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Developing strategies to improve diabetes management in college-going young adults

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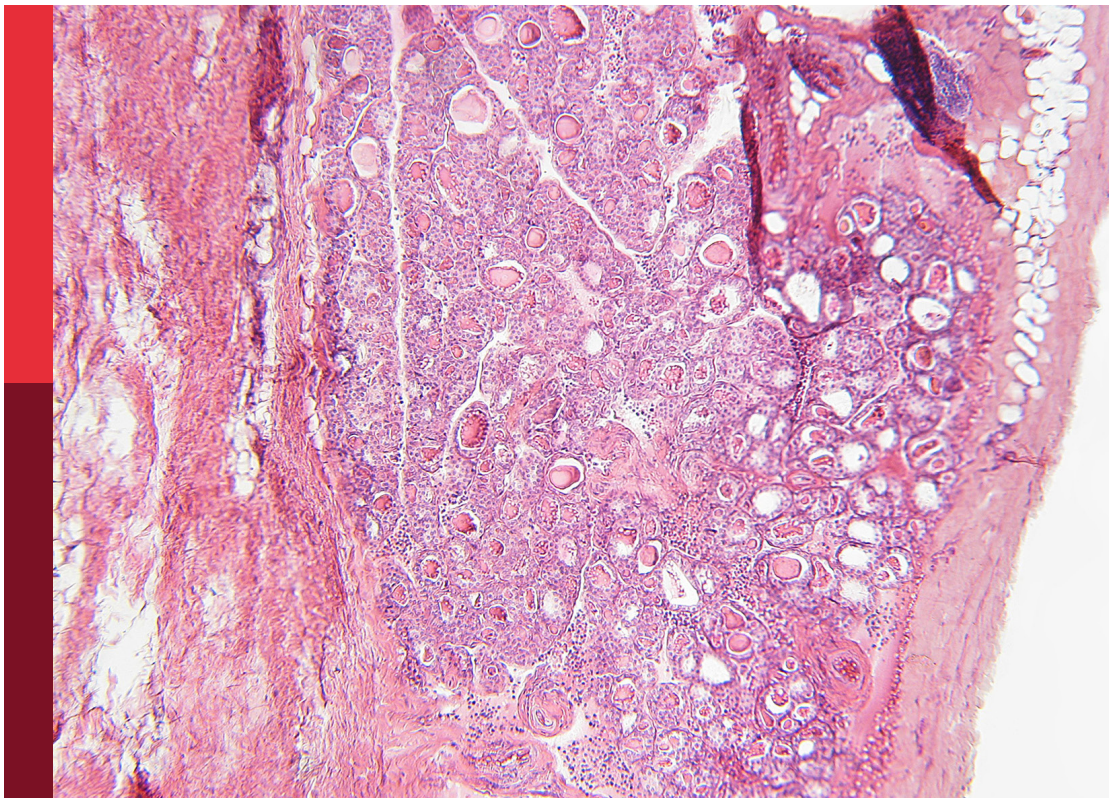

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Developing strategies to improve diabetes management in college-going young adults

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Editorial: Developing strategies to improve diabetes management in college-going young adults

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KEYWORDS

type 1 diabetes mellitus (T1DM), glucose sensing, community engagement, diabetes workshop, diet, lifestyle

Editorial on the Research Topic
Developing strategies to improve diabetes management in college-going young adults

Introduction

Navigating the transition from life at home to university can be often challenging, more so for the new generation of students entering university after a COVID-ravaged high school period. This change is even more difficult for students with Type 1 diabetes mellitus (T1DM), who must navigate this social and physical transition while assuming increasing responsibility for their diabetes care. These pressures may sometimes lead to disengagement from medical care (1). The number of young adults affected by T1DM is increasing globally. Studies have even suggested that SARS-CoV-2 may be the underlying cause of new-onset T1DM (2–4). Thus, there is an urgent need for research into T1DM, particularly focusing on creating treatment options, clinical services, and social support for college-going patients. This Research Topic aims to bridge some of the gaps in the current understanding of T1DM in young adults and provide a roadmap for creating more youth-friendly treatment and support options.

Themes highlighted in the Research Topic

Creating treatment options for college students with T1DM

T1DM is a condition in which the body can no longer produce sufficient amounts of its own insulin (5). The most common treatment for a person with T1DM is insulin

supplementation (6). This is administered through injections at set times of the day and mealtimes (prandial). For optimal management, people with T1DM need to be aware of the ratio of their prandial insulin dose to carbohydrate intake, premeal blood glucose levels, and anticipated physical activity. They should also be capable of measuring their blood glucose levels and managing insulin dosing under various circumstances. All of this can become overwhelming for a person juggling academic and social pressures.

Alternatively, a person with T1DM may use an insulin pump. Currently, the most advanced commercially available technology for glucose monitoring and automatic delivery is a single-hormone (SH [insulin]) closed-loop system, also known as an artificial pancreas (AP). Even this advanced technology, might induce periods of hypoglycemia, especially, during intense physical activity. A study in this Research Topic by Lindkvist et al. tested whether their new dual-hormone (DH [insulin and glucagon]) closed-loop system can provide better glycemic control. Their results indicate that the DH system is not as efficient as the SH system. Another study in this Research Topic, conducted by Liu et al. has created an intelligent controller that may be combined with AP technology for better control of blood glucose levels. In-silico tests show that the controller is effective in preventing hyperglycemia, however, it does not attenuate the risk of hypoglycemia.

An uncomplicated medication routine might alleviate pressure on college students with T1DM. In this Research Topic, Lagunas-Rangel et al. report that a triple-drug combination therapy consisting of gamma-aminobutyric acid (GABA) together with a dipeptidyl-peptidase-4 inhibitor (DPP-4i), and a proton pump inhibitor (PPI), has a superior therapeutic effect in improving diabetes parameters in NOD mice, an animal model for human T1DM. The authors comment that their inexpensive triple-drug regimen is an accessible treatment option for T1DM. Another article in this Research Topic by Almutair and Almulhem reports that treatment with Semaglutide, a GLP-1RA, administered weekly over 4 months, led to a notable improvement in an 18-year-old, non-obese female diagnosed with Hepatocyte nuclear factor-1B maturity-onset diabetes of the young (MODY).

T1DM management through diet and weight management

In addition to medication, maintaining a healthy lifestyle can play a crucial role in the management of T1DM. The study by Li et al. shows that high sugar intake in the diet can be linked to the development of T1DM in NOD mice. Obesity is another factor often associated with diabetes. Sheng et al. monitored the body mass index (BMI), waist circumference (WC), and waist-height ratio (WHtR) of 12,823 normoglycemic individuals to begin with, for 12 years. They report that WC is highly correlated with the occurrence of diabetes in the short term (2-5 years), while WHtR is correlated with the occurrence of diabetes in the long term (6-12 years).

