviation from the actual situation. However, the 2019 GBD study has applied robust statistical methods to solve this problem; (2) the GBD data are updated slowly, currently only up to 2019. Therefore, the use of previous data may not reflect the latest disease trends; 3) the burden of T2DM attributable to a high BMI could not be divided into the burden of T2DM due to overweight and obesity due to the lack of relevant data; (4) complications from T2DM are not taken into account in estimating the burden of T2DM attributable to a high BMI; (5) the study does not include children and individuals aged<20 years; 6) we did not take in account the difference in the burden of T2DM attributable to a high BMI between different provinces, different socioeconomic development, different ethnic groups, and rural and urban groups in China since these data were not available. Future research should focus on a comparative analysis of these factors to guide the development of appropriate health policies and programs and promote the realization of Healthy China 2030; and 7) the age-period-cohort model only considers the population level and ignores individual differences, which could lead to an ecological fallacy when extrapolating the overall trend to individuals.

Conclusions

T2DM attributable to a high BMI has caused a serious disease burden for the Chinese population, mainly among middle-aged and older adults. The burden of T2DM attributable to a high BMI has increased significantly in China in the last 30 years and has grown more in men than in women. According to the joinpoint analysis, the men's ASMR and ASDR of T2DM due to a high BMI increased approximately linearly from 1990 to 2019, while the women's ASMR and ASDR fluctuated. Between 2004 and 2015, there was even a decrease in the ASMR among women. The relative risk of T2DM mortality attributable to a high BMI continued to increase with age and period but decreased with the birth cohort. However, the effects of age and cohort on the DALY rate showed a bell-like and a flip horizontal J-shaped curve trend, respectively, but the impacts of the period on the DALY rate gradually increased. Our findings will provide a better epidemiological basis for future T2DM and high BMI management. The growing burden of T2DM due to a high BMI in China urgently requires close collaboration between policymakers, researchers, and healthcare professionals to develop gender- and age-based public health guidelines on prevention strategies, early diagnosis, and effective management of T2DM, overweight, and obesity. Additionally, education campaigns on healthy lifestyle choices and regular physical activity must be promoted to prevent the onset of T2DM, overweight, and obesity.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

J-LW and X-CZ designed the study and interpreted the results. J-LW analyzed the data and performed the statistical analysis. X-CZ supervised the study. W-JY and L-YZ double-checked all the data. J-LW, CH and X-CZ wrote the initial manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1193884/full#supplementary-material

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Prediabetes is associated with a higher serum neurofilament light chain level in adolescents

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Objective: Serum neurofilament light chain (sNfL) level, which is a biomarker indicative of neuroaxonal damage and cognitive impairment, has been reported in several neurological diseases. There has been a lack of studies on the association between sNfL levels and prediabetes in adolescents. This study investigated whether sNfL levels were higher in adolescents with prediabetes undergoing elective orthopedic surgery.

Methods: The sNfL level was measured in 149 adolescents aged from 12 to 18 years who underwent elective orthopedic surgery at the Hunan Children's Hospital (18 with and 131 without prediabetes). We evaluated the association between prediabetes and sNfL level after adjusting for age, sex, and triglycerides using a multivariable linear regression model.

Results: The prevalence of prediabetes in adolescents was 12.08%. Univariate logistic regression analysis showed that prediabetes was related to sNfL. In multivariate logistic regression analysis, the association between prediabetes with sNfL levels remained significant after adjustment for age, sex, and triglyceride. The relationship between the two was further visualized by a smoothed curve.

Conclusions: Prediabetes is associated with a higher sNfL. Further large-scale and prospective studies are needed to verify the clinical application of sNfL as a monitoring biomarker for adolescent prediabetes in adolescents and to evaluate the performance of sNfL in predicting the incidence of neuropathy and cognitive dysfunction in adolescents with prediabetes.

KEYWORDS

prediabetes, adolescents, sNfL, cognitive dysfunction, retrospective cohort study

Background

Due to increasing obesity, diseases previously almost exclusively found in adults, such as prediabetes and type-II diabetes(T2D), are now frequently diagnosed among adolescents (1). Prediabetes is a term that generally refers to an intermediate state of abnormal glycemia. In the United States, the National Health and Nutrition Examination Surveys have shown that the prevalence of prediabetes among adolescents is approximately 18.0%

(2). Meanwhile, prediabetes is a pressing clinical and public health issue, as studies have reported that the highest risk of diabetes, major adverse cardiovascular events (MACE), and chronic kidney disease occur in individuals with prediabetes (3, 4). As a precursor of T2D, prediabetes is more severe in adolescents than in adults because the accelerated period of progression from prediabetes to T2D (5, 6) could lead to an early onset of complications and adverse events that affect patients' quality of life and long-term outcomes. The increasing prevalence of prediabetes in adolescents has now become one of the major public health concerns worldwide (7).

Neurofilaments are considered major cytoskeletal components of neurons and are classified into light, medium, and heavy chains according to the size of the proteins. Neurofilament proteins are enriched in axons, simultaneously providing mechanical support and maintaining axon homeostasis (8, 9). Neurofilaments can be released from damaged or diseased axons in significant amounts into the blood and cerebrospinal fluid (CSF), and therefore their elevated levels are often used as potential biomarkers to indicate various neurological diseases (10, 11). CSF is the most frequently used biofluid for measuring neurofilament light chain (NfL) levels in neurodegenerative diseases, but because of the invasiveness of lumbar puncture as well as the pain and distress associated with CSF collection, it is impractical to obtain CSF from adolescents, who require strict instruction (12). The application of novel highly sensitive analytical methods has made it possible to measure low levels of NfL in blood with high accuracy and reproducibility. As several studies have demonstrated close correlations between CSF and sNfL (13, 14), it is practical to study sNfL in a wide range of neurological disorders. However, to properly explain sNfL levels in relation to disease states, it is also important to consider factors relevant to this protein change. Recent studies have shown that sNfL may be affected by some factors such as age, systolic blood pressure (SBP), and body mass index (BMI) (15–17).

Traditionally, neuropathy has been considered a microvascular complication that occurs in patients with a long history of diabetes. More recently, however, it has been reported that neuropathic complications may develop as early as the time of diagnosis of diabetes mellitus (18–20). The prevalence of documented neuropathy in individuals with prediabetes is approximately 11%-25% (18). Prediabetes has been increasingly recognized as an important factor leading to neuropathy. In particular, emerging data and epidemiologic studies support that prediabetes is a risk factor for mild cognitive impairment (21–23).

There is a lack of studies on prediabetes in adolescents. This study was designed to identify the main contributors to the sNfL level in perioperative adolescents and to assess whether a higher sNfL level was related to adolescents with prediabetes in elective orthopedic surgery. The current data analyzed in our study were obtained from Hunan Children's Hospital.

Methods

The data for this study were collected from the electronic medical records (2021-2022) of the Hunan Children's Hospital. This retrospective study was approved by the Ethics Committee of Hunan Children's Hospital [No. HCHLL-20230-43]. Informed consent was waived due to the observational nature of the study.

This study contained anonymous demographic, medical, surgical, and laboratory information from adolescents undergoing elective orthopedic surgery in the Department of Anesthesiology. All adolescent patients (aged from 12 to 18 years old) had a random preoperative plasma glucose or hemoglobin A1c. Exclusion criteria were patients undergoing emergency surgery, age <12 years old, ASA grade VI, no sNfL data, or no plasma glucose. The present analysis was exempted due to the deidentified dataset of the study. The study was conducted following a pre-specified protocol and statistical plan that was not disclosed prior to data analysis. This manuscript was prepared according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Laboratory tests and clinical data

Patient data were collected. This included age, sex, and body mass index (BMI), calculated as weight in kilograms divided by height in meters squared.

Laboratory methods were used to measure hemoglobin A1c (HbA1c), plasma glucose, high-density lipoprotein (HDL), systolic blood pressure (SBP), sNfL, cholesterol, and triglyceride levels. The single point insulin sensitivity estimator (SPISE) was calculated using the following formula: [600* HDL^0.185/(TG^0.2* BMI^1.338)] (24).

Definition of prediabetes

Prediabetes was defined in accordance with the American Diabetes Association criteria when any of the following conditions were met: (1) hemoglobin A1c (HbA1c) level≥5.7% and <6.5% (25); and (2) a random plasma glucose≥100 mg/dL and <160mg/dL (26).

Measurement of sNfL

Frozen serum was thawed on ice and then spun prior to NfL measurement using the Simoa HD-X Analyzer (Quanterix) and Single Molecule Array (Simoa) technology according to the manufacturer's instructions. The assay for these samples had a lower limit of detection of 0.038 pg/mL, a lower limit of quantitation (LLOQ) of 0.174 pg/mL, a dynamic range of 0–2,000 pg/mL, and a coefficient of variation of 7% at the LLOQ. Measurements were performed in duplicate and lots were completed by an investigator blinded to all clinical data, including outcome measures. Samples with a between-measurement coefficient of variation >20% were repeated according to standard practice.

Statistical analysis

All the analyses were conducted using IBM SPSS (version 22.0) and R (version 4.12.0) software. Data were presented as median (M)

and interquartile range (IQR). The Wilcoxon rank-sum test was used to compare continuous variables with non-normal distributions, and the chi-squared test was used to compare the composition ratio of classified data. The association between prediabetes and sNfL level was modeled using multivariate linear regression analysis. The selection of covariates to be included in the model was based on data from previous studies in which age, sex, prediabetes, and triglycerides were found to be associated with sNfL level. A two-tailed value of $P<0.05$ was considered statistically significant.

Results

Features of the study population

The process of patient selection was shown in Figure 1. A total of 149 patients were eligible for analysis, including 131 participants without prediabetes and 18 patients with prediabetes (12.08%). All the adolescents were grouped according to whether they had prediabetes before surgery. The overall characteristics of the study population were shown in Table 1. The median [IQR] sNfL of adolescents with and without prediabetes before surgery was 13.500 mmol/L and 9.750 mmol/L, respectively, with a statistically significant difference (P=0.029). Other factors including gender, age, BMI, cholesterol, triglycerides, HDL, SBP, and SPISE were not significantly different between the two groups.

Univariable analysis of sNfL

As shown in Table 2, when considering the entire cohort, the univariable analysis showed that prediabetes (RR, 4.147; 95%CI, 0.584-7.710, P=0.024) was associated with sNfL. Apart from prediabetes, other factors, such as gender, age, BMI, cholesterol, triglycerides, HDL, SBP, and SPISE, were irrelevant to sNfL. In Figure 2, a smoothed curve was applied to visualize the relationship between sNfL and the prevalence of prediabetes in adolescents, indicating that the prevalence of prediabetes was correlated with sNfL.

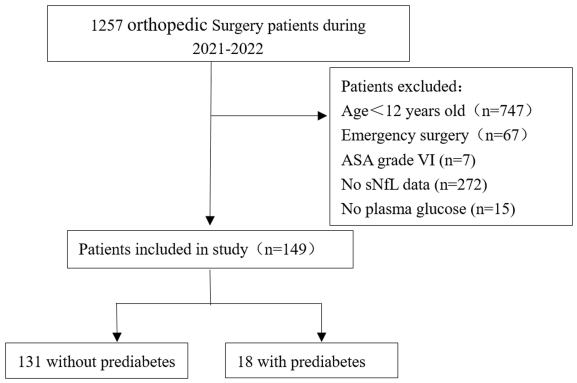

FIGURE 1
Flowchart of patients' selection.

The sNfL level as an independent predictor in adolescents taking elective orthopedic surgery

The association between prediabetes and sNfL level was modeled using multivariate linear regression analysis. The results were shown in Table 3. Multiple regression analysis revealed that prediabetes (RR, 4.172; 95%CI, 0.602-0.742, P=0.023) was positively correlated with sNfL independent of other factors in all patients.

Sensitivity analysis for sNfL

As shown in Table 4, various variables were divided into subgroups, which were subjected to regression analysis to investigate the relationship between the subgroup variables and sNfL using sensitivity analysis. We found that low cholesterol (RR, 10.585; 95%CI, 3.616-17.554, P=0.005), low triglyceride (RR, 13.124; 95%CI, 6.160-20.089, P<0.001), and high HDL (RR, 9.593; 95%CI, 2.964, 16.222, P=0.006) were associated with sNfL.

Discussion

In the present study, we evaluated the association between a higher sNfL level and prediabetes in adolescents undergoing elective orthopedic surgery. According to the American Diabetes Association criteria, the prevalence of prediabetes in adolescents in our study was 12.08%. The current univariate analysis showed a relation between prediabetes and sNfL. After adjustment for age, gender, and triglyceride, the results showed that the association between prediabetes and sNfL level was still significant. The smoothed curve further visualized the relationship between the two. Our findings demonstrated showed a higher sNfL level in adolescents who had prediabetes, providing neurochemical evidence for subclinical axonal damage and underlying cognitive impairment in prediabetes adolescents. Such a finding could help improve interventions to promote the reversion of prediabetic states to normal glucose tolerance.

Although several studies have examined sNfL levels in patients with various neurological disorders, to the best of our knowledge, this was the first study to report the association of sNfL levels with prediabetes in adolescents. Importantly, the association between prediabetes and sNfL remained significant after adjustment for sex, age, and triglycerides. Previous studies have shown a positive association between sNfL levels and age (27, 28), as a marked increase in sNfL levels has been found to be associated with older age, particularly in those over 60 years of age (29). Our results did not over 60 years of age. Similarly, there was no independent association between sex and sNfL in our study, which was generally over 60 years of age studies (30, 31).

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TABLE 1 Characteristics of participants according to quartiles of serum neurofilament light chain (sNfL) levels.

Variables	Without prediabetes (N=131)	With prediabetes (N=18)	P-value
Male,%	51.50	48.01	0.511
Age, years	14.636 ± 1.593	14.556 ± 1.854	0.960
sNfL, mmol/L	9.750 (7.275-14.175)	13.500 (9.850-20.775)	0.029*
BMI, kg/m^2	23.005 ± 1.382	23.260 ± 1.555	0.470
Cholesterol, mmol/L	4.770 ± 0.988	5.106 ± 1.177	0.188
Triglyceride, mmol/L	92.000 (62.000-134.500)	102.000 (72.250-146.750)	0.662
HDL, mmol/L	47.000 (41.000-58.000)	51.500 (40.500-54.500)	0.808
SBP, mmHg	110.500 ± 12.011	112.667 ± 11.003	0.470
SPISE	7.542 ± 1.386	7.460 ± 1.338	0.813

BMI, Body Mass Index; HDL, High-Density Lipoprotein; SBP, Systolic Blood Pressure; SPISE, Single-Point Insulin Sensitivity Estimator.
*: P<0.05.

TABLE 2 Univariable analysis of sNfL.

Exposure	Statistics	sNfL, RR (95%CI)	P-value
Prediabetes	18 (12.08%)	4.147 (0.584, 7.710)	0.024*
Age	14.427 ± 1.620	0.312 (-0.416, 1.040)	0.402
SPISE	7.532 ± 1.376	-0.240 (-1.099, 0.618)	0.584
BMI	23.035 ± 1.401	-0.158 (-1.001, 0.686)	0.714
Cholesterol	4.810 ± 1.014	-0.664 (-1.825, 0.497)	0.264
Triglyceride	122.320 ± 106.169	0.006 (-0.005, 0.018)	0.256
HDL	49.173 ± 11.957	-0.022 (-0.120, 0.077)	0.670
SBP	110.760 ± 11.881	0.085 (-0.014, 0.184)	0.093
Male	76 (51.01%)	-2.480 (-6.510, 1.550)	0.230

SPISE, Single-Point Insulin Sensitivity Estimator; BMI, Body Mass Index; HDL, High-Density Lipoprotein; SBP, Systolic Blood Pressure.
*: P<0.05.

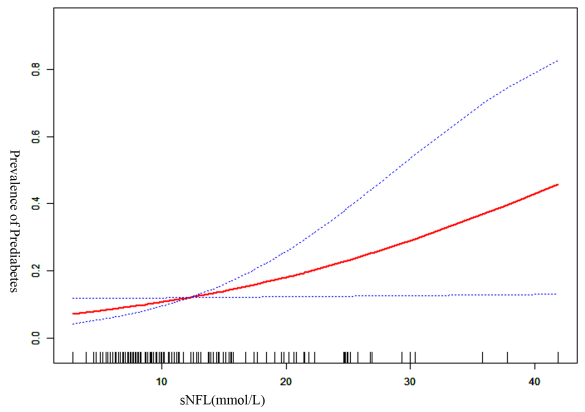

FIGURE 2
Smoothed curve of the sNfL and Prevalence of Prediabetes.

Neurofilaments are released into the extracellular space following neuroaxonal damage, and we found that prediabetes was associated with a higher sNfL. Our study was the first to validate a higher sNfL as a potential biomarker of mild nerve axon damage or milder peripheral neuropathy in prediabetic adolescents. It was well known that neuropathy is one of the major causes of complications in the diabetic population, however, but some studies reported that peripheral neuropathy may develop in humans with prediabetes before overt hyperglycemia (19, 32, 33), suggesting that peripheral nerve injury may occur in the early stages of the disease with milder glycemic dysregulation. In general, prediabetic neuropathy is milder than diabetic neuropathy and primarily affects nerve fibers that mediate sensory function (34, 35). Currently, there is increasing scientific evidence indicating that sNfL is correlated with neurodegenerative diseases (36) and peripheral neuropathies in both humans and animals (37–40). Some studies have observed that diabetes is associated with sNfL (41–43) and that individuals recently diagnosed with diabetes provide new evidence that a higher sNfL is related to diabetic sensorimotor polyneuropathy and peripheral nerve dysfunction (44). Our findings extend previous observations by demonstrating the association between sNfL and prediabetes in adolescents. A recent study showed that the level of sNfL is elevated 6 years before the clinical onset of multiple sclerosis (MS), implying that damage to nerve axons has already begun during the long prodromal period before the diagnosis of MS (45). Similar to this study, we showed that in an intermediate state of abnormal glycemia, that is, prediabetes, started to appear potential or minor nerve axon damage began to appear before the diagnosis of diabetes based on the changes in sNfL.

By correlating sNfL with adolescent prediabetes, our study indirectly demonstrated that there may be an underlying cognitive impairment in adolescent prediabetes. Previous studies have shown that performance in some domains of cognitive

TABLE 3 Multivariable linear regression model evaluating predictors of serum neurofilament light chain (sNfL) levels in the studied population.

Variables	RR	95%CI	P-value
(Intercept)	7.222	-7.831, 13.851	0.184
Age	0.269	-0.465, 1.004	0.473
Prediabetes	4.172	0.602, 0.742	0.023*
Male	-2.222	-6.222, 1.779	0.278
Triglyceride	0.006	-0.005, 0.017	0.304

*:P<0.05.

TABLE 4 Sensitivity Analysis for sNfL.

Subgroups	N	sNfL, RR (95%CI)	P.value
SPISE Tertile			
Low	44	2.230 (-2.823, 7.283)	0.392
Middle	42	5.072 (-3.979, 14.123)	0.279
High	63	4.994 (-0.086, 10.073)	0.059
BMI Tertile			
Low	50	4.408 (-0.772, 9.587)	0.102
Middle	50	4.591 (-2.605, 11.788)	0.217
High	49	4.136 (-2.320, 10.593)	0.215
Cholesterol Tertile			
Low	50	10.585 (3.616, 17.554)	0.005*
Middle	50	5.592 (-4.199, 15.382)	0.268
High	49	-0.285 (-4.287, 3.717)	0.890
Triglyceride Tertile			
Low	50	13.124 (6.160, 20.089)	<0.001*
Middle	50	-0.540 (-6.949, 5.869)	0.870
High	49	0.967 (-4.088, 6.021)	0.709
HDL Tertile			
Low	49	1.324 (-4.439, 7.087)	0.655
Middle	51	2.668 (-3.441, 8.776)	0.396
High	49	9.593 (2.964, 16.222)	0.006*
SBP Tertile			
Low	44	2.230 (-2.823, 7.283)	0.392
Middle	42	5.072 (-3.979, 14.123)	0.279
High	63	4.994 (-0.086, 10.073)	0.059
Gender			
Female	73	3.781 (0.021, 7.541)	0.051
Male	76	8.362 (-2.301, 19.024)	0.150

*:P<0.05.

function appears to be impaired in patients at early stages of the disease, including prediabetes (46, 47). In addition, prediabetic status or progression to the diabetic phase may promote the reversion of mild cognitive impairment (MCI) to normal cognition (48). Current evidence increasingly supports the use of sNfL level as a biomarker for cognitive dysfunction (49–52). Our study found that measuring sNfL in adolescents with prediabetes could help identify young people at risk of developing cognitive impairment. Therefore, it was necessary to measure sNfL in prediabetic adolescents to provide an early opportunity to reverse the prediabetic state to a normal blood glucose state and prevent more serious cognitive impairment.

Strengths

Our study had several strengths. It was the first study of the relationship between sNfL and prediabetes in adolescents, and such a selection of the study population allowed analyses of associations without the confounding effect of age-related comorbidities. In addition, sNfL levels were measured with a highly reproducible method, and serum samples from cases and controls were analyzed simultaneously in a blinded and randomized fashion, effectively avoiding potential artifactual differences in sNfL concentrations.

Limitations

Several limitations should also be acknowledged. First, the retrospective design made it impossible for us to know the predictive value of sNfL, and the retrospective study implies the possibility of missing data. Second, we lacked cognitive function tests and objective measurements of sensorimotor neuropathy, so more studies are needed to thoroughly illustrate the relevant contents. Finally, although several variables were adjusted in the analysis, we cannot completely exclude the possibility of residual confounding.

Conclusion

In conclusion, our study showed that the association between prediabetes and sNfL was significant even after adjustment for several covariates. A higher serum NFL level was associated with prediabetes in adolescents. It also suggested the possibility of potential neurological damage and cognitive impairment in prediabetic adolescents. Further large-scale and prospective studies are needed to verify the clinical application of sNfL as a monitoring biomarker for prediabetes, and we encourage future studies to evaluate the performance of sNfL in predicting the incidence of neuropathy and cognitive dysfunction in adolescents with prediabetes.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Hunan Children's Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

ZC contributed to the conception and design of the study. T-CP performed data collection and statistical analysis. L-PW collated and interpreted the results and wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Telehealth model versus in-person standard care for persons with type 1 diabetes treated with multiple daily injections: an open-label randomized controlled trial

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Objective: Increasing evidence indicates that the telehealth (TH) model is noninferior to the in-person approach regarding metabolic control in type 1 diabetes (T1D) and offers advantages such as a decrease in travel time and increased accessibility for shorter/frequent visits. The primary aim of this study was to compare the change in glycated hemoglobin (HbA_{1c}) at 6 months in T1D care in a rural area between TH and in-person visits.

Research design and methods: Randomized controlled, open-label, parallel-arm study among adults with T1D. Participants were submitted to in-person visits at baseline and at months 3 and 6 (conventional group) or teleconsultation in months 1 to 4 plus 2 in-person visits (baseline and 6 months) (TH group). Mixed effects models estimated differences in HbA_{1c} changes.

Results: Fifty-five participants were included (29 conventional/26 TH). No significant differences in HbA_{1c} between groups were found. Significant improvement in *time in range* (5.40, 95% confidence interval (CI): 0.43-10.38; $p < 0.05$) and in *time above range* (-6.34, 95% CI: -12.13- -0.55;$p < 0.05$) in the TH group and an improvement in the Diabetes Quality of Life questionnaire (EsDQoL) score (-7.65, 95% CI: -14.67 - -0.63; $p < 0.05$) were observed. In TH, the costs for the participants were lower.

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Conclusions: The TH model is comparable to in-person visits regarding HbA_{1c} levels at the 6-month follow-up, with significant improvement in some glucose metrics and health-related quality of life. Further studies are necessary to evaluate a more efficient timing of the TH visits.

KEYWORDS

type 1 diabetes, metabolic control, telehealth, emerging technologies, chronic complications

Introduction

Advances in technology have irrupted strongly in the life and care of persons with type 1 diabetes in the last decade (1), also for those who are not users of insulin infusion pumps (2). As examples, different smartphone applications led to register data of self-monitoring blood glucose (SMBG) with finger-stick glucose (FSG), which are remotely available for clinicians. Other persons with diabetes are users of real-time continuous glucose monitoring (CGM) or flash glucose monitoring (FGM) devices that provide a standardized ambulatory glucose profile, which is also remotely available (2). Therefore, SMBG and CGM/FGM data can be easily downloaded to review patterns and make adjustments in treatment during a telehealth (TH) consultation (2–4). Two consequences of the availability of such data are, on the one hand, the possibility of a closer treatment adjustment, which may result in an improvement of metabolic control; and on the other hand, the need for in-person visits has been reduced since the data are available online, thus favoring the TH.To date, few randomized studies have been reported on the impact of TH on the control of type 1 diabetes. Increasing evidence suggests that TH is noninferior to the in-person approach regarding metabolic control assessed by glycated hemoglobin (HbA_{1c}), but data on glucose metrics are very limited due to the low inclusion of participants with glucose monitoring sensors (5–9).

Moreover, TH could show some advantages; for example, interacting with persons in their natural environment offers more personalized care, a decrease in traveling time to outpatient clinics and increased accessibility for shorter and more frequent visits, increasing the time that persons have available to address competing needs, such as family, work and social demands (1, 3, 8–11). This last point has been even more important in rural zones, such as our area in Alt Penedès, where distances are longer and time spent traveling is greater. In addition, the coronavirus disease 2019 (COVID-19) pandemic highlighted TH as a need, given the increased risk of virus infection in in-person care at hospitals and traveling restrictions (12–14).

Nevertheless, most studies have been conducted in participants with FSG determinations, and there is little evidence of the application of telemedicine in persons with CGM or FGM, devices that provide much more information on the glycemic profile and that allow guidelines to be adjusted more appropriately, even remotely.

For all of the above, we propose TH as a noninferiority approach in the management of persons with type 1 diabetes who use classic multiple daily injections insulin therapy with or without FGM attended in a rural area, with fewer in-person visits.

The primary aim of the present study was to compare, in type 1 diabetes persons assisted in a rural area, the change in HbA_{1c} at 6 months between TH and in-person visits. As secondary objectives, we compared the change in HbA_{1c} at the 3-month follow-up, glucose metrics (time in range (TIR); time below range (TBR); time above range (TAR); glucose management indicator (GMI); glycemic variability (CV)), hypoglycemic events, direct and indirect costs, Diabetes Quality of Life questionnaire (EsDQoL), and participant satisfaction.

Research design and methods

Study design and participants

This is a randomized controlled study, open-label, parallel arms, among adult persons with type 1 diabetes (ClinicalTrials.gov identifier NCT04758884). The two arms of the study were the control arm, in which participants were submitted to standard in-person visits in the outpatient clinic, and the experimental arm, in which participants were submitted to teleconsultation (phone-call or video-call). Insulin bolus adjustments were made in both groups using the SocialDiabetes® App, which is a virtual platform that acts as a bolus calculator and allows changes (in the ratio and in the sensitivity factor) to be made remotely. In addition, this application allows users to generate a message to request, automatically, a telematic visit with the doctor in case they need it. A simple randomization was performed at a baseline in-person clinical visit (1:1), stratified by flash glucose monitoring (FGM) system use. The follow-up period was 6 months.

Eligible participants were persons with type 1 diabetes who visited the outpatient clinic in Hospital Comarcal de l'Alt Penedès between January 2021 and June 2021. Inclusion criteria were persons over 18 years with type 1 diabetes of at least 6 months duration, with internet access mobile phone, and trained to use the SocialDiabetes® App. All participants were receiving multiple daily injections of insulin therapy. For participants with the FGM system, 2 months of use was required before randomization. The exclusion criteria were severe ketoacidosis in the previous 3 months, severe or

recurrent hypoglycemic events, need for diabetes education support, or lack of consent to participate.

The required sample size for bilateral contrast was estimated using an α value of 0.05, a β value of 0.2 and a common standard deviation for HbA1c of 0.6% (4.2 mmol/mol) to detect a difference in HbA1c $\geq$ 0.5% (3.1 mmol/mol) and taking into account a dropout rate of 10%, 27 participants per arm would be needed.

Basal assessment and definitions

At the baseline in-person clinical appointment, we enrolled the participants after verification of the inclusion criteria compliance and the exclusion criteria. Epidemiological data, working data and diabetes history were recorded. Diabetes complications were diagnosed in accordance with the American Diabetes Association criteria 2021: microangiopathy was diagnosed in the presence of retinopathy, neuropathy and/or nephropathy (15); macroangiopathy was established in the presence of coronary heart disease (CHD), cerebrovascular disease, or peripheral arterial disease (16). In addition, the duration of diabetes, treatment, insulin dose and presence of hypoglycemia were recorded. Mild hypoglycemia was defined as a capillary blood glucose < 70 mg/dl, serious hypoglycemia was defined as a capillary blood glucose < 54 mg/dl, and severe hypoglycemia as a severe event characterized by altered mental and/or physical functioning that requires assistance from another person for recovery (4). Sensor-using participants were asked to check sensor-measured hypoglycemia in capillary glucose, to report only those confirmed in capillary blood. Thus, hypoglycemic events were self-reported by each participant in the form of an estimated number of hypoglycemic events per month. In those participants who use an FGM system, different glucose metrics were reported (TIR, TAR, GMI, CV, number and level of hypoglycemic events) (4).

Anthropometric parameters (weight, height, body mass index (BMI), waist circumference, hip circumference) were measured using standardized methods. Blood samples were obtained from all participants for the measurement of basal glucose, HbA_{1c}, total cholesterol, low-density lipoprotein cholesterol (LDLc), high-density lipoprotein cholesterol (HDLc), and triglycerides. The albumin/creatinine ratio was determined in a random urine sample.

Health-Related Quality of Life (HRQoL) was evaluated through EsDQoL questionnaire (17) completed by participants.