Maintaining a healthy lifestyle can be tricky in college; the tendency for quick meals and late-night snacking can lead to hyperglycemia and obesity. Explaining the implications of such behavior can be key to preparing young adults with T1DM for college life.

T1DM management through education, social support and sustained monitoring

A study in this Research Topic by Wolf et al. provides an account of the workshop they created and implemented to prepare young adults with T1DM and their parents, for life in college. In addition to valuable insights into academic life, the workshop provided opportunities to interact with endocrinologists and nutritionists. One key piece of advice was to set up a diabetes care provider in their new place of residence as soon as possible.

Monitoring health parameters is key to managing T1DM. Catamo et al. in their study involving 324 subjects with T1DM aged <21 years, show that markers of diabetic complications, such as dyslipidemia, can occur in individuals as young as 11. Long waiting times for specialist appointments might aggravate such conditions, leading to complications. Dupenloup et al. discuss an interesting alternative. A new, telemedicine-based care model based on the use of a remote patient monitoring tool that analyzes continuous glucose monitoring data and identifies individuals likely to benefit from contact with the diabetes care team. Dupenloup et al. evaluated the financial impact of adopting this tool by pediatric endocrinology clinics and reported that it is financially sustainable with insurance. This can be a medical intervention tool that students rely on while waiting to obtain specialist appointments.

Mental health is another important concern for people with T1DM as well as their family members. In their review, Chen et al. discuss the high prevalence of depression among parents of children/adolescents with T1DM. The authors state that higher instances of social discrimination, marginalization and stigma experienced by these patients and their parents might contribute to this observation. A workshop such as the one created by Wolf et al. can provide opportunities for interaction and social support for people with T1DM and their parents.

Summary and future directions

Easy and reliable treatment options will go a long way in providing support to students with T1DM. However, we are still far from effective insulin alternatives. In this Research Topic, Liu

et al. discuss the use of traditional Chinese medicine to regulate the microbiome of people with diabetes as a treatment option. Immunotherapy has been another avenue of research (7, 8). While we await a breakthrough, this Research Topic highlights the importance of preparation through education and counseling, access to health monitoring, and support for a healthy lifestyle in the smooth transition of young adults with T1DM from home to their place of higher education.

Author contributions

MS: Conceptualization, Writing – original draft, Writing – review & editing. ZL: Writing – review & editing. AM: Writing – review & editing.

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

References

1. Hynes L, Byrne M, Dinneen SF, McGuire BE, O'Donnell M, Mc Sharry J. Barriers and facilitators associated with attendance at hospital diabetes clinics among young adults (15-30 years) with type 1 diabetes mellitus: a systematic review. *Pediatr Diabetes*. (2016) 17:509–18. doi: 10.1111/pedi.12198

2. Unsworth R, Wallace S, Oliver NS, Yeung S, Kshirsagar A, Naidu H, et al. New-onset type 1 diabetes in children during COVID-19: multicenter regional findings in the U.K. Diabetes Care. *Diabetes Care* (2020) 43(11):e170–1. doi: 10.2337/dc20-1551

3. Boddu SK, Aurangabadkar G, Kuchay MS. New onset diabetes, type 1 diabetes and COVID-19. *Diabetes Metab Syndr*. (2020) 14:2211–7. doi: 10.1016/j.dsx.2020.11.012

4. Wang Y, Guo H, Wang G, Zhai J, Du B. COVID-19 as a trigger for type 1 diabetes. *J Clin Endocrinol Metab*. (2023) 108:2176–83. doi: 10.1210/clinem/dgad165

5. Katsarou A, Gudbjörnsdottir S, Rawshani A, Dabelea D, Bonifacio E, Anderson BJ, et al. Type 1 diabetes mellitus. *Nat Rev Dis Primers*. (2017) 3:17016. doi: 10.1038/nrdp.2017.16

6. Janež A, Guja C, Mitrakou A, Lalic N, Tankova T, Czupryniak L, et al. Insulin therapy in adults with type 1 diabetes mellitus: a narrative review. *Diabetes Ther*. (2020) 11:387–409. doi: 10.1007/s13300-019-00743-7

7. Bresson D, von Herrath M. Immunotherapy for the prevention and treatment of type 1 diabetes: optimizing the path from bench to bedside. *Diabetes Care*. (2009) 32:1753–68. doi: 10.2337/dc09-0373

8. Jo S, Fang S. Therapeutic strategies for diabetes: immune modulation in pancreatic β Cells. *Front Endocrinol (Lausanne)*. (2021) 12:716692. doi: 10.3389/fendo.2021.716692

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Triple drug therapy with GABA, sitagliptin, and omeprazole prevents type 1 diabetes onset and promotes its reversal in non-obese diabetic mice

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Previous studies have reported that dual drug combinations consisting of γ-aminobutyric acid (GABA) together with a dipeptidyl-peptidase-4 inhibitor (DPP-4i), also a DPP-4i with a proton pump inhibitor (PPI), could improve pancreatic β-cell function and ameliorate diabetes in diabetic mice. In this study, we sought to determine if a triple drug combination of GABA, a DPP-4i and a PPI might have superior therapeutic effects compared with double drug therapies in the prevention and reversal of diabetes in the non-obese diabetic (NOD) mouse model of human type 1 diabetes (T1D). In a diabetes prevention arm of the study, the triple drug combination of GABA, a DPP-4i, and a PPI exhibited superior therapeutic effects in preventing the onset of diabetes compared with all the double drug combinations and placebo. Also, the triple drug combination significantly increased circulating C-peptide and serum insulin levels in the mice. In a diabetes reversal arm of the study, the triple drug combination was superior to all of the double drug combinations in reducing hyperglycemia in the mice. In addition, the triple drug combination was the most effective in increasing circulating levels of C-peptide and serum insulin, thereby significantly reducing exogenous insulin needs. The combination of GABA, a DPP-4i and a PPI appears to be a promising and easily scalable therapy for the treatment and prevention of T1D.