Follow-up program

After enrollment in the baseline visit, participants were randomized 1:1 in the following: a) Control group: participants were submitted to standard in-person visits in the outpatient clinic at months 3 and 6 after randomization; b) TH group: participants were submitted to teleconsultation at months 1, 2, 3 and 4 after randomization. Most of the teleconsultations were made by phone-call. Participants in both groups had the possibility of teleconsulting when necessary through the SocialDiabetes® App.

Teleconsultations in months 1, 2 and 4 included the recording of the number and level of hypoglycemia events and glycemic data, if available. At the month 3 visit, we also registered laboratory data in both the control and experimental groups. At the month 6 in-person visit, we added all these records plus anthropometric parameters and the EsDQoL quality of life questionnaire. At the closing visit, participants also completed a nonstandardized satisfaction questionnaire.

A visiting time of 30 minutes was assigned to all in-person visits, whereas it was 10 minutes in teleconsultations. All extra visits (in-person and/or telematics) performed for participant needs or medical criteria during the follow-up period were registered.

Analysis of total costs

Costs for the National Health System (NHS) were calculated, according to the standard rates at the time of the study, in prices 80 euros for each in-person visit and 48 euros for teleconsultation assistance. The time spent with the endocrinologist was calculated as 30 minutes assigned to in-person visits and 10 minutes assigned to teleconsultations. Extra visits (in-person or teleconsultation) have been taken into account for the calculation of costs in each study arm.

Direct costs for participants assessed were both time losses and transportation costs. Time losses were calculated according to 4 hours for in-person visits (30 minutes for visits plus 3 hours traveling plus 30 minutes waiting) and 30 minutes for teleconsultation. Transportation costs assuming that participants would travel with their cars were calculated based on distance traveling (kilometers (km) to hospital), an average price for petrol in Spain equal to 1.255€ per liter in 2021, and a consumer car average of 5 liter for km. These costs were estimated based on information from the international statistical portal Statista (Spain).

Indirect costs for participants assessed were both abstention rate and productivity losses. Abstention rates were calculated based on total time spent by working active participants and/or companions with respect to total working hours during the follow-up period assigned by collective conveners. Productivity losses were calculated taking into account the average national salary for Spain in 2021, according to the Spanish National Institute of Statistics, for these active-working participants.

Total direct and indirect costs for participants were calculated as the sum of petrol expenses and productivity losses for each participant.

Statistical analysis

Statistical analysis was based on all valid data of randomized participants according to per-protocol analysis. For all variables, normality was evaluated by qqplot and the Shapiro-Wilk test. Descriptive analysis used frequencies and percentages (categorical variables), means and standard deviation (symmetric distributed continuous variables), and median and interquartile range (skewed continuous variables). Parametric Student's t test was used to

evaluate the difference between means, and a nonparametric Mann-Whitney U test was used to evaluate the difference between medians. The chi-square test was employed to assess the association between categorical variables. The mixed effects models evaluated the impact of the intervention over time on the primary outcome, HbA_{1c} at 6 months. We included a binary indicator for intervention group assignment and a group-by-time interaction term in the models to compare improvement over time between the intervention and usual care groups.

Statistical analyses were performed using the R 4.1.3 statistical package (R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). For all statistical tests, all comparisons were bilateral, and data with a p value of less than 0.05 were considered statistically significant.

Ethics

The protocol was concordant with current and relevant guidelines and regulations, and the Ethics Committee of Hospital Universitari de Bellvitge approved it (protocol Reference: PR040/20; 17/SEP/2020). Written informed consent was obtained from all participants after they were provided with a full explanation of the purpose and nature of the study procedures.

Results

Study population

Among 59 persons who accepted participation in the study, four were lost during the follow-up period (2 in the control group and 2 in the TH group; dropout rate 6.78%). Therefore, the final study population consisted of 55 participants, 29 assigned to the conventional group and 26 to the intervention group (Figure 1). Demographic, clinical and biochemical variables and diabetes-related variables of this cohort are shown in Table 1.

Metabolic control

At the 6-month follow-up, the mean HbA_{1c} was 7.66% (± 0.82) in the conventional group and 7.55% (± 0.79) in the intervention group. Our mixed effect regression models evaluated the effect of the intervention on metabolic control variables over time (3 months and 6 months) (Table 2). We found no significant differences in HbA_{1c} at 6 months (main outcome) or at 3 months.

Regarding glucose metrics at the end of the follow-up period, TIR/TAR/TBR in the conventional group was 60.4 (± 15.8)/37.0 (± 16.0)/2.00 [1.00-4.00]% and 61.6 (± 14.6)/35.5 (14.3)/2.00 [1.00-4.00]% in the intervention group. We found significant improvement in TIR (5.40, 95% confidence interval (CI) 0.43-10.38; $p < 0.05$) and TAR (-6.34, 95% CI: -12.13- -0.55;$p < 0.05$) in the intervention group at 6 months. We did not find significant changes in TBR.

Safety outcomes

At the 6-month follow-up, the mean number of mild hypoglycemic events was 11.7 (± 15.4) in the conventional group and 12.2 (± 9.90) in the intervention group. Non severe hypoglycemic events occurs in any participant during the follow-up. The number of serious hypoglycemic events during the follow-up period was almost negligible. No significant differences in the number of mild hypoglycemic events at 3 or 6 months were found (Table 2).

Cost analysis

The cost analysis of TH versus conventional care assistance for the 6-month follow-up period is shown in Table 3. There were no significant differences between the extra visits of both groups (+100 min in the conventional group vs. +90 min in the TH group). Costs for the NHS and time spent with the endocrinologist were significantly higher in the intervention group than in the

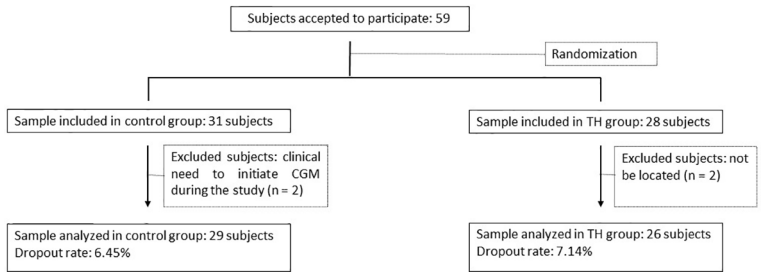

FIGURE 1
Flow Chart. TH, telehealth; continuous glucose monitoring (CGM).

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TABLE 1 Baseline characteristics of the study population.

	Conventional group n = 29	Intervention group n = 26
Demographic variables		
Male/Female, n (%)	15 (51.7)/14 (48.3)	13 (50.0)/13 (50.0)
Age, years	50.1 (± 12.5)	52.5 (± 12.4)
Employed, n (%)	21 (77.8)	18 (69.2)
Unaccompanied, n (%)	28 (96.6)	21 (95.5)
Distance to hospital, km	13.3 [3.61-17.3]	11.2 [0.00-17.0]
Clinical and biochemical variables		
BMI, kg/m^2	26.7 [23.0-30.0]	27.7 [24.1-29.6]
Waist circumference, cm	93.3 (± 17.7)	95.3 (± 17.1)
Hip circumference, cm	104 (± 11.6)	105 (± 11.6)
HTA, n (%)	9 (31.0)	5 (19.2)
eGDR, mg/kg min	8.05 (± 1.70)	8.37 (± 1.85)
HbA_{1c},		
%	7.61 (± 0.69)	7.52 (± 0.72)
mmol/mol	60 (± 5.2)	59 (± 5.5)
Triglycerides, mg/dL	99.7 [83.0-137]	74.8 [56.9-92.6]
Total cholesterol, mg/dL	190 [172-209]	183 [173–201]
LDLc, mg/dL	109 (± 27.8)	97.8 (± 20.6)
HDLc, mg/dL	64.4 (± 18.6)	65.5 (± 17.3)
Albumin/creatinine, mg/g	5.00 [3.00-10.0]	5.00 [4.00-8.50]
Diabetes related variables		
Age at onset, years	30.1 (± 13.0)	28.0 (± 13.9)
Diabetes evolution, years	20.0 (± 10.5)	24.5 (± 12.2)
Microangiopathy, n (%)	12 (41.4)	10 (38.5)
Macroangiopathy, n (%)	4 (13.8)	1 (3.85)
FGM, n (%)	24 (82.8)	25 (96.2)
GMI, %	7.21 (± 0.49)	7.36 (± 0.60)
TIR, %	61.8 (± 13.5)	58.1 (± 14.2)
TBR, %	2.00 [1.00-4.00]	2.00 [2.00-4.00]
TAR, %	34.4 (± 13.3)	38.8 (± 14.5)
Basal insulin (UI/day)	24.0 [17.0-34.0]	21.0 [16.5-29.5]
Prandial insulin (UI/day)	22.0 [13.8-27.0]	19.0 [14.3-29.5]
SGLT-2 inhibitors, n (%)	4 (13.8)	3 (11.5)
EsDQoL, points	81.5 [66.2-86.5]	74.0 [61.8-81.2]

Data are presented as n (%), means (± SDs) or medians [interquartile ranges].
BMI, body mass index; HbA_{1c}, glycated hemoglobin; LDLc, low-density lipoprotein cholesterol; HDLc, high-density lipoprotein cholesterol; FGM, flash glucose monitoring; GMI, glucose management indicator; TIR, time in range; TBR, time below range; TAR, time above range; SGLT-2, sodium-glucose cotransporter-2.

conventional group, whereas time losses for participants were lower in the TH group. No significant differences were observed in transportation costs or indirect costs (abstention rate and productivity losses) for participants, although a trend to be higher in the conventional group was found. We describe a lower expense in total participant losses (transportation costs plus productivity losses) in the TH group.

Participant perception

At the 6-month follow-up, the median score in EsDQoL was 79.0 [73.0–88.0] and 65.0 [56.0–81.5] in the conventional and intervention groups, respectively. A mixed effect regression model evaluated the effect of the intervention on the EsDQoL questionnaire results over time (6 months) (Table 2). We found significant differences in the intervention group at 6 months, with a decrease in the total EsDQoL score (-7.65, 95% CI -14.67 – -0.63; $p < 0.05$) reflecting better HRQoL.

Regarding participant satisfaction, in both groups, the majority preferred alternation between conventional in-person visits and teleconsultations (42% in the control group vs. 46% in the intervention group). The main concern about TH was noncompliance with visiting hours (6.5% in the control group versus 11% in the intervention group). The main advantages of TH were no need to travel to the hospital followed by time savings (26% and 16% in the control group versus 32% and 25% in the intervention group, respectively) (Figure 2).

Conclusions

The best findings of the present study were that the use of TH in persons with type 1 diabetes assisted in a rural area achieved similar metabolic control to conventional management, with a slight improvement in glucose metrics and HRQoL and a lower cost for the affected individuals.

This is the first clinical trial aimed at comparing TH versus in-person visits among adults with type 1 diabetes treated with multiple insulin doses that includes a high percentage of participants using FGM. The development of new technologies in type 1 diabetes and the situation caused by the COVID-19 pandemic has precipitated the use of TH, although strategies have been tried for some time. Recently, advocating for prioritizing TH over in-person care and considering a hybrid model of in-person and TH for people with diabetes has been proposed (3). Despite some systematic reviews and meta-analyses about improving type 1 diabetes management with mobile tools suggesting promising results in terms of a decrease in HbA_{1c} values, these remain inconclusive (5). Few previous studies, which mainly based telemedicine on the adjustment of insulin treatment by FSG, have also demonstrated a noninferiority approach of TH in type 1 diabetes care regarding metabolic control and safety events (7, 8, 11, 18, 19). Indeed, the PLATEDIAN, TELEDIABE and TeleMed studies showed no statistically significant differences in HbA1c and mild hypoglycemic events (7, 8, 11). In most studies mentioned

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TABLE 2 Effect of the intervention on metabolic control outcomes and on the participants' perception outcomes.

	β	95% CI	p value
Metabolic control and safety			
at 3 months:			
HbA_{1c}	0.00	-0.30 – 0.31	0.989
TIR	3.60	-1.34 – 8.54	0.152
TBR	1.28	-0.86 – 3.43	0.239
TAR	-4.89	-10.63 – 0.85	0.095
Mild hypoglycemia	1.00	0.90 – 1.10	0.949
at 6 months:			
HbA_{1c}	-0.01	-0.32 – 0.29	0.929
TIR	5.40	0.43 – 10.38	**0.034**
TBR	0.98	-1.18 – 3.14	0.373
TAR	-6.34	-12.13 – -0.55	**0.032**
Mild hypoglycemia	1.01	0.88 – 1.15	0.882
Participant's perception			
EsDQoL at 6 months	-7.65	-14.67 – -0.63	**0.033**

Effect estimates are regression coefficients (β) for assessed metabolic control variables and for the EsDQoL questionnaire.
HbA_{1c}, glycated hemoglobin; TIR, time in range; TBR, time below range; TAR, time above range.
Bold values are statistically significant P.

above, the representation of FGM/FGM users was low, so glucose metrics were not reported and were not assessed as outcome variables. The use of glucose monitoring systems greatly facilitates telemedicine because it provides much more information and different parameters that can be adjusted during visits. Virtual platforms such as the SocialDiabetes® App make it possible to readjust the parameters of the bolus calculator remotely, facilitating telematic visits and helping to ensure that the proposed therapeutic changes are correctly applied. In the present study, a high proportion of participants were users of FGM, and monitoring data were evaluated as outcomes. For the first time, an improvement in TIR and TAR in the TH group has been confirmed, which can be attributed to a pattern of shorter but more frequent follow-up visits. Although the improvement may be clinically modest, it remains to be proven in a longer-term study whether the changes we have observed in these glucose metrics will be reflected in HbA_{1c} levels.

Beyond the results in metabolic control and safety, TH offers an alternative to persons in rural areas where geographic isolation represents an obstacle for traveling to hospital centers. Nonetheless, although some studies have included rural areas, none have assessed the efficiency of TH for type 1 diabetes care (7, 8, 11). In the present study, total costs for participants were significantly lower in the TH group, but transport losses did not reach statistical significance. A similar cost analysis was performed in the TeleMed study (11); unlike our results, and although that study was not conducted in a rural area, transportation costs were significantly lower in the intervention group (11), reaching statistical significance due to the greater number of visits. Regarding NHS costs, they described that, compared with the control group, the TH group required less healthcare time for the professionals (11). The main difference from the present study was that in the TeleMed study, participants in both groups were submitted to the same number of visits, but in the present study, participants in the intervention group received more medical interactions, which could have limited the economic benefits of TH. Indeed, the results of our study showed a higher cost for the NHS with a reduction in participant´s costs. In this sense, it is necessary to make some clarifications. First, the cost value assigned to virtual visits has been calculated on an approximate basis, since their real value has yet to be recatalogued after the COVID-19 pandemic has caused this type of visit to increase significantly (14). On the other hand, the visit made in person in the sixth month to the TH group was carried out as part of the protocol to determine the anthropometric variables and close the trial. It should be noted that once the results have been analyzed, we have modified our usual practice, and this visit is virtually performed. Therefore, currently, the costs for the NHS would be comparable between groups, the endocrinologist's time commitment would be 20 minutes less, and the cost savings for the affected individuals would be even greater than those shown in the present study.

Precisely the fact that the present study was conducted in a geographically dispersed area may have influenced a better perception of HRQoL, which was not reflected in all previous

TABLE 3 Analysis of costs.

	Conventional group n = 29	Intervention group n = 26	p value
Costs for the NHS, euros	250 (33.5)	362 (29.7)	**<0.001**
Time spent for endocrinologist, minutes	90.0 [90.0;90.0]	100 [100;100]	**<0.001**
Time losses for participant, hours	12.4 (1.64)	10.2 (0.82)	**<0.001**
Transportation costs, euros	6.59 (7.93)	3.46 (4.06)	0.069
Abstention rate	1.20 (0.70)	0.87 (0.55)	0.053
Productivity losses, euros	154 (89.1)	111 (69.8)	0.053
Total participant's losses, euros	160 (92.0)	115 (70.6)	**0.043**

NHS, National Health System; min., minutes.
Bold values are statistically significant P.

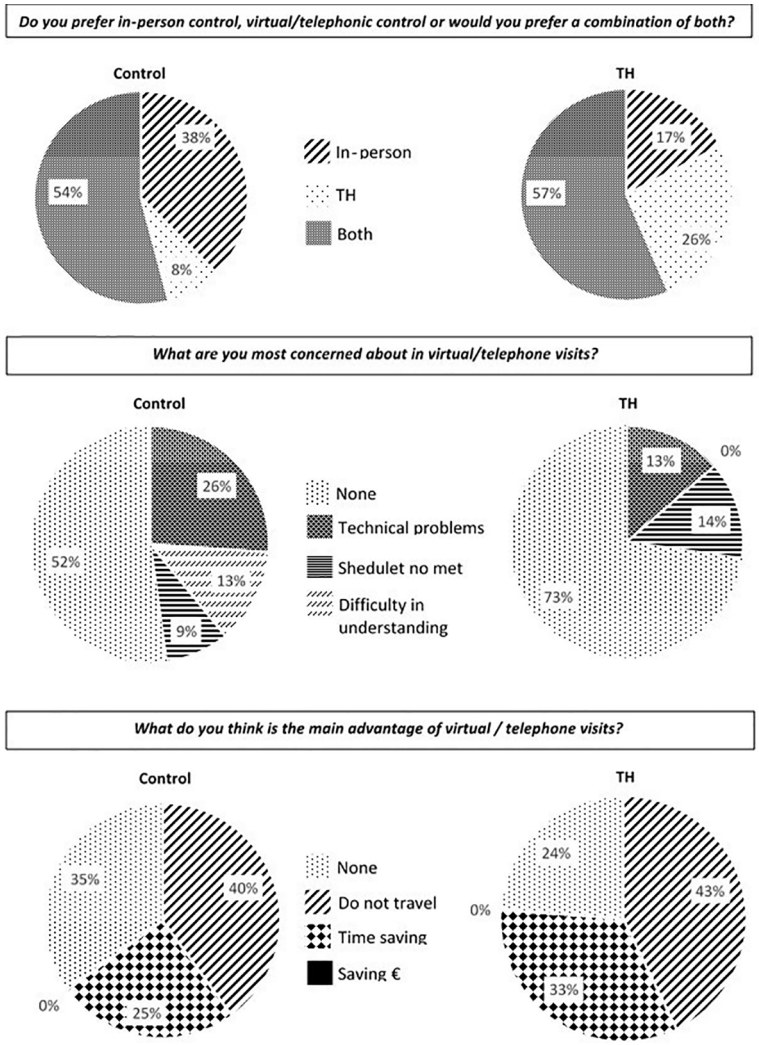

FIGURE 2
Participant satisfaction. TH, telehealth.

studies (7, 11) but in some (20). Therefore, our study provides emerging evidence of HRQoL improvement with TH. The authors consider that the way TH is implemented may have a direct impact on its acceptance by participants. It seems that app that allow the physician to directly modify the treatment regimen, without the user having to make the changes on his or her own, may be more widely accepted.

Regarding TH acceptance, in the CoYoT1 pilot study, participants reported high levels of satisfaction with the virtual clinic compared to a traditional in-person visit through a nonstandardized satisfaction survey. However, the CoYoT1 pilot study only included young adults aged 18-25 with type 1 diabetes (10), who are generally more familiar with new technologies. Similarly, in the TELEDIABE study, all the participants in the teleconsultation group (age 36 ± 12 years) reported a high level of comfort, and the majority also reported an improvement in diabetes management (8). Our results indicate that TH acceptance could be extensible to older adults (age 52.5 ± 12.4 years in the intervention group), although this age range could be less comfortable with new technologies. The accessibility that the SocialDiabetes® app allows, as well as the lack of need to travel to the hospital and the time savings, seem to contribute to the good acceptability of telemedicine by persons with type 1 diabetes.

The major strength of the current investigation is the prospective and randomized design used to describe causality relations in the presented findings. However, our study had some limitations that deserve mentioning: due to the study design, the time expended for endocrinologists in the TH group was higher than that for endocrinologists in the control group (100 minutes vs. 90 minutes, excluding extra visits), so the time spent on medical care is not directly comparable, and the results obtained in this sense must be carefully interpreted; the follow-up period may be too short to achieve significant differences in direct costs of transportation and indirect costs for participants; it may not be long enough to confirm that the advantages of TH on some glucose metrics and participant satisfaction are maintained over time; and finally, the participant satisfaction questionnaire used in this study was not standardized.

To conclude, the TH model represents a safe and well-accepted alternative to conventional in-person visits for chronic care among adults with type 1 diabetes assisted in a rural area, which entails lower costs for affected individuals. It offers comparable care in terms of HbA_{1c} at 6 months of follow-up, with significant improvement in some glucose metrics (TIR, TAB) and in health-related quality of life (EsDQoL). We consider it necessary to develop a standardized recommendation of the most efficient timing of teleconsultations for chronic care of persons with type 1 diabetes. Further studies are necessary to evaluate a more efficient timing of the TH visits.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Hospital Universitari de Bellvitge (protocol Reference: PR040/20; 17/SEP/2020). The patients/participants provided their written informed consent to participate in this study.

Author contributions

SB researched the data and wrote the manuscript. JC contributed to the study concept and design, researched the data, analyzed and interpreted the data, wrote/reviewed/edited the manuscript, and supervised the study. YI reviewed/edited the manuscript. EC contributed to the discussion and reviewed/edited the manuscript. GL contributed to the discussion and reviewed/edited the manuscript. JP-B contributed to the discussion and reviewed/edited the manuscript. FC reviewed/edited the manuscript. HC reviewed/edited the manuscript. JF contributed to the discussion and reviewed/edited the manuscript. DB contributed to the study concept and design, researched the data, analyzed and interpreted the data, and reviewed/edited the manuscript. JC and DB are the guarantors of this work and, as such, had full access to all the data in this study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Effect of sodium-glucose transporter 2 inhibitors on sarcopenia in patients with type 2 diabetes mellitus: a systematic review and meta-analysis

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Objective: Sarcopenia has been recognized as the third category of disabling complications in patients with type 2 diabetes mellitus(T2DM), in addition to micro- and macrovascular complications. Sodium-glucose co-transporter 2 (SGLT2) inhibitors are innovative glucose-lowering treatments that have been shown to reduce body weight and enhance cardiovascular and renal outcomes. However, there is vigilance that SGLT2 inhibitors should be taken cautiously because they target skeletal muscle and may raise the risk of sarcopenia. Herein, we conducted a meta-analysis of randomized controlled trials to evaluate the effects of SGLT2 inhibitors on sarcopenia in patients with T2DM.

Method: Relevant studies were obtained from PubMed, Embase, Medicine, Cochrane, and Web of Science databases to determine eligible studies until February 2023, without any language restrictions. A random effects model was utilized irrespective of heterogeneity, and the I^2 statistic was used to evaluate study heterogeneity. The differences in results were measured using the weighted average difference (WMD) of the continuous data, along with a 95% confidence interval (CI).

Results: A total of 25 randomized controlled trials with 2,286 participants were included. SGLT2 inhibitors significantly reduced weight-related changes and fat-related changes, including body weight(BW) (WMD= -2.74, 95% CI: -3.26 to -2.23, P<0.01), body mass index(BMI) (WMD= -0.72, 95% CI: -0.95 to -0.49, P<0.01), waist circumference(WC) (WMD= -1.60, 95% CI: -2.99 to -0.22, P=0.02), fat mass (FM)(WMD= -1.49, 95% CI: -2.18 to -0.80, P<0.01), percentage body fat(PBF) (WMD= -1.28, 95% CI: -1.83 to -0.74, P<0.01), visceral fat area(VFA)(WMD= -19.52, 95% CI: -25.90 to -13.14, P<0.01), subcutaneous fat area(SFA)(WMD= -19.11, 95% CI: -31.18 to -7.03, P=0.002), In terms of muscle-related changes, lean mass(LM)(WMD= -0.80, 95% CI: -1.43 to -0.16, P=0.01), and skeletal muscle mass(SMM) (WMD= -0.38, 95% CI: -0.65 to -0.10, P=0.007), skeletal muscle index (SMI) (WMD= -0.12, 95% CI: -0.22 to -0.02, P=0.02)were also significantly reduced. In addition, body water likewise decreased significantly (WMD=-0.96, 95% CI: -1.68 to -0.23, P=0.009).

Conclusions: As one of the most widely used hypoglycemic, SGLT2 inhibitors have beneficial effects on FM and BW weight loss in T2DM, such as BW, BMI, WC, FM, PBF, VFA, and SFA. However, the negative influence on muscle mass paralleled the reduction in FM and BW, and the consequent increased risk of sarcopenia warrants high attention, especially as patients are already predisposed to physical frailty.

Clinical Trial Registration: https://www.crd.york.ac.uk/prospero/#myprospero, identifier PROSPERO (No.CRD 42023396278).

KEYWORDS

sodium-glucose cotransporter 2 inhibitors, sarcopenia, type 2 diabetes mellitus, muscle mass, meta-analysis

1 Introduction

Sarcopenia is a syndrome that is common in elderly populations and is defined by age-related muscle mass loss, muscle strength decreased, and/or poor physical performance, all of which lead to functional decline, disability, frailty, and falls (1).

The European Working Group on Sarcopenia in Older People updated the clinically relevant definition and established an agreement on sarcopenia's diagnostic standards in 2018, which encompass three main components: muscle quantity, muscle strength, and physical performance, and assessed by LM or SMM, assessed by hand grip strength, and assessed by gait speed or a short physical performance battery, respectively (2). Crucially, the guideline underscores that the reduction of SMM and LM represents a critical foundation for diagnosing sarcopenia in a clinical setting.

Moreover, Sarcopenia has been implicated as a serious consequence of T2DM (3). T2DM is a metabolic disorder characterized by insulin resistance, elevated advanced glycation end-products (AGEs), proinflammatory factors, and oxidative stress. These factors can disrupt normal cellular processes and result in microvascular and macrovascular complications, ultimately leading to cell death. As a result, individuals with T2DM may experience reductions in muscle mass, strength, and function, potentially precipitating the onset of sarcopenia (4). Kim et al (5) showed that patients with DM had a three times higher chance of developing sarcopenia than those without DM. Researchers and medics have been paying more attention to sarcopenia because of its serious impact on the quality of life of elderly patients and have therefore been recognized as the third category of disabling complications in patients with T2DM, in addition to micro- and macrovascular complications (6). DM is currently one of the most prevalent chronic non-communicable diseases globally, presently affects 537 million adults worldwide, and by 2045, it's expected to affect 783 million people (7). It is widely recognized that hypoglycemic medications are pivotal in treating T2DM. However, glucose-lowering drugs that target skeletal muscle have the potential to impact SMM and function in T2DM patients.

SGLT2 inhibitors are gaining attention as novel oral hypoglycemic agents due to their distinct mechanism of decreasing proximal tubular glucose reabsorption and increasing urine glucose excretion, which has been shown to lower body weight and improves cardiovascular and renal outcomes (8, 9). Based on these important pharmacological effects, SGLT2 inhibitors are included in international authoritative diabetes guidelines and are widely used in clinical practice (10). However, there are cautions about using SGLT2 inhibitors, as they may raise the incidence of sarcopenia, especially in senior T2DM patients. Currently available studies published in this context have yielded inconclusive results. Therefore, it is necessary to conduct a comprehensive systematic review and meta-analysis of randomized controlled trials (RCTs) to assess the effects of SGLT2 inhibitors on sarcopenia in T2DM patients, to ensure medication safety and enhance the general health of elderly patients.

2 Materials and methods

2.1 Study design and search strategy

This meta-analysis was carried out following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and was registered with PROSPERO (No. CRD 42023396278). We extensively examined the databases of PubMed, Embase, Medicine, Cochrane, and Web of Science for literature published before February 2023 using the following keywords: "Sodium-Glucose Transporter 2 Inhibitors", "dapagliflozin", "canagliflozin", "empagliflozin", "ipragliflozin", "luseogliflozin", "tofogliflozin", "ertugliflflozin", "sotagliflozin", "sarcopenia", "muscle mass", "skeletal muscle", "randomized controlled trials". Manual searches were conducted on all found articles. To find additional material, we manually searched the references of relevant papers.

2.2 Study selection

We screened articles according to the following inclusion and exclusion criteria: Inclusion criteria: 1) All participants enrolled in

the study were clinically diagnosed with T2DM and aged ≥18 years; 2) All chosen studies must be RCTs with SGLT2 inhibitors as the treatment and a placebo or another type of hypoglycemia medication as the control; 3) The outcomes should be sarcopenia relevant indicators, such as LM, SMM, SMI, gait speed, grip strength. Exclusion criteria: 1) studies with incomplete or inaccessible study data; 2) studies with unavailable primary outcome indicators; 3) duplicate literature studies; 4) non-RCT type research; and 5) experimental animal studies.

2.3 Data extraction and quality assessment

Study screening and data extraction from the relevant literature was carried out separately by two reviewers (ZS and WYD), when there were disagreements, a third researcher was consulted to reach a consensus. The following data were extracted:1) study characteristics (first author, publication year, country, intervention, sample size, follow-up time); 2) intervention characteristics (drug name, dose, duration of treatment, comparison, etc.); 3) primary outcome indicators (LM, SMM, SMI, gait speed, grip strength); and 4) secondary outcome indicators (BW, BMI, WC, FM, PBF, VFA and SFA.

According to the following seven criteria, the Cochrane Risk of Bias tool was used to evaluate the risk of bias: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome data, incomplete outcome data, selective reporting, and other biases. Each study was classified as a "low risk", "high risk" or "unclear risk" of bias.