KEYWORDS

type 1 diabetes (or diabetes), NOD mice, insulin, GABA, DPP-4 inhibitor, PPI

Introduction

Type 1 diabetes (T1D) is a disease characterized by a lack of insulin production by pancreatic islet β-cells due to their autoimmune destruction (1, 2). In the United States, the overall pediatric incidence of T1D is approximately 25 per 100,000 per year and increased by 2% to 3% per year during the period 2002-2012 (3). T1D develops mainly in childhood or adolescence and, to date, there is no known way to reverse or prevent it (4). Treatment options are very limited and most T1D patients rely solely on lifelong insulin therapy for survival (5). The high costs of new technologies based on advanced insulin treatments reduce their universal accessibility (6). Procedures such as islet or pancreas transplantation cannot be considered scalable and consistently reliable treatment options, due to a number of limiting factors including their extreme complexity, donor availability, requirement for life-long immunosuppression, and high cost (7). Indeed, only autologous stem cell-derived islet transplants are considered as a future therapeutic option (6). With early diagnosis of the disease, some β-cell functions persist for a short period of time, although this is insufficient to maintain normoglycemia without exogenous insulin injection (8). Even minimally preserved β-cell function can prevent the development of diabetic complications (9, 10). Therefore, any therapy that can offer protection and maintenance of residual β-cell function is of paramount importance.

Repurposing of approved medical compounds and finding synergistic drug combinations has been identified as a viable and easily scalable treatment option for complex diseases with no available effective medications, such as T1D (11).

Therapeutic agents for type 2 diabetes such as DPP-4i are considered safe and have been extensively researched for decades (12). DPP4-i were shown to inhibit approximately 80–90% of DPP-4 activity (13–15) leading to a 2–3-fold elevation in glucagon-like peptide-1 (GLP-1) (16),thereby prolonging the stimulation of GLP-1 receptors (GLP-1R) and activating key transcription factors for β-cell growth and survival (17). Furthermore, drugs in the DPP4-i family also reduce inflammation (insulitis), and enhance pancreatic β-cell regeneration (18). Addressing immunomodulation in T1D, DPP-4i have also been reported to effectively target autoimmunity through down-regulating Th1-like immune cells, up-regulating Th2-type cytokines, promoting Treg cell proliferation, and decreasing IL-17 production (19).

Effects of DPP-4i drugs in diabetic animal models showed some promise through improving islet neogenesis and β-cell survival (20–24). Selected clinical studies have reported on the therapeutic effects of DPP-4i in humans with T1D, specifically in the reduction of insulin requirements and inhibition of glucagon secretion (25, 26). However, these findings remain controversial and did not translate successfully in T1D human clinical trials, showing no significant improvement in blood glucose, C-peptide, or HbA1c levels (27, 28).

γ-aminobutyric acid (GABA) is an important neurotransmitter that was found to induce membrane hyperpolarization and suppress glucagon secretion by pancreatic islet α-cells whereas in β-cells it induced membrane depolarization and increased insulin production (29). Importantly, GABA has an overall anti-inflammatory effect on the immune system and was shown to suppresses lymphocyte proliferation through GABA-A receptors (30). Additionally, induction and activation of the GABA-B receptor in human islets modulates insulin secretion (31). GABA has also been reported to induce α-cell transdifferentiation into β-like cells through the GABA-A receptor (32).

The therapeutic efficacy of GABA was significantly improved with the addition of the DPP-4i, sitagliptin in murine pancreatic islets and the combination increased β-cell mass and proliferation as well as reduced β-cell apoptosis (33).

Another family of well-researched drugs, PPI, inhibit H+-K+-ATPase and increase the concentration of gastrin (a stimulant for pancreatic β-cell regeneration) in blood and pancreatic tissue (34). Combining a DPP-4i with a PPI was reported to enhance the synergistic effect of the two drugs, restore normoglycemia in NOD mice (35) and induce β-cell neogenesis from human pancreatic duct cells implanted in NOD-severe combined immunodeficient mice (36).

Here, we sought to determine if there might be an improved therapeutic effect of the oral administration of a three-drug combination consisting of sitagliptin (a DPP-4i), omeprazole (a PPI) and GABA as a novel pharmacological treatment of T1D. We compared the effects of the triple drug combination with those of the double combinations and placebo, both before and after diabetes onset in NOD mice.

Material and methods

Animal ethics

The experimental procedures were carried out in accordance with the Decision of the Council of the Eurasian Economic Commission No. 81 dated 03/11/2016 “On the approval of the rules of good laboratory practice of the Eurasian Economic Union in the field of circulation of medicines”; GOST R 33044-2014 “Principles of good laboratory practice”; US FDA Good Laboratory Practice (GLP) Regulations for Non-clinical Laboratory Studies (21 CFR Part 58); guidelines of the European Federation of Laboratory Animal Science Associations, based on European Union legislation (Directive 2010/63/EU), as well as the NIH Guide for the care and use of laboratory animals, local laws and policies. The procedures were approved by the ethics committee of the I. M. Sechenov First Moscow State Medical University (Approval Code PRC-043 with start date October 28, 2020).

Animals

Female non-obese diabetic (NOD)/ShiLtJ mice (The Jackson Laboratories, IMSR_JAX:001976) weighing 18-20 grams and 10-11 weeks old were used in this study. At the time of initiation of therapy, the animals had reached the age of 17-18 weeks. The animals were kept in polycarbonate cages with sterilized non-coniferous softwood sawdust bedding under controlled conditions of temperature (23°C ± 3) and humidity (55% ± 15) and with a light-dark cycle of 12:12 hours. Water and standard chow (Safe, D04) were available *ad libitum* at all times.

Group formation, drug preparation and administration

NOD mice were identified as diabetic by blood glucose (BG) monitoring starting at 10–11 weeks of age twice a week. Diabetes was defined as a random BG >13.9 mmol/L in two consecutive measurements on different days. The randomization was initiated after 30% of the cohort were diagnosed with diabetes. Thereafter, BG was measured on four consecutive days to reaffirm the diabetes diagnosis, and randomization in five groups was performed.

NOD mice between 17 and 18 weeks of age were equally divided into 5 groups: 1. Placebo, 2. GABA, sitagliptin, and omeprazole (designated A+B+C), 3. GABA and sitagliptin (designated A+B), 4. GABA and omeprazole (designated A +C), and 5. Sitagliptin and omeprazole (designated B+C). After that, two study arms were formed based on BG levels in the mice: a) a diabetes prevention arm that involved 94 NOD mice before the onset of diabetes and b) a diabetes reversal arm that involved 51 NOD mice with confirmed T1D status. For the treatments, GABA 250 mg (Aminalon) and sitagliptin 100 mg (Januvia) film-coated tablets, as well as omeprazole 20 mg (Omeprazole Teva) enteric capsules were used. The pharmaceutical forms were dissolved in a 1% starch solution and were delivered by oral gavage to the mice twice a day in a volume of 1 mL in the morning at 10:00-11:00 and in the afternoon at 18:00-19:00. The doses used were GABA 300 mg/kg, sitagliptin 30 mg/kg, and omeprazole 15 mg/kg. The placebo group received 1% starch solution. The drugs were administered for 70 days.