2.4 Statistical analysis

The weight mean difference (WMD) with 95%CI was used to quantify the pooled effects for continuous variable outcomes. All statistical analyses were performed using the RevMan5.4 software. The degree of heterogeneity in studies was evaluated using the I^2 statistic. Studies with I^2 statistics between 25% and 50% were regarded as having low heterogeneity, studies with I^2 statistics between 50% and 75% as having moderate heterogeneity, and studies with I^2 statistics above 75% as having high heterogeneity. A random-effects model was used in all studies, followed by either subgroup or sensitivity analysis to explicate the source of heterogeneity. Publication bias was assessed using funnel plots. $P< 0.05$ was considered statistically significant.

3 Results

3.1 Study selection

A total of 462 articles were selected based on the search strategy, of which 98 duplicate studies were removed using EndNote 20 software, 242 studies were excluded based on their titles and abstracts, and 122 studies were evaluated further for full-text examination. 25 studies total were eventually included in the meta-analysis. The detailed process is shown in Figure 1.

3.2 Studies characteristics and quality assessment

The included 25 research characteristics are shown in Supplementary Table 1 (11–35). The intervention group consisted of a range of SGLT2 inhibitors, including dapagliflozin (ten studies), canagliflozin (five studies), empagliflozin (five studies), ipragliflozin (five studies), and tofogliflozin (one study). Meanwhile, the control groups received other hypoglycemic drugs, including metformin, glimepiride, pioglitazone, dipeptidyl peptidase-4 inhibitors (DPP-4 inhibitors), and Glucagon-like peptide-1 receptor agonists (GLP-1RAs). The follow-up period ranged from 8 to 104 weeks, with most studies lasting 24 weeks. Furthermore, all studies were high-quality parallel grouping studies according to the Cochrane Risk Bias Tool. As shown in Supplementary Figure 1.

3.3 Meta-analysis of outcomes

3.3.1 Weight-related changes: BW, BMI, WC

20 studies reported on changes in BW in a total of 1,644 participants, of which 831 were treated with SGLT2 inhibitors and 813 were not. The meta-analysis showed that patients treated with SGLT2 inhibitors experienced a significant decrease in body weight compared to the control group (WMD= -2.74, 95% CI: -3.26 to -2.23, $P<0.01$) (Figure 2A), with low heterogeneity among the studies (I^2 = 38%). 12 studies reported BMI, comprising 498 SGLT2 inhibitor users and 475 non-users. The results suggest that treatment with SGLT2 inhibitors resulted in a statistically significant decrease in BMI when compared to other drugs (WMD= -0.72, 95% CI: -0.95 to -0.49, $P<0.01$) (Figure 2B), and no heterogeneity existed between the studies

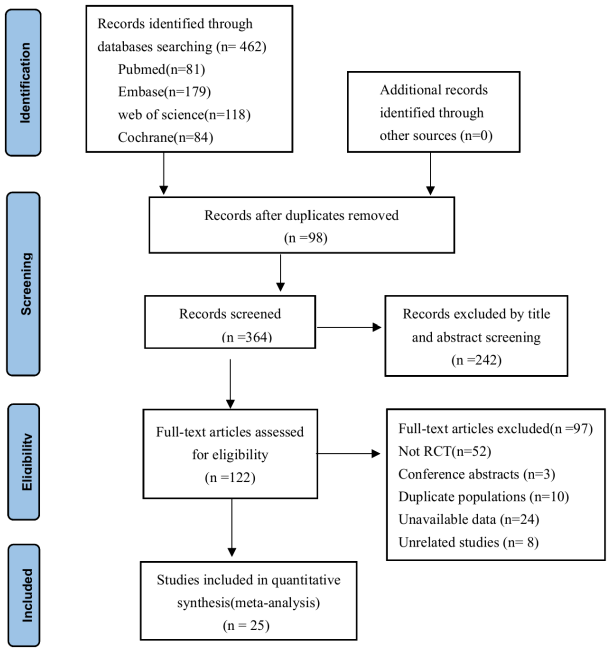

FIGURE 1
Flow chart of studies selected for the meta-analysis.

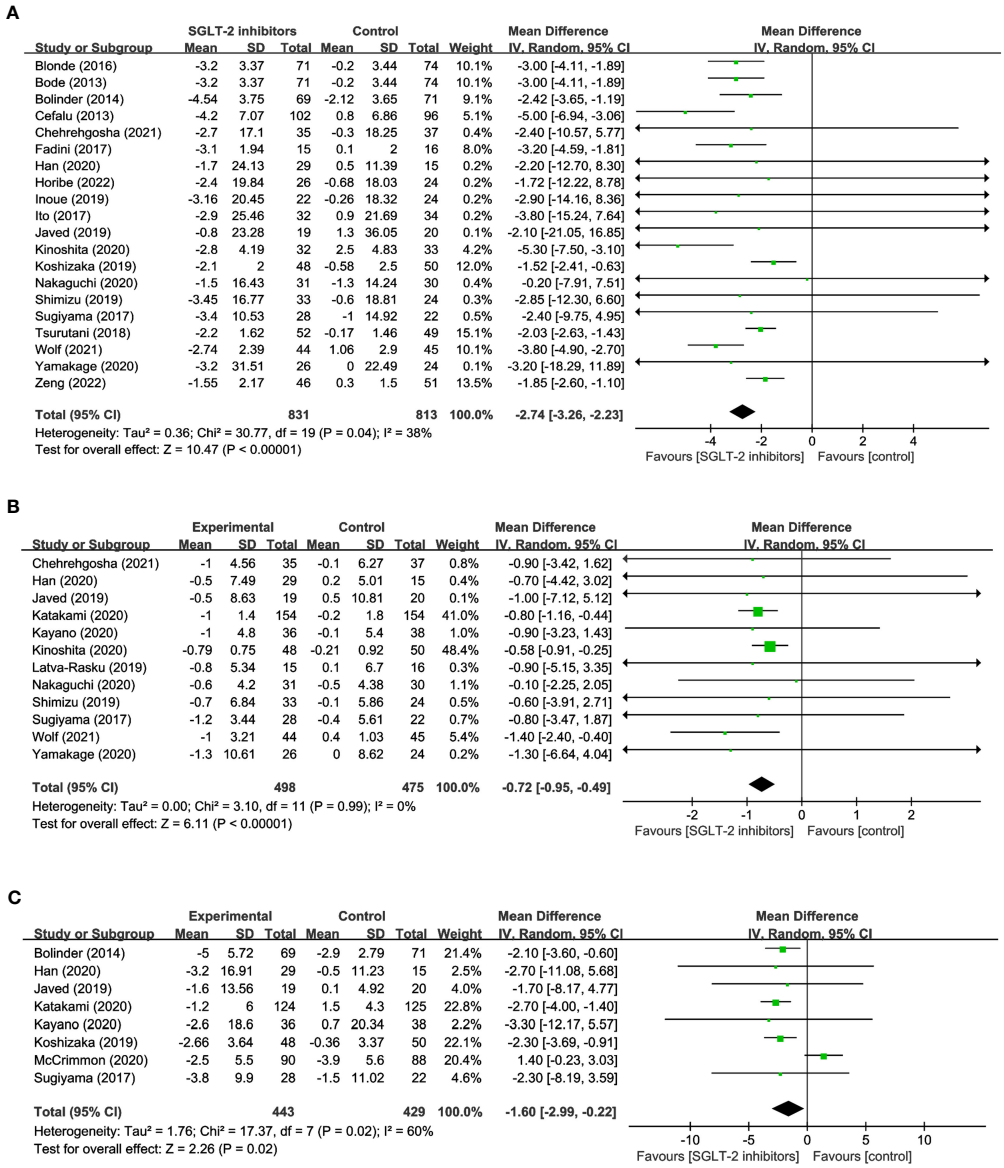

FIGURE 2
Forest plots of **(A)** BW, **(B)** BMI, and **(C)** WC.

(I^2 = 0%). 8 studies reported WC, with 443 using SGLT2 inhibitor and 429 non-use. In addition, when compared to the control group, patients in the SGLT2 inhibitor-treated group had a significantly smaller WC (WMD = -1.60, 95% CI: -2.99 to -0.22, P=0.02) (Figure 2C), however, there was considerable heterogeneity among the studies, (I^2 = 60%). These results offer crucial information about the efficacy of SGLT2 inhibitors in reducing weight and can aid in the development of evidence-based interventions for obesity management.

3.3.2 Fat-related changes: FM, PBF, VFA, SFA

13 studies involving 1,034 participants were analyzed in FM, with 526 using SGLT2 inhibitors and 508 non-users. And the results indicated that SGLT2 inhibitors significantly reduced FM when compared to other antihyperglycemic drugs (WMD = -1.49, 95% CI: -2.18 to -0.80, P<0.01) (Figure 3A), albeit with moderate heterogeneity (I^2 = 47%). 8 studies explored the impact of SGLT2 inhibitors on PBF in 610 participants. Results suggested that SGLT2 inhibitors dramatically decreased PBF in comparison to the control group (WMD = -1.28, 95% CI: -1.83 to -0.74, P<0.01) (Figure 3B), with no observed heterogeneity (I^2 = 0%). 9 studies were identified that reported measuring VFA in a total of 488 individuals, with 227 using SGLT2 inhibitors and 261 non-users. The findings indicated that SGLT2 inhibitors greatly decreased VFA compared to other anti-glycemic drugs (WMD= -19.52, 95% CI: -25.90 to -13.14, P<0.01) (Figure 3C), with no heterogeneity among the studies (I^2 = 0%). Besides, the effects of SGLT2 inhibitors on SFA were evaluated in 7 trials in 210 SGLT2 inhibitor users and 191 non-

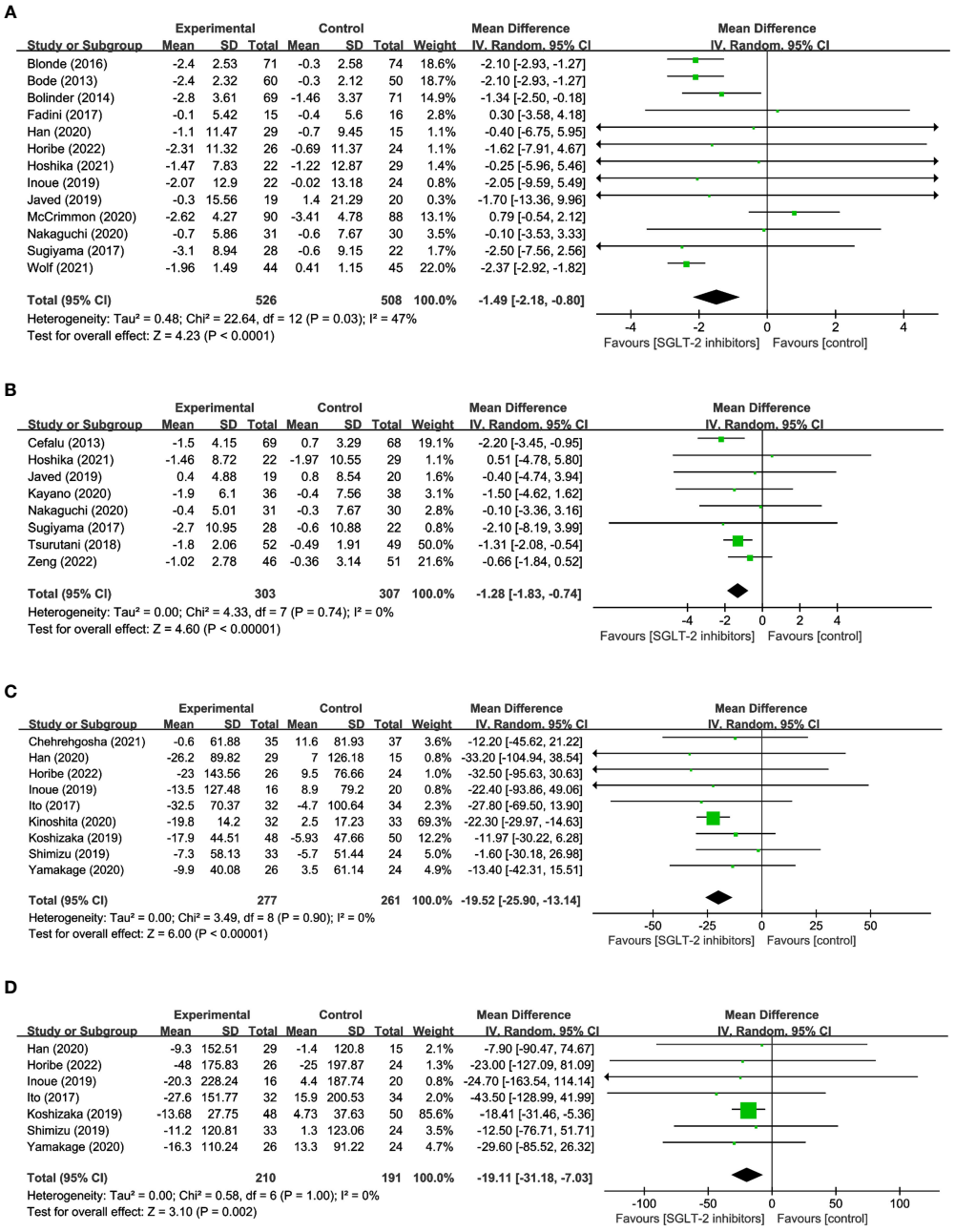

FIGURE 3
Forest plots of **(A)** FM, **(B)** PBF, **(C)** VFA, and **(D)** SFA.

users. The outcomes additionally demonstrated that SGLT2 inhibitors markedly decreased SFA more than the control group (WMD = -19.11, 95% CI: -31.18 to -7.03, P=0.002) (Figure 3D), with no heterogeneity between the studies (I^2 = 0%). The aforementioned findings indicate that SGLT2 inhibitors may be a more efficient alternative for managing fat-related alterations in people with hyperglycemia, as they have demonstrated efficacy in reducing FM, PBF, VFA, and SFA. These results suggest that SGLT2 inhibitors could be a viable option for managing metabolic complications associated with hyperglycemia-related conditions.