TABLE 1 Dosing and administration of insulin.

Blood glucose range	Dose of insulin glargine
30.0- 33.3 mM/L	1.5 units/day
> 33.3 mM/L in a single measurement	1.5 units - morning 1 unit - evening
> 33.3 mM/L in two consecutive measurements	1.5 units - morning 1.5 units in the evening

Along with the drugs, the severely diabetic mice were given insulin glargine (Lantus) according to the specific "Sliding scale" protocol shown in Table 1. The insulin therapy was only administered to mice in severe hyperglycaemia with blood glucose levels over 30 mmol/L. Mice with blood glucose lower than 30 mmol/L were not treated with exogenous insulin injections.

Blood glucose determination

Non-fasting blood glucose levels in mice were measured three times per week for 70 days with an AccuCheck Performa glucometer (Roche) and AccuCheck Performa test strips (Roche) following the supplier's instructions. Blood was taken from the tail vein.

Weighing

The mice were weighed once per week for 70 days with an M-ER 122ACFJR-600.01 LCD balance (Mertech).

Plasma collection

Approximately 700 µL of blood was sampled from the ophthalmic sinus of fasted mice (4 hours without food) using tubes with K+EDTA in the afternoon (12-2 PM) at weeks 2, 5 and 10. Plasma was obtained by centrifuging the blood for 10 minutes at 3500 rpm at 4°C. The plasma samples were frozen and kept at -20°C until the measurement of the different parameters.

C-peptide determination

A 96-well plate (Corning, 3590) was incubated for 16-18 hours at 40°C with C-peptide polyclonal antibodies (Invitrogen, PA5-85595) diluted in 20 mM carbonate buffer (Panreac, 571648.201638) at a concentration of 200 ng/100 µL. Unbound immunoglobulins were removed by three serial washes with PBST (1X PBS and 0.01% Tween 20). To block non-specific binding sites, the plate was incubated during 30 minutes at 37°C with 200 µL of blocking buffer (BB) composed of 20 mM Tris-HCl buffer (Panreac, 141940.1211), 0.14 M NaCl (Panreac, 141659.1214) and 0.5% skimmed bovine milk powder (Sigma, M7409). Then, three serial washes with PBST were performed and 100 µL of BB containing increasing concentrations of C-peptide (0, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250 ng/mL) were added to 12 wells to create a curve. In parallel, 100 µL of test plasma diluted 3000-fold with BB was added to the other wells. Plates were incubated for 30 minutes at 37°C. The

contents of the wells were removed and washed 3 times with 200 µL of 20 mM Tris-HCl buffer containing 0.05% Tween-20 (Panreac, 162312.1611). 100 µL of a BB containing biotin-labeled rabbit polyclonal anti-C-peptide antibody (Sigma, H1759) at a concentration of 200 ng/100 µL was added to the wells and incubated for 30 minutes at 37°C. After incubation, the contents of the wells were aspirated and peroxidase-conjugated avidin (Sigma, A3151) was added and incubated for 30 minutes at 37°C. The contents of the wells were aspirated, the wells were washed 4 times with 200 µL of 20 mM Tris-HCl containing 0.05% Tween 20 and subsequently 100 µL of 0.1 M sodium acetate buffer (Panreac, 141008.1611), 0.1 mg/mL tetramethylbenzidine (Sigma, T8768), and 0.003% H_2O_2 (Sigma-Aldrich, H1009) were added to the wells. To stop the reaction, 50 µL of 2M H_2SO_4 (chemically pure, KhimMed LLC) was added to the wells and the optical density at 450 nm was measured. The concentration of C-peptide in the samples was determined based on the curve created.

Insulin determination

Serum insulin determination was performed with a protocol similar to that of C-peptide, except that in this case polyclonal insulin antibodies (Bioss, BS-0056R) were used.

Glucagon determination

Serum glucagon levels were measured with the mouse GCG/glucagon ELISA kit (LSBio, F5904) according to the supplier's instructions.

Statistical analysis

Data processing was performed in GraphPad Prism v. 9.1 (GraphPad Software, San Diego, California USA) and using Python with the libraries: NumPy, Scipy, Statsmodels, Lmfit, Lifelines, Pandas, Matplotlib, Seaborn, Scikit-postthoc. The Kruskal-Wallis test with Dunn's *post hoc* was used to compare three or more groups. The false discovery rate method, the two-stage Benjamini-Hochberg procedure, was used as the method to control the level of significance. To represent the change in glucose concentration and mass, the median was used as a measure of central tendency and the interquartile width as a measure of dispersion. To visualize between-group differences for other data, "whisker boxes" representing the median, interquartile width, and range were used. To describe the cumulative insulin consumption curve, an approximation to the sigmoid shape was applied:

$$y = \frac{C_1}{\left(1 + e^{-C_2 x - C_3}\right)}$$

optimize the parameters of the curve, the Levenberg-Marquardt algorithm was used using the initial values C1 = 500, C2 = 0.02, C3 = 25. Model quality was assessed using probability plots and Cramer-von Mises and Kolmogorov-Smirnov goodness-of-fit tests. According to the criteria, the model adequately describes the data under study. Using the sigmoid model, the days on which there was a decrease in the rate of insulin consumption were determined. The significance level of 5% ($\alpha = 0.05$) was chosen as the threshold to reject the null hypothesis in all the tests used.

Results

Combined use of GABA, sitagliptin, and omeprazole prevents the onset of diabetes in NOD mice

To assess the potential additive therapeutic effects of the triple combination of GABA, sitagliptin, and omeprazole (A+B+C) in preventing the onset of diabetes in NOD mice, mice 17 -18 weeks of age were administered placebo, double, and triple combination therapies for 10 weeks.

Mice in all groups gradually gained weight with age, and there were no significant differences between the groups (Figure 1A).

At randomization (week 0), median blood glucose levels were similar (6.4 – 6.7 mmol/L) among the groups (Figure 1B). By week 10, the median blood glucose level for group A+B+C (8.2 mmol/L) was significantly lower than that in the placebo group (18.7 mmol/L, $p<0.001$) and all double combinations. Also, blood glucose levels in groups A+B (8.9 mmol/L, $p<0.001$), A+C (10.6 mmol/L, $p<0.001$), and B+C (9.2 mmol/L, $p<0.001$) were significantly lower than in the placebo group (Figure 1C).