3.3.3 Muscle-related changes: LM, SMM, SMI

12 studies were conducted to assess the effects of SGLT2 inhibitors on LM using DXA involved in 1,101 participants. The overall analysis indicated a significant reduction in LM with SGLT2 inhibitors compared to other antihyperglycemic drugs (WMD= -0.80, 95% CI: -1.43 to -0.16, P=0.01) (Figure 4A), with a moderate degree of heterogeneity observed among the studies (I^2 = 65%). Similarly, 12 studies involving 340 SGLT2 inhibitor users and 337 non-users were evaluated for SMM, and the results revealed a significant reduction in SMM with SGLT2 inhibitors compared to other antihyperglycemic drugs (WMD = -0.38, 95% CI: -0.65 to -0.10, P=0.007) (Figure 4B),

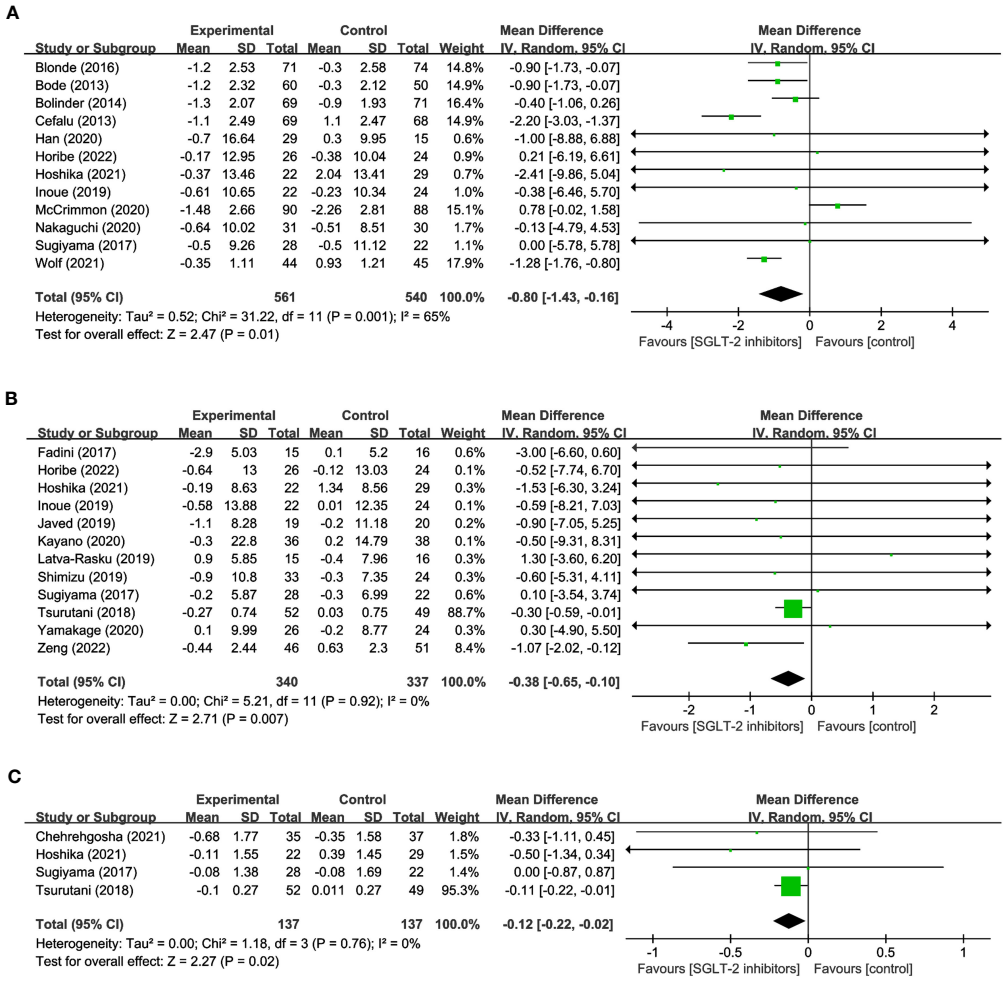

FIGURE 4
Forest plots of **(A)** LM, **(B)** SMM, and **(C)** SMI.

with no heterogeneity observed (I^2 = 0). Furthermore, 4 studies including 137 SGLT2 inhibitor users and 137 non-users were analyzed to assess SMI using BIA, and the results indicated a significant reduction in SMI with SGLT2 inhibitors compared to other antihyperglycemic drugs (WMD= -0.12, 95% CI: -0.22 to -0.02, P=0.02) (Figure 4C), and no heterogeneity was found among the studies (I^2 = 0). These findings imply that SGLT2 inhibitors may negatively impact LM, SMM, and SMI, and should be considered when developing treatment plans for individuals with hyperglycemia-related conditions.

3.3.4 Fluid-related changes: body water

6 studies evaluated s body water in 161 SGLT2 inhibitor users and 164 non-users. The results revealed a significant reduction in body water with SGLT2 inhibitors compared to other hypoglycemic drugs (WMD = -0.96, 95% CI: -1.68 to -0.23, P=0.009) (Figure 5),

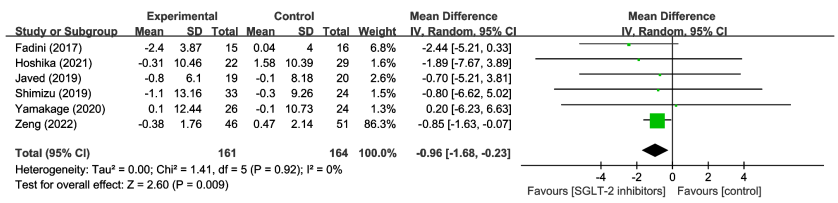

FIGURE 5
Forest plot of body water.

with no heterogeneity observed ($I^2 = 0$). The results imply that it is critical to take into account the potential loss of body fluids when using SGLT2 inhibitors.

3.4 Sensitivity analysis and subgroup analysis

Sensitivity analyses were carried out to identify the causes of heterogeneity. When McCrimmon's study was removed, the heterogeneity in terms of WC, FM, and LM was significantly decreased. The findings indicated that SGLT2 inhibitors significantly reduced WC (WMD= -2.39, 95% CI: -3.17, -1.61, P<0.01), FM (WMD = -2.08, 95% CI: -2.46, -1.71, P<0.01), and LM (WMD = -1.10, 95% CI: -1.50, -0.70, P<0.01), with lower effects than GLP-1RAs, but the differences were not statistically significant WC (WMD = 1.40, 95% CI: -0.23, 3.03, P=0.09) (Figure 6A), FM (WMD= 0.79,95% CI: -0.54, 2.12, P=0.25) (Figure 6B), and LM (WMD = 0.78, 95% CI: -0.02, 1.58, P=0.06) (Figure 6C). Due to low heterogeneity, other outcomes including BW, BMI, SMM, SMI, VFA, SFA, PBF, and Body water were not tested further.

3.5 Publication bias

Publication bias was assessed using funnel plots (Figure 7), which showed that the scatter points pertaining to each study were mainly dispersed on the midline or largely symmetrically distributed.

4 Discussion

We demonstrated that SGLT-2 inhibitors may increase the risk of sarcopenia in diabetic patients. As we found, in addition to greatly lowering BW and FM in T2DM patients, SGLT2 inhibitors also significantly lowered LM, SMM, and SMI and consequently increased the risk of sarcopenia.

There exist multiple bidirectional relationships between T2DM and sarcopenia, whereby the presence of one condition may elevate the likelihood of developing the other and make it a significant public health concern (6). T2DM represents a state of accelerated metabolic aging, and a portion of its associated frailty risk may stem from an escalated decline in muscle mass and function. Decrease in muscle mass and function, which are linked to reduced muscle strength and endurance, also lead to a higher risk of falls and physical frailty (36, 37). Consequently, Elderly diabetics with combined sarcopenia experience more pronounced metabolic abnormalities, suboptimal nutritional status, and increased susceptibility to developing osteoporosis and falls, which decrease quality of life and increase mortality (38). Hence, the<<Guideline for the management of diabetes mellitus in the elderly in China (2021 edition)>>recommends healthcare practitioners promptly evaluate sarcopenia in all older patients with diabetes (39).

SGLT2 inhibitors are novel antihyperglycemic drugs that decrease proximal tubular glucose reabsorption, which raises urine sugar excretion and lowers blood glucose levels. These drugs have received high attention due to their glucose-dependent mechanisms of action, and pose a low risk of hypoglycemia, particularly when used without insulin or sulphonylureas (40). Importantly, SGLT2 inhibitors also reduce body weight, blood pressure, urine protein, and uric acid, and improve adipocyte dysfunction in visceral adipose tissue, resulting in lower leptin, vastatins, fibrinogen activator inhibitor-1, and higher lipocalin levels, effectively promoting lipolysis and reducing visceral fat, thereby achieving a cardiovascular benefit (41, 42). To sum up, SGLT2 inhibitors are recommended by the guidelines for the following chronic diseases: diabetes, obesity, cardiovascular disease, and kidney disease. However, Sarcopenia may be a major concern and the most significant barrier to SGLT2 inhibitor use (43). SGLT2 inhibitor-promoted activation of gluconeogenesis resulting from the decrease in insulin levels and increase in glucagon levels, which may lead to lipolysis in adipose tissue and proteolysis in skeletal muscle, could supply amino acids to the liver and potentially contribute to sarcopenia (44). Clinical studies of sarcopenia caused by SGLT2 inhibitors in T2DM patients have been published. Typically, Nagai Y. et al. found that ipragliflozin reduced the weight of FM and the LM (45). Conversely, other studies have shown that dapagliflozin dramatically decreased FM but had no impact on lean tissue mass (46). Currently, it is unknown whether SGLT2 inhibitors exert a deleterious impact on sarcopenia. Thus, it is crucial to evaluate the effect of SGLT2 inhibitors on sarcopenia in T2DM patients. Previous studies have indicated that sarcopenia involves a complex interplay of metabolic dysregulation, insulin resistance, fat infiltration, fibrosis, and neural activity. Interestingly, there is considerable overlap in the etiology of sarcopenia, obesity, and T2DM, with obesity-related insulin resistance being one of the primary pathogenic mechanisms underlying T2DM and potentially contributing to sarcopenia's underlying mechanisms (47, 48). Parallel to insulin resistance, fat infiltration contributes significantly to impairments in muscle quality and function. Thus, weight loss is a crucial goal in the management of obesity-associated chronic metabolic diseases, including T2DM, and pharmacological interventions that promote weight loss are attractive and feasible. Previous research has shown that SGLT2 inhibitors help with both BW and FM weight loss, with Kawata T et al. estimating that body fat accounts for 50% to 75% of SGLT2-induced weight loss (49). While BMI and WC represent quick, convenient, and reliable measures of obesity, they are relatively broad indicators that do not account for fat distribution and thus have limited helpfulness in predicting the risk of T2DM and sarcopenia (50). To further our understanding of sarcopenia in T2DM patients, this meta-analysis employs multiple body composition measurements including FM, BFM, VAT, and SAT, and confirmed the role of SGLT2 inhibitors in reducing BW, BMI, WC, FM, and BFM, which agreed with the results of earlier researches. In addition to reducing FM, SGLT2 inhibitors decrease VAT and SAT. Although the exact mechanism by which SGLT2 inhibitors reduce adipose tissue is unknown, some studies have shown that they promote a metabolic switch from carbohydrate oxidation to fatty acid oxidation, boosting the liver's and adipose tissues' fatty acid oxidation to potentially prevent lipid accumulation (43). Furthermore, they facilitate energy loss through a sustained increase in glucose excretion in urine, which may boost

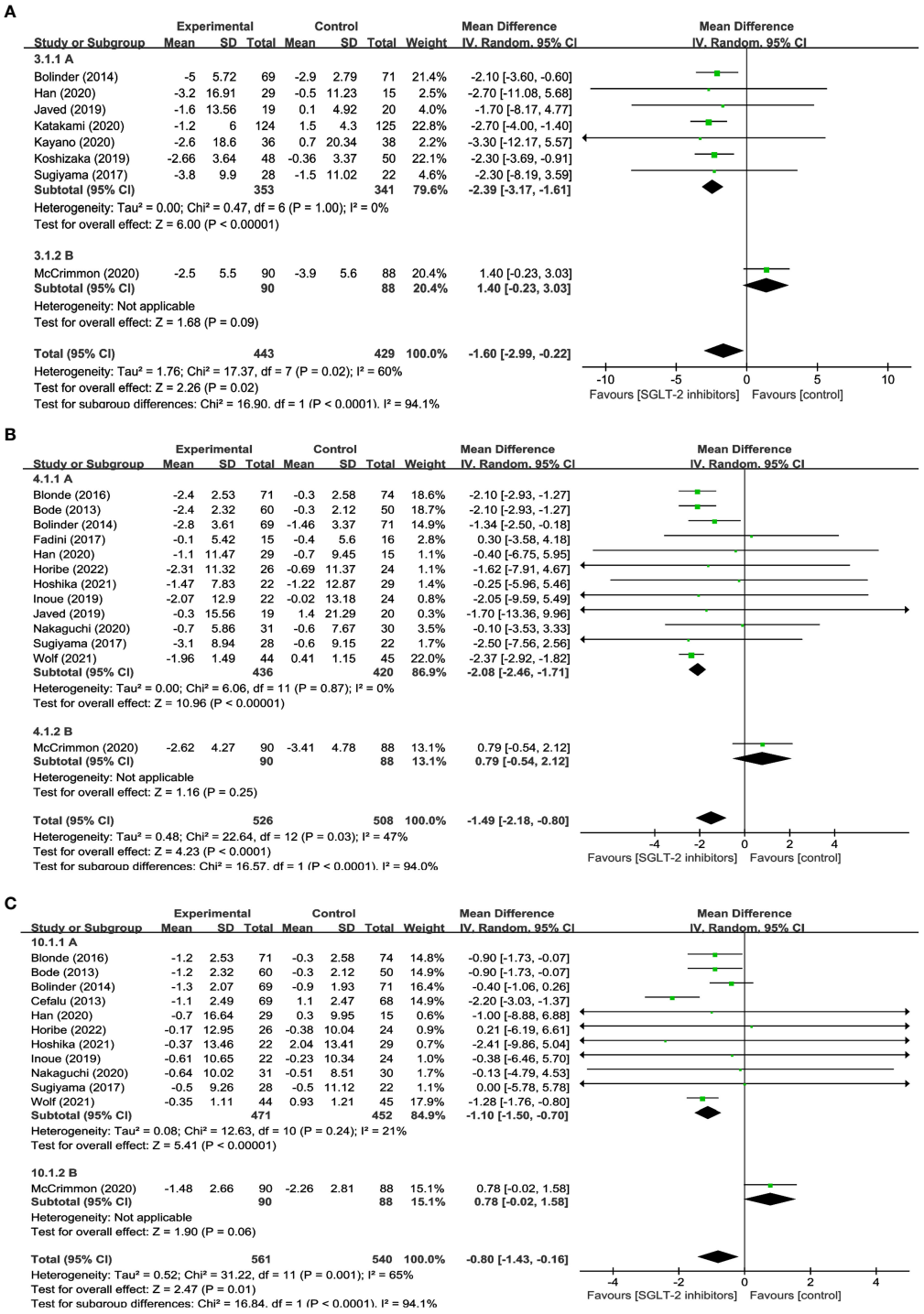

FIGURE 6
Subgroup analysis of **(A)** WC, **(B)** FM, and **(C)** LM.

b-oxidation in the liver and visceral fat, enhance liver fat metabolism, and decrease VAT and SAT levels (51). In addition, our meta-analysis revealed that SGLT2 inhibitors dramatically enhanced body water loss in comparison to other conventional glucose-lowering treatments, which prior meta-analyses had not mentioned (52, 53). It could be explained because the unique hypoglycemic mechanism of SGLT2 inhibitors through urinary glucose excretion takes away some water while excreting sugar, which may contribute to weight loss. However, it is worth mentioning that studies also have reported instances of ketosis and euglycemic ketoacidosis caused by dehydration and insulinogenic during the use of SGLT2 inhibitors (54). As a potentially severe adverse reaction, ketosis demands our utmost attention when using SGLT2 inhibitors.