Figure 1D shows that 12 out 18 (67%) mice in the placebo group had developed diabetes (blood glucose >13.9 mmol/L in two repeated measurements) by week 10, whereas only 5 out of 18 (28%) of mice in the A+B+C group were diabetic at that time ($p=0.04$, A+B+C *vs.* placebo). Diabetes also developed in 8 out of 19 (42%) mice in A+B and A+C groups and in 8 out of 20 (40%) mice in B+C group, but this was not significantly different from the 67% diabetes development in the placebo group at 10 weeks.

To monitor the effects of the treatments on pancreatic β-cell function, plasma C-peptide and serum insulin levels were measured at weeks 2, 5 and 10. Plasma C-peptide levels in the placebo group dropped from 1.13 nmol/L at 2 weeks to 0.38 nmol/L at 10 weeks, whereas all other groups showed elevation of the median C-peptide level. However, only A+B+C ($p<0.001$) and A+B ($p=0.031$) combination therapies showed significant increases in C-peptide levels compared with the placebo group at week 10 (Figure 1E). Serum insulin levels were also increased

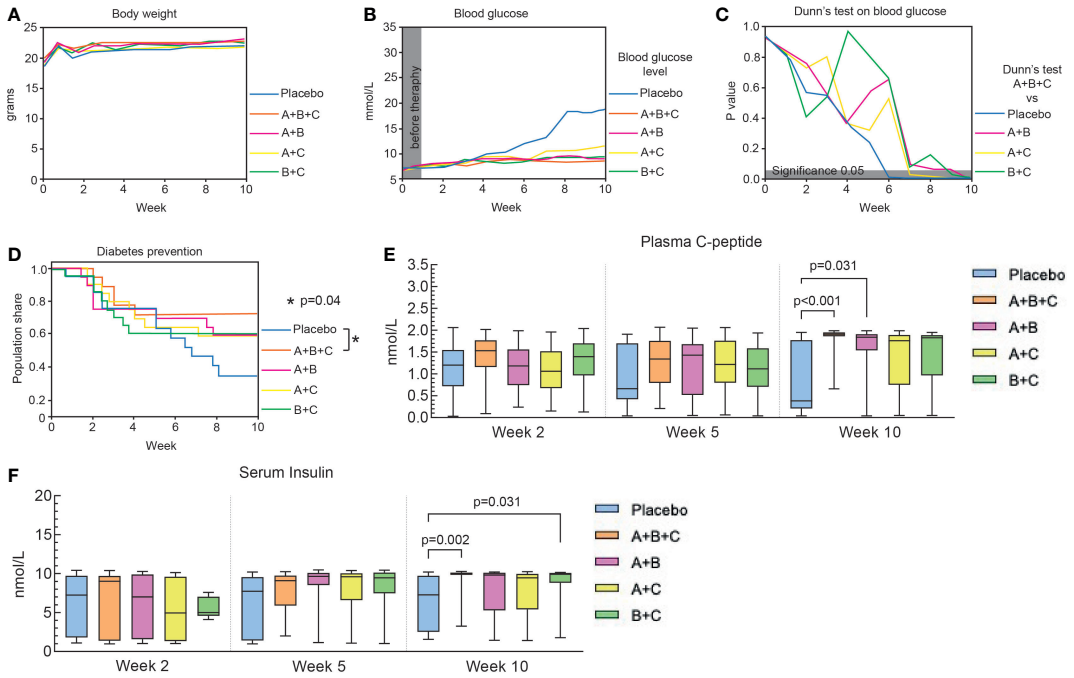

FIGURE 1
Triple therapy (A+B+C) prevents diabetes onset in NOD mice. This arm of the experiments was performed on 94 mice that had not developed diabetes at the start of drug therapy (blood glucose<13.9. mmol/L). The placebo, A+B+C, A+B and A+C groups included 19 mice each and the B+C group 18. **(A)** Changes in body weight of the mice in each group are shown as median values. The data were analyzed with the Kruskal-Wallis test with Dunn's *post hoc*. No significant changes were found between the groups. **(B)** Changes in median blood glucose levels in each group over 10 weeks. **(C)** Blood glucose levels of the A+B+C group were compared with the other groups at different times using the Kruskal-Wallis test with Dunn's *post hoc*. The gray area corresponds to a level of significance (p = 0.05). **(D)** Kaplan-Meier curves of the proportion of mice that were prevented from diabetes development. There was a significant difference between the A+B+C group and the placebo group (p = 0.04). No statistical differences were found when comparing the placebo group with the dual therapy groups (A+B [p=0.22], A+C [p=0.23], B+C [p=0.22]). **(E)** Plasma C-peptide levels were analyzed with the Kruskal-Wallis test with Dunn's *post hoc*. Significant differences were found by comparing the placebo group with A+B+C (p<0.001) and with A+B (p= 0.031) groups at week 10. **(F)** Serum insulin levels were analyzed with the Kruskal-Wallis test with Dunn's *post hoc*. Significant differences were found by comparing the placebo group with the A+B+C (p=0.002) and with the B+C (p=0.031) groups at week 10. Plasma C-peptide and serum insulin levels are shown as box-whisker plots representing median values with interquartile width and range.

significantly in A+B+C (p = 0.002) and B+C (p = 0.031) groups compared with the placebo group (Figure 1F). Notably, A+B+C was the only group to show significant increases in both C-peptide and insulin levels compared with the placebo group.

Combined use of GABA, sitagliptin, and omeprazole promotes diabetes reversal in NOD mice

To evaluate the potential additive therapeutic effects of the triple combination of GABA, sitagliptin, and omeprazole (A+B+C) in treating NOD mice 17-18 weeks of age with confirmed diabetes (blood glucose >13.9 mmol/L), the mice were administered placebo, double, and triple combination therapies for 10 weeks.

There were no significant differences in weight changes with aging in the different groups (Figure 2A).

At randomization (week 0), median blood glucose levels were similar (26.0 – 28.0 mmol/L) among the groups (Figure 2B). At week 10 there was a highly significant difference in the median blood glucose level in the A+B+C group (14.5 mmol/L) compared with the placebo group (33.3 mmol/L, p=0.001). Also, blood glucose in the A+B+C group (14.5 mmol/L) was significantly lower than that in all double combination groups: A+B (28.1 mmol/L, p<0.001), A+C (28.6 mmol/L, p<0.001), and B+C (30.2 mmol/L, p<0.001) (Figure 2C).