Some research has shown that using SGLT2 inhibitors reduces LM along with weight reduction. Outstandingly, Bolinder J et al. found that

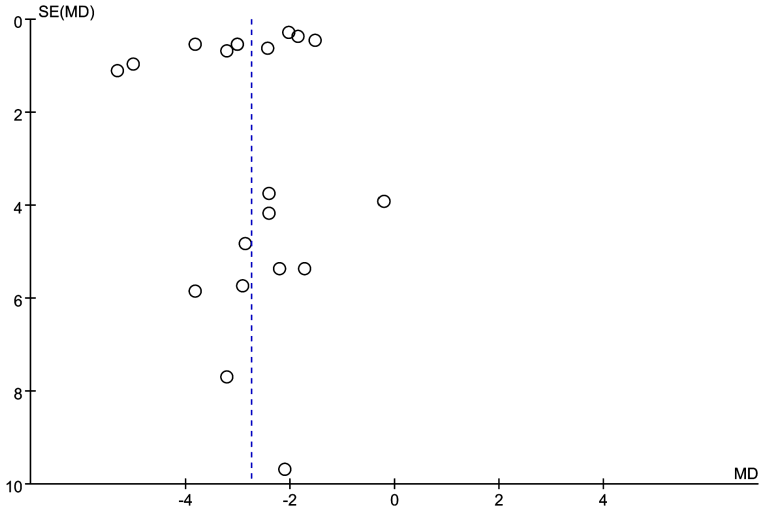

FIGURE 7
Funnel plot of BW.

approximately 2/3 of the weight loss brought on by SGLT2 inhibitors was responsible for a decrease in FM, while a decrease in LM was responsible for the remaining 1/3 (55). Our meta-analysis confirmed that SGLT2 inhibitors considerably reduced both LM and SMM when compared to other traditional hypoglycemic therapies, which was in line with the conclusions of previous studies. In this meta-analysis, we analyzed 12 studies that reported changes in BW and LM, and the loss of LM accounted for between 10% and 40% of the BW lost, with an average of around 30%, roughly consistent with Bolinder J's conclusion. Considering the subtle differences between LM and SMM, LM was measured by Dual-energy x-ray absorptiometry, which includes muscle, organs, and body water, whereas SMM was measured by bioelectrical impedance analysis. Our meta-analysis further assessed the BW due to SMM, interestingly, it found that SMM accounted for around 1/3 of weight loss, and the reduction in SMM accounts for a non-negligible proportion of the weight loss with SGLT2 inhibitors. Furthermore, we also verified the benefits of SGLT2 inhibitors in lowering SMI that were not previously included in meta-analyses (52). It is worth mentioning that SMI, which is defined as SMM/height^2 or SMM/BMI, is an underappreciated evaluation metric in sarcopenia research. As stated previously, although total body SMM or LM measurements can be used to estimate muscle quantity, the relationship between muscle mass and body size is crucial; people with larger frames often have greater muscle mass, so SMM can be adjusted for body size, such as using height squared (SMM/height^2) (2). In comparison, SMI may be a stronger predictor of sarcopenia in T2DM patients than SMM or LM and should be viewed as a crucial outcome metric in upcoming clinical studies. In conclusion, as one of the most widely used glucose-lowering drugs, although it brings many positive benefits, the potential LM and SMM loss linked to SGLT2 inhibitors-induced weight loss warrants attention. A faster decrease in skeletal muscle and the concomitant rise in the risk of sarcopenia is concerning, especially because those who receive these medicines are frequently already at a higher risk of physical frailty (56). Therefore, we believe it is critical to implement some strategies to protect skeletal muscle while using SGLT2 inhibitors, such as improved nutrition and resistance training (57). It is essential to emphasize that skeletal muscle absolute mass is not the sole component to consider; skeletal muscle strength and physical performance remain critical to performance in the presence of sarcopenia and have an impact on an individual's quality of life. Nevertheless, only one study evaluating the grip strength of SGLT2 inhibitors *vs* other glucose-lowering medications was included in this meta-analysis, more research into the effects of SGLT2 inhibitors on skeletal muscle strength and athletic performance is required.

Both SGLT2 inhibitors and GLP-1RAs have displayed positive effects on body composition measurements including FM, WC, and LM. Within this meta-analysis, semaglutide has demonstrated superiority in reducing FM and WC when compared to SGLT2 inhibitors, however, the difference was not statistically significant. It is noteworthy that semaglutide exhibited a greater reduction in LM than SGLT2 inhibitors, although there was no statistically significant difference in LM reduction between the two medications. As with other GLP-1RAs, liraglutide also reduces LM in patients with T2DM, although it does not confer any additional advantage over SGLT2 inhibitors in this regard. This meta-analysis includes only two studies that compare the body composition of GLP-1RAs and SGLT2 inhibitors, and the differences between these two drugs are currently unclear. However, the potential negative consequences of LM induced by GLP-1RAs and SGLT2 is warrants attention. Further research is necessary to comprehensively evaluate the differences in body composition changes resulting from the use of these drugs.

The highlight of this meta-analysis was the comprehensive evaluation of the effects of SGLT2 inhibitors on T2DM patients regarding body composition, not only the positive of weight loss, such as BW, BMI, WC, FM, VFA, SFA, but also the negative influence on muscle mass, and consequent increased risk of sarcopenia. However, the followings are this article's limitations: First, the sample size of the few RCTs that did meet the criteria was small. Second, the majority of these studies only had 24-week follow-up

durations, the long-term effects of the SGLT2 inhibitors are also unknown, necessitating ongoing monitoring. Third, due to limited data, only one major indicator of muscle mass was included in this article on sarcopenia; additional RCTs are required to further validate the influence of SGLT2 inhibitors on skeletal muscle strength and physical performance in sarcopenia.

5 Conclusion

SGLT2 inhibitors have positive effects on weight loss in T2DM, including BW, BMI, WC, FM, VFA, and SFA, and the SGLT2 inhibitors therapy results in weight loss that is predominantly derived from FM. However, the negative influence on muscle mass is parallel to the reduction in FM and BW, and the consequent increased risk of sarcopenia is noteworthy, especially as patients are already predisposed to physical frailty. Therefore, SGLT2 inhibitors as one of the most widely used hypoglycemic agents should be considered for both benefits on weight loss and harmful muscle reduction of sarcopenia. It is imperative to conduct large-sample and long-term follow-up studies to better understand the risk of sarcopenia and explore strategies for preserving lean mass and improving physical function.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

The study was conceived and designed by SZ and DZ. The literature search, data extraction, and statistical analysis were carried out by SZ, ZQ, DS, and YW. SZ write the original draft, while DZ, YW, DS, and DZ severely review and edited it. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1203666/full#supplementary-material

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Low-dose esketamine with sufentanil for postcesarean analgesia in women with gestational diabetes mellitus: a prospective, randomized, double-blind study

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Background: Pregnant women with gestational diabetes mellitus (GDM) require more analgesics after cesarean delivery than those who do not have GDM. Uncontrolled pain following cesarean delivery is a major problem in women with GDM. We investigate the efficacy of low-dose esketamine combined with sufentanil intravenous patient-controlled analgesia (PCA)for postcesarean analgesia in women with GDM.

Methods: One hundred forty pregnant women with GDM were enrolled participate in this randomized controlled trial and were randomized into two groups (70 in each group). The esketamine (S) group was given esketamine +sufentanil + ondansetron, and the control (C) group was given sufentanil +ondansetron. The primary outcome is sufentanil consumption at 24 hours postoperatively, the secondary outcomes are sufentanil consumption at 6 hours postoperatively, pain scores at 6, 24 and 48 hours postoperatively.

Results: Compared with group C, group S had significantly lower sufentanil consumption at 6 and 24 hours postoperatively (P= 0.049 and P<0.001), significantly lower activities VAS(pain during activities)scores at 6 hours postoperatively, rest and activities VAS (pain at rest and pain during activities) scores at 24 hours postoperatively, and activities VAS scores at 48 hours postoperatively(P=0.022, P =0.002, P=0.001 and P=0.007). Compared to group C, the time to bowel function return was significantly shorter in group S. There was no significant difference in rest VAS (pain at rest) scores at 6 and 48 hours postoperatively (P>0.05). The time to first lactation was not significantly different between the two groups (P>0.05). There was no significant difference in neonatal neurobehavioral scores between the two groups (P>0.05).

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Conclusion: Compared to sufentanil PCA, adding low dose of esketamine significantly reduced the consumption of sufentanil while providing equally effective post cesarean analgesia in the patients with gestational diabetes.

KEYWORDS

gestational diabetes, cesarean delivery, intravenous patient-controlled analgesia, esketamine, prospective study

1 Background

GDM is a common complication of pregnancy, and the incidence of GDM rapidly increased in recent years (1). Pregnant women with GDM tend to deliver by cesarean section, and despite the short duration of hyperglycemia in GDM patients, it is still a risk factor for postcesarean pain (2), some studies have found that postcesarean pain is more severe in women with GDM than in those without GDM, and postoperative pain requires more sufentanil to achieve satisfactory analgesia after surgery (3, 4). Clinicians should focus on postoperative analgesic management in patients with GDM to improve the effectiveness of postoperative analgesia.

Esketamine is a noncompetitive antagonist of the N-methyl-D-aspartate receptor (NMDA receptor), which binds to the NMDA receptor and blocks the NMDA receptor from binding to glutamate to exert analgesic and antinociceptive effects (5). In clinical trials (6), esketamine was used as an adjuvant for postoperative pain control, reducing opioid consumption and prolonging the duration of analgesic action.Some studies (7, 8) found that GDM and the onset of postpartum depression are related, that inflammatory pathways are involved in the pathogenesis of GDM-related postpartum depression, and that intravenous patient-controlled esketamine has a preventative effect on postpartum depression (9). Suppa et al. (10) suggested that preoperative use of low-dose esketamine is safe for women, and that intravenous use of low-dose esketamine after cesarean section can reduce maternal morphine use after lumbar neuraxial anesthesia for cesarean section. Esketamine has no adverse effects on uterine blood flow, maternal or fetal hemodynamics (11). Thus esketamine is suitable for postcesarean analgesia in women with GDM. However, more clinical data are still needed to confirm this idea.

We hypothesized that esketamine might produce better analgesia and higher patient satisfaction than conventional anesthetics after cesarean section in women with GDM. To test our hypothesis, we designed this prospective randomized controlled study to evaluate the effect of low-dose esketamine with sufentanil for intravenous patient-controlled analgesia as an analgesic regimen in GDM women.

2 Materials and methods

2.1 Study design

This prospective study was approved by the Ethics Committee of Hunan Provincial Maternal and Child Health Hospital [No. 2020-S068], and the study participants signed the informed consent form and was conducted between March 1, 2021 and December, 2022.

2.2 Population selection criteria

Full-term singleton pregnancies with GDM (those with ASA scores of I-II) were included. Exclusion criteria: (1) patients with ASA scores of III and above; (2) patients with contraindications to neuraxial anesthesia; (3) patients with a history of opioid dependence; (4) patients who were diagnosed as having diabetes mellitus before pregnancy. Withdrawal criteria: (1) any clinical adverse event; (2) severe pain; (3) pregnant woman or family member who were unwilling to complete the study.

2.3 Interventions

We screened 150 patients with GDM undergoing elective cesarean section, and then 140 patients were enrolled based on inclusion, exclusion and withdrawal criteria. They were randomly divided into the esketamine (S) group, esketamine 0.5 mg/kg + sufentanil 150ug + ondansetron 4mg and the control (C) group, sufentanil 150ug + ondansetron 4mg, with 70 in each group.

Maternal blood glucose was checked on the morning of the cesarean section. Maternal age, height, weight, gestational week and cesarean section data were recorded. The patients fasted from solid foods for >6 hours and from alcohol for 2 hours before the operation, and no premedication was administrated before the surgery. Patients were educated how to use the PCA pump (FORNIA pump model CPE-101, Zhuhai California Medical Equipment Co., Ltd.) and how to assess pain using a Visual Analog Scale (VAS) before surgery.

After entering the operation room, noninvasive blood pressure (NBP), electrocardiogram (ECG), HR, and SPO2 were standard monitored. Oxygen was administered via nasal catheter at 2L/min, compound sodium chloride was injected. The anesthesiologist adjusted the infusion rate according to the patient's circulatory status. All patients were placed in the left decubitus position, received CSEA at L3-4 space using the needle-through-needle technique. After the epidural space was identified, a spinal needle was used to puncture the dura mater and enter the subarachnoid space, with 15mg ropivacaine was diluted with cerebrospinal fluid to 2 ml for intrathecal injection, and the epidural catheter was

immediately inserted cephalad 3–4 cm. The patients were positioned supine and tilted 15° to the left until fetal extraction. The operation was started when T6 was blocked, and additional ropivacaine can be added to the epidural if necessary. The mean intraoperative blood pressure was maintained at ≥65 mmHg, and if the mean blood pressure fell below 65 mmHg, methoxamine was administered and repeated as needed; in addition, in the case of sinus bradycardia (heart rate <50 bpm), 0.3 mg of intravenous atropine was administered and repeated as needed. After delivery, flurbiprofen ester 50 mg and ondansetron 4 mg are administered intravenously. The epidural catheter was removed at the end of the procedure. The duration of the procedure and the blood loss were recorded. After surgery, use PCA pump. Group S received esketamine 0.5mg/kg + sufentanil 150μg + ondansetron 4mg, and group C received sufentanil 150μg + ondansetron 4 mg, both were diluted to 100ml with saline, the sufentanil concentration was1.5ug/mL, the maintenance dose was 2ml/h, PCA bolus 2 ml at lock out interval of 15 minutes. If the VAS score was ≥7, flurbiprofen was readministered and the patient was excluded from the group. Research assistant who was blinded to the randomization recorded the patients' PCA pump use (sufentanil consumption), and any adverse effects, such as nausea, vomiting, dizziness, and hallucinatory symptoms. Pain was assessed using the score VAS at 6, 24, and 48 hours postoperatively, with "0 " indicating no pain and "10 " indicating the most severe pain imaginable. The time to bowel function return, time to first lactation, and neonatal neurobehavioral scores were recorded.

2.4 Outcomes

The primary outcome was sufentanil consumption at 24 hours postoperatively, and secondary outcomes were sufentanil consumption at 6 hours postoperatively, VAS scores at rest and during activities at 6, 24, and 48 hours postoperatively, time to bowel function return(the time to pass flatus as the sign of bowel function return), time to first lactation, adverse effects such as postoperative nausea, vomiting, dizziness, and hallucinations, and neonatal neurobehavioral scores.

2.5 Sample size

The primary endpoint of this study was the consumption of sufentanil at 24 hours after surgery. We used a two-tailed test with $\alpha = 0.05$ and $\beta = 0.1$ based on the pre-experimental 24-hour postoperative sufentanil consumption in groups S (95.1 ± 5.5) and C (99.3 ± 7.0), with a minimum of 60 patients required in each group. Ultimately, each group included 75 patients, taking into account a 20% attrition rate.