Combined use of GABA, sitagliptin, and omeprazole decreases insulin requirements and increases C-peptide and insulin levels in diabetic NOD mice

Insulin therapy was used to manage severely hyperglycemic mice (blood glucose > 30 mmol/L) in all treatment groups, and

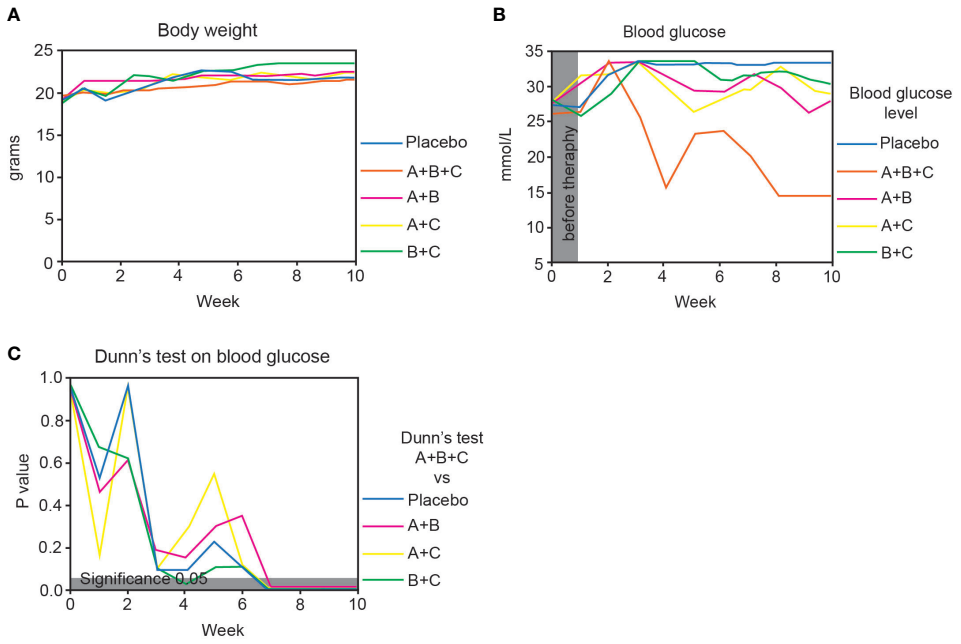

FIGURE 2
Triple therapy (A+B+C) promotes diabetes reversal in NOD mice. This arm of the experiments was performed on 51 mice that had developed diabetes at the start of drug therapy (blood glucose >13.9 mmol/L). The placebo, A+B, A+C and B+C groups included 10 mice each and the A+B+C group 11. **(A)** Changes in body weight of the mice in each group are shown as median values. The data were analyzed with the Kruskal-Wallis test with Dunn's *post hoc*. No significant changes were found between the groups. **(B)** Changes in median blood glucose levels in each group over 10 weeks. **(C)** Blood glucose levels of the A+B+C group were compared with the other groups at different times using the Kruskal-Wallis test with Dunn's *post hoc*. The gray area corresponds to a level of significance (p = 0.05).

the total insulin intake was measured to determine the efficacy of the treatments in reducing insulin requirements.

By week 10 in the diabetes reversal arm of the experiments, the triple therapy (A+B+C) group had fewer mice dependent on insulin therapy compared with all other groups. The A+B+C group had the largest proportion of mice that discontinued insulin treatment: 9 of 10 mice (90%) compared with 7 of 9 mice (78%) in group A+B, 6 of 9 mice (67%) in group A+C, 3 of 9 mice (33%) in group B+C, and only 1 of 8 mice (13%) in the placebo group.

It is important to additionally point out that in the reversal arm of the study only mice with BG levels over 30 mmol/L were treated with exogenous insulin injections in accordance with "Sliding scale" protocol (Table 1). 5 mice in the reversal arm of the study did not reach BG levels higher than 30 mmol/L and, therefore, were not treated with exogenous insulin injections during the experiment.

On Figure 3 sigmoid approximation of cumulative insulin intake shows that the A+B+C group's decrease in exogenous insulin demands occurred earlier (day 25) than in the placebo group (day 47) and the dual therapy groups: A+B (day 33), A+C (day 41), and B+C (day 38). As expected, the placebo group had the highest insulin demands.

Plasma C-peptide and serum insulin levels were measured at weeks 2, 5 and 10 to monitor the effects of the combination treatments on pancreatic β-cell function. Soon after the beginning of treatments (week 2), median C-peptide levels were similar (0.17-0.25 nmol/L) among the groups (Figure 4A). By week 10, the C-peptide level in group A+B+C increased more than 5-fold to 0.93 nmol/L and was significantly higher than in the placebo group (0.06 nmol/l, p=0.003) and the B+C group (0.07 nmol/l, p=0.002), both of which saw decreases in C-peptide levels from weeks 2 to 10. C-peptide levels increased slightly from 2 to 10 weeks in groups A+B (0.43 nmol/l, p=0.216) and A+C (0.38 nmol/l, p=0.521), but did not reach significance compared with the placebo group (Figure 4A).

Serum insulin levels were similar (1.29-1.76 μU/ml) among the groups at week 2 (Figure 4B). By week 10,insulin levels in groups A+B+C increased more than 6-fold to 8.75μU/ml and this was significantly higher than in the placebo group (1.65 μU/ml, p=0.003), the B+C group (1.79 μU/ml, p=0.002) and the A+C group (2.68 μU/ml, p=0.041, but did not reach significance compared with group A+B (2.92 μU/ml, p=0.49). The A+B+C group was the only group to show a significant increase in serum insulin compared with the placebo group (Figure 4B), which is compatible with this A+B+C group having

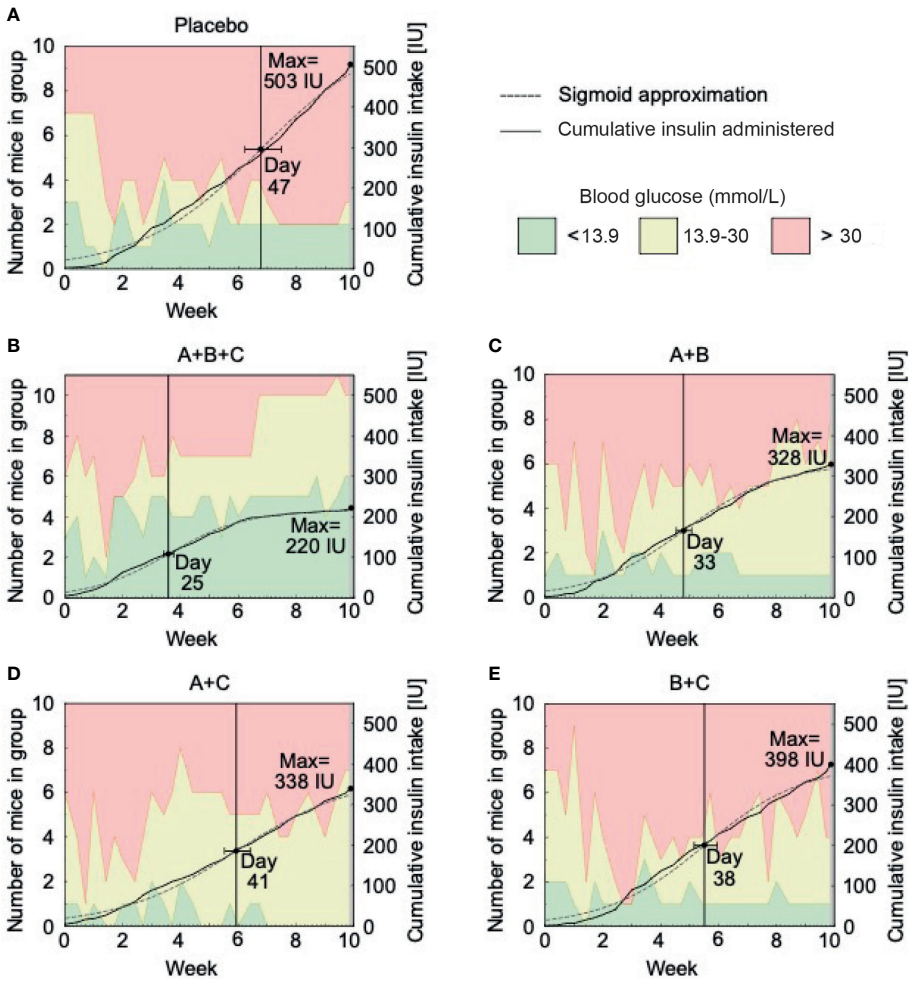

FIGURE 3
Insulin requirements decrease with triple therapy (A+B+C) in NOD mice. This arm of the experiment was performed on 51 mice with confirmed diabetes (blood glucose >13.9 mmol/L) at the start of drug therapy. The placebo **(A)**, A+B **(C)**, A+C **(D)** and B+C **(E)** groups included 10 mice each and the A+B+C **(B)** group included 11 mice. The graphs show the number of mice within the different glucose levels (green area represents normoglycemic mice with blood glucose levels below 13.9 mmol/l, yellow represents hyperglycemic mice without insulin therapy with blood glucose levels between 13.9 - 30.0 mmol/l and red represents severely hyperglycemic mice on insulin therapy with blood glucose levels above 30.0 mmol/l) and the cumulative amount of insulin administered to them during each week of the study. Additionally, a sigmoid approximation model of the exogenous insulin used in each group and the anticipated day of a reduced insulin administration intensity are displayed.

required significantly less exogeneous insulin therapy than all other groups (Figure 3).

Serum glucagon levels were measured once in week 10. (Figure 4C). Serum glucagon tended to increase in the A+B+C group and the insulin/glucagon ratio (Figure 4D) was significantly higher in mice in the A+B+C group than in the placebo group (p=0.005).

Discussion

In this study, we found that a triple drug combination therapy consisting of GABA, a DPP-4i and a PPI (designated A+B+C) has a superior therapeutic effect in improving diabetes parameters in NOD mice, an animal model for human T1D. The A+B+C triple drug combination was superior to all the double drug combinations in preventing diabetes onset, and this was accompanied by significant increases in circulating levels of C-peptide and insulin. In addition, the triple drug combination was most effective at lowering blood glucose levels in severely diabetic mice and was the only drug combination to significantly increase C-peptide and insulin levels in the mice, together with decreased requirements for exogenous insulin therapy.

To determine the appropriate duration of the experiment, various parameters, including renewal of heterogeneous cells in the mouse pancreas (37) and previous studies with GABA, DPP-4i, and PPI (32, 33, 35) were taken into account to

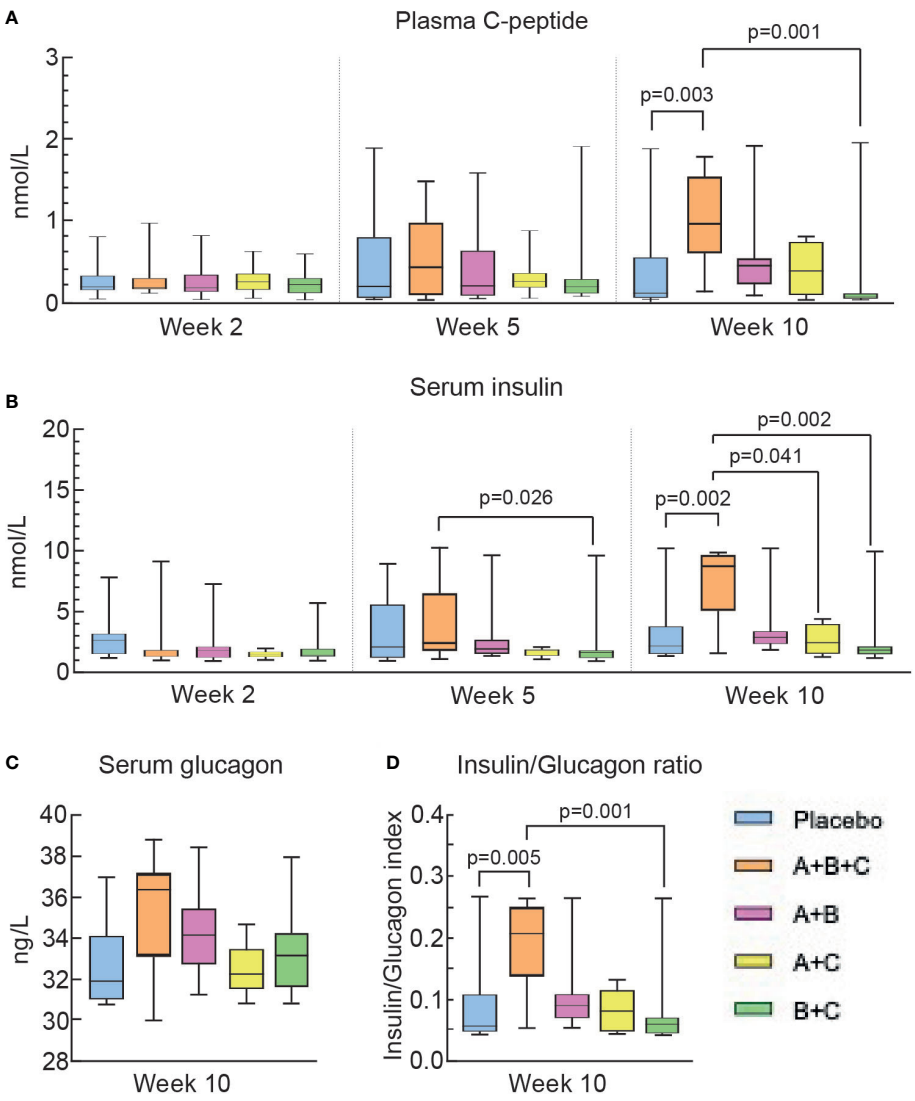

FIGURE 4
Triple therapy (A+B+C) increases C-peptide and insulin levels in diabetic NOD mice. Differences in the levels of plasma C-peptide **(A)**, serum insulin **(B)**, serum glucagon **(C)**, and the insulin/glucagon ratio **(D)** were analyzed using the Kruskal-Wallis test with Dunn's *post hoc*. The box-whisker plots represent median values with interquartile width and range.

demonstrate the efficacy of the long-term therapy administration in mouse T1D models. It was concluded that in order for the study to yield conclusive evidence on the efficacy of the combination treatment in NOD mice, the triple combination therapy should be administered for 70 days or 10 weeks.

In this study, the NOD mouse with spontaneous autoimmune diabetes (38, 39) was deliberately chosen to assess the potential of additive therapeutic effect of drug combinations in T1D. A major strength of the NOD model for human T1D compared to diabetes induced by chemical substances (STZ, alloxan, dithizone) (38, 40, 41) is the similarity to human T1D, as the NOD mice develop pancreatic islet β-cell autoantibodies (42) and their islets are infiltrated with CD4+ and CD8+ lymphocytes (40).

Insulin replacement therapy is required for all individuals with T1D and consists of multiple daily injections (MDI) of basal and prandial insulin or a continuous subcutaneous insulin infusion (43). To determine whether any of the combination treatments could decrease or completely remove the requirement for insulin therapy, our NOD model study was designed to resemble T1D disease management in humans. In our study, exogenous insulin therapy was introduced to control hyperglycemia and ensure the survival of severely diabetic NOD mice with BG >30 mmol/L (41, 42), similar to the necessity of insulin therapy in human patients with T1D. The "Sliding Scale"

protocol for insulin administration was necessary to keep the hyperglycemic animals alive throughout the experiment in all groups and to observe the effects of treatments during the long-term administration of the therapeutic agents and placebo. Importantly, the insulin therapy was only administered to mice in severe hyperglycaemia with BG levels over 30 mmol/L.

In the reversal arm of our study, we found that mice treated with the triple drug combination of GABA+DPP-4i+PPI received the least amount of exogenous insulin compared to all other dual drug combinations or placebo but had significantly higher circulating levels of C-peptide and endogenous insulin.

T1D clinical parameters and biomarkers were found to accurately correlate with T1D pancreas pathology (44). C-peptide, the molecule produced in an equimolar concentration to insulin, has become an established insulin secretion biomarker. Circulating levels of C-peptide in diabetic patients are widely used to determine the effects of interventions designed to preserve or improve residual β-cell function (45, 46), and the C-peptide response is often evaluated as the primary outcome in T1D intervention trials (10, 47, 48). Our findings, in the present study, of significant and superior actions of the triple drug combination of GABA, a DPP-4i and a PPI (A+B+C) to increase C-peptide and insulin levels, compared with the double drug combinations, both in diabetes prevention and reversal, supports the conclusion that the triple therapy improved diabetes by actions leading to improved islet β-cell function. Similar to other T1D murine studies investigating single agents and combination therapies (32, 33, 35), our experiment was conducted on non-stimulated β-cells in diabetic mice, and glucose tolerance testing was not included in the scope of our research. Immunohistochemical studies of pancreatic islet β-cell mass will be needed to evaluate possible regeneration and preservation of β cells after treatment with these different drug combinations.

T1D is a disease with numerous distinct pathogenic pathways underlying its clinical manifestation. We believe that the prevention of diabetes and its reversal may be explained by reports that the three therapeutic agents used in this study target different processes that occur in T1D.

The first component of the A+B+C combination, GABA, functions as an important transmitter within the islets, acting to regulate islet cell function. It promotes regeneration of β-cells, counters their apoptosis induced by cytokines, drugs, and other stressors, and has anti-inflammatory and immunoregulatory activities (49, 50). Furthermore, studies have indicated that GABA induces α-cell to β-cell transdifferentiation, beginning with the conversion of pancreatic duct cells to α-cells (32).

The second component of the A+B+C combination, sitagliptin, inhibits DPP-4, an enzyme strongly expressed on pancreatic islet cells and on the surface of immune cells (51). DPP-4 inhibition exerts positive effects on insulin secretion, β-cell survival and proliferation (52). Like other DPP-4i, sitagliptin prevents the degradation of the incretin hormone GLP-1 and thus prolongs stimulation of GLP-1 receptors (GLP-1R) with subsequent sustained elevation of cAMP and activation of the PI3K/AKT signaling pathway, which together activate key transcription factors for cell growth and survival (17). GLP-1 lowers blood glucose levels by stimulating insulin secretion from pancreatic β-cells in a glucose-dependent manner, while simultaneously inhibiting glucagon secretion from α-cells (53).

In addition, DPP-4i have been hypothesized to treat autoimmune T1D because they down-regulate Th1 cells, up-regulate Th2 cells and cytokines and promote Treg proliferation (19). Studies show that inhibition of CD26/DPP4 leads to decreased T-cell activation, proliferation, and migration, as well as increased GLP-1-mediated uptake of GABA by immune and endocrine cells (54, 55). We chose a dose of 30 mg/kg for the DPP-4i, sitagliptin, because this was the dose used by He and colleagues (56) to significantly downregulate serum IL-1β and IL-12 in NOD mice compared to placebo control.

Previously, the combination of GABA and sitagliptin was shown to promote regeneration of β-cell and reduce their apoptosis in the mouse model of streptozotocin (STZ)-induced β-cell injury (33) and in human islets transplanted into immunodeficient mice with STZ-induced diabetes (57). Subsequently, it was reported that these results are due, in part, to an additive effects of the agents to activate the PI3K/AKT pathway, stimulate cAMP-β-catenin signaling, reduce TxNIP activity, and promote SIRT1 and α-Klotho expression (55, 58). In the diabetes reversal arm of the current study, we observed that the combination of GABA and sitagliptin reduced blood glucose levels and lowered exogenous insulin demand, however the GABA and sitagliptin combination was significantly less effective than the triple combination therapy and did not significantly increase C-peptide and insulin levels in the mice.

We attribute the limited effects of the GABA and sitagliptin combination in our study, when compared to that of other studies (33, 57) to important differences between chemically-induced diabetes in the mice in those studies, and spontaneous autoimmune diabetes in the NOD