2.6 Randomization and blinding

The investigators used the SAS statistical software package on a computer to generate random numbers in a 1:1 ratio to determine groups S and C. Neither the blinded investigators (the surgeon and the anesthesia resident physician) nor the patients were aware of the study groupings. Before the patients entered the operating room, the attending anesthetist opened the envelope, and the patients were assigned to group S or C according to the randomization entry number. Another anesthetist resident physician did not know the group assignment, and performed the subsequent anesthesia and the postoperative follow-up. A CONSORT diagram shows the participant flow (Figure 1).

2.7 Statistical analysis

SPSS 25.0 was used for the statistical analysis, and a P value < 0.05 was considered statistically significant. Continuous variables were analyzed with the Mann-Whitney U test or independent samples t test, and the Kolmogorov-Smirnov test was performed first to confirm whether the data were normally distributed. Normally distributed measurements are expressed as the mean ± standard deviation, and non-normally distributed variables are expressed as the median (interquartile range). Categorical variables were compared using the chi-square test or Fisher's exact test and expressed as percentages. Statistical analyses were performed using SPSS software version 25.0. Two-sided p values less than 0.05 were considered statistically significant.

3 Results

A total of 150 women with GDM were screened in this study from March 2021 to December 2022. Based on exclusion criteria, 10 women with GDM were excluded prior to randomization (6 with GDM combined with gestational hypertension, 2 with contraindications to neuraxial anesthesia, and 2 with a prepregnancy diagnosis of diabetes mellitus). A total of 140 women with GDM were included in the study, 70 in each group (Figure 1).

3.1 Demographic data

There were no significant differences in the basic information of the two groups, including age, height, weight, gestational week, number of cesarean deliveries, preoperative glucose, and duration of surgery (Table 1).

3.2 Comparison of the postoperative analgesic effects between groups

Compared with group C, sufentanil consumption was significantly reduced in group S at 6 and 24 hours postoperatively (P=0.049 and P<0.001),

The activities VAS scores at 6 hours, the rest and activities VAS scores at 24 hours, the activities VAS scores at 48 hours were significantly lower in group S (P=0.022, P=0.002, P=0.001 and

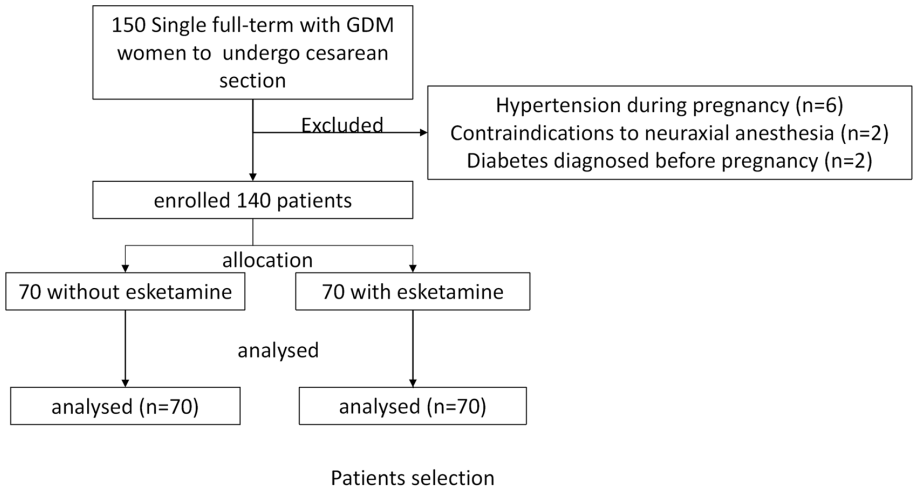

FIGURE 1
Flow chart of participants.

P=0.007), with no significant difference in rest VAS scores at 6 hours and at 48 hours postoperatively (Table 2).

3.3 Comparison of the postoperative adverse effects between groups

Compared with group C, the time to bowel function return (P=0.031) was significantly shorter in group S, and there was no significant difference in the time to first lactation (Table 3). There was no significant difference in the neonatal neurobehavioral scores between the two groups (Table 3).

There was no significant difference in the postoperative adverse effects (nausea, vomiting, and dizziness) between the two groups (Table 3).

4 Discussion

This randomized controlled trial evaluated the postoperative analgesic regimen for GDM women undergoing cesarean delivery. Our results showed that low-dose esketamine combined with sufentanil PCA in group S not only reduced the amount of sufentanil used at 6 and 24 hours postoperatively in GDM women undergoing cesarean delivery, but also significantly lowered the rest VAS scores at 24 hours postoperatively and significantly lowered the activities VAS scores at 6, 24, and 48 hours postoperatively compared with group C.

Many previous studies (12–14) have evaluated esketamine in combination with different opioids by different routes of administration for postoperative analgesia in different surgical types. Han et al. (9) studied the effect of intravenous patient-

TABLE 1 Demographic data.

Variables	Group C	Group S	P-value
	N=70	N=70	
Age, years	32.1 ± 3.9	31.7 ± 3.9	0.511
Duration, mins†	65 (55-80)	70 (60-80)	0.199
BMI, kg/m^2	28.4 ± 3.0	28.0 ± 2.8	0.349
Preoperative blood glucose, mmol/L†	5.4 (5.1-6.2)	5.3 (4.9-5.8)	0.459
Gestational week			0.512
37	17 (24.3%)	16 (22.9%)	
38	37 (52.9%)	32 (45.7%)	
39	16 (22.9%)	22 (31.4%)	
Nulliparous			0.175
yes	42 (60.0%)	34 (48.6%)	
no	28 (40.0%)	36 (51.4%)	

†median(Q1-Q3).

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TABLE 2 Comparison the postoperative analgesic effects between Groups.

Groups	Group C	Group S	P-value
	N=70	N=70	
Sufentanil consumption(P6H), ug†	24.0 (24.0-27.0)	24.0 (24.0-26.3)	0.049
Sufentanil consumption(P24H), ug†	93.0 (90.0-98.3)	84.0 (81.0-87.0)	<0.001
VAS Rest, P6H†	2.0 (2.0-2.8)	2.0 (2.0-2.0)	0.235
VAS Motion, P6H†	3.0 (3.0-3.0)	3.0 (3.0-3.0)	0.022
VAS Rest, P24H†	3.0 (3.0-4.0)	3.0 (2.0-3.0)	0.002
VAS Motion, P24H†	5.0 (5.0-6.0)	5.0 (4.0-5.0)	0.001
VAS Rest, P48H†	2.0 (2.0-3.0)	2.0 (2.0-2.0)	0.675
VAS Motion, P48H†	3.0 (3.0-4.0)	3.0 (3.0-3.0)	0.007

†Median(Q1-Q3); P6H, Postoperative 6h; P24H, Postoperative 24h; P48H, Postoperative 48h;VAS, Visual Analog Scale.

controlled analgesia with esketamine on postpartum depression and found that esketamine reduced postoperative pain after cesarean delivery. Another recent study found (15) that intraoperative intravenous administration of 0.25 mg/kg esketamine relieved pain during exercise 24 hours after cesarean delivery. In addition, a retrospective study by Ye Wang et al. (16) showed that esketamine controlled pain after cesarean delivery. Although the effectiveness of esketamine combined with opioids for postcesarean analgesia is well established, there are limited data on analgesia in women with GDM who have more severe postcesarean pain than non-GDM women, thus the effect of esketamine in women with GDM is unclear. Our study further evaluated the effect of low-dose

TABLE 3 Comparison the postoperative adverse effects between Groups.

Groups	Group C	Group S	P-value
	N=70	N=70	
NBNA scores(2 day) †	38 (37- 39)	38 (37- 39)	0.461
Postoperative blood glucose(4h), mmol/L	5.6 ± 0.8	5.4 ± 0.6	0.025
First ambulation, day†	2 (2-2)	2 (2-2)	0.319
First lactation, n (%)			0.627
<24h	15 (21.4)	16 (22.9)	
24-48h	43 (61.4)	46 (65.7)	
>48h	12 (17.1)	8 (11.4)	
Bowel function return time, n (%)			0.031
<24h	25 (35.7)	37 (52.9)	
24-48h	29 (41.4)	27 (38.6)	
>48h	16 (22.9)	6 (8.6)	
Nausea, n (%)			0.73
no	65 (92.9)	66 (94.3)	
yes	5 (7.1)	4 (5.7)	
Vomit, n (%)			1
no	67 (95.7)	67 (95.7)	
yes	3 (4.3)	3 (4.3)	
Dazzle, n (%)			1
no	68 (97.1)	68 (97.1)	
yes	2 (2.9)	2 (2.9)	

†Median(Q1-Q3); NBNA, neonatal neurobehavioral.

esketamine combined with sufentanil intravenous patient-controlled analgesia in GDM women with postcesarean analgesia. A dose of 0.5 mg/kg esketamine for PCA reduced the sufentanil dose, reduced pain scores, and improved analgesia in GDM patients, thereby reflecting the effectiveness of esketamine for pain control in such cases.

Esketamine, the dextroisomer of ketamine, has a higher affinity for NMDA receptors and U-opioid receptors and is used at only 1/2 the dose of ketamine. Previous studies (17) have shown that ketamine at 0.75 mg/kg-1.5 mg/kg did not provide analgesic effects in the biological phase during the postoperative period. Ketamine provided analgesia only at very high doses. One study reported (18), that the effect of small doses of esketamine on NMDA receptors was not considered analgesic but antinociceptive. Animal experiments (19) found that 10 mg/kg ketamine failed to induce an analgesic effect in rats; thus, the response to injurious stimuli was measured by nociceptometry, and although the ketamine dose did not have a sufficient direct anti-injurious effect, it effectively attenuated the development of acute tolerance to alfentanil and inhibited alfentanil-induced nociceptive hyperalgesia. In this experiment, esketamine was administered at a dose of 0.5 mg/kg in an intravenous patient-controlled analgesia pump at an infusion dose of 0.01 mg/kg/h. This dose was not sufficient to provide analgesic effects in the biological phase during the postoperative period, but it increased the analgesic effect while reducing the postoperative sufentanil consumption, which might have been related to its attenuation of opioid analgesic tolerance. Moreover, the effect of esketamine on pain-induced central sensitization might be related to its attenuation of opioid analgesia. The effect of esketamine on pain-induced central sensitization might also be a factor in the reduction in opioid demand. Furthermore, there is a large body of literature (20–22) showing that the use of low-dose ketamine during a cesarean section could enhance analgesia by antagonizing opioid-induced nociceptive sensitization, thereby reducing the use of postoperative morphine. This explains the low opioid requirement in the esketamine group. In addition, it was found that (23), esketamine has anti-inflammatory effects, inhibits pro-inflammatory cytokines, and enhances the production of anti-inflammatory mediators. GDM's pathological process is an inflammatory response, which worsens women's pain (24), the anti-inflammatory effect of esketamine, through the inhibition of inflammatory factors, can reduce the development of inflammation associated with GDM postoperatively, and may have a certain effect on the occurrence of pain. But future studies are needed to establish more precise correlations and conclusions regarding the interactions between these drugs.

The presence of hyperglycemia in gestational diabetes patients undergoing surgery had an adverse effect on gastrointestinal function, and this study showed that the proportion of mothers in group S with bowel function return times <24h and 24-48h was significantly higher than that in group C. This result may be related to the fact that esketamine effectively relieved the adverse stress caused by pain, and that esketamine reduced the amount of sufentanil, thus controlling the resulting gastrointestinal adverse effects and facilitating the recovery of bowel function after surgery. However, we need to monitor more indicators related to gastrointestinal function to corroborate this result, and the specific mechanism needs to be further investigated.

Postpartum lactation was mainly the result of the combined action of prolactin and lactogen, and the results of our study showed no significant difference in the time to the first lactation between the two groups. Consistent with the findings of a previous study (10) esketamine exposure did not decrease the patient's ability to breastfeed or the duration of breastfeeding. There was also no significant difference in neonatal neurobehavioral scores between the two groups, indicating that low-dose esketamine intravenous patient-controlled analgesia did not increase neonatal risk. This was consistent with the 2020 guidelines for anesthesia and sedation for breastfeeding women (25), which categorize ketamine as safe for breastfeeding women. A previous study (10) showing that low-dose esketamine used for postoperative pain management after cesarean section had no effect on the neonate during the observed duration of continuous breastfeeding.

The incidence of nausea, vomiting, dizziness, and hallucinations in this study was not significantly different between the two groups. Consistent with the findings of a previous study (9), the addition of low-dose esketamine to sufentanil intravenous patient-controlled analgesia was well tolerated and did not increase the risk of hallucinations.

This study has some limitations. First, esketamine is contraindicated in patients with severe hypertension and abnormal thyroid function; therefore, the population selected for this study did not include mothers with complicated pregnancies. Second, we did not measure esketamine levels in breast milk, and although studies have reported that low-dose esketamine has no effect on breastmilk or newborns, it is possible to monitor esketamine levels in breast milk in subsequent studies. None of the newborns in this study had any adverse outcomes.

5 Conclusion

In conclusion, based on the results from our study, it can be concluded that low-dose esketamine combined with sufentanil for intravenous patient-controlled analgesia can reduce the amount of sufentanil and enhance the analgesic effect after cesarean section in women with GDM, providing new data for the analgesic protocol after cesarean section in women with GDM.

The combination of low-dose esketamine with sufentanil for PCA can enhance the analgesic effect.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Hunan Provincial Maternal and Child Health

Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

LC and TH designed the study. TH and QC organized the data. JZ and JH analyzed the data and wrote the first draft of the manuscript. AL, WX, ZL, and ZP revised the manuscript. All the authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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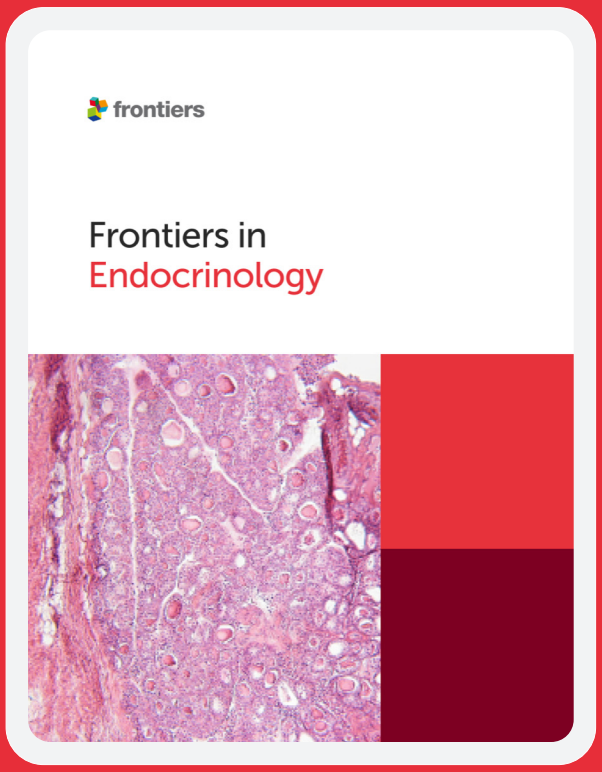

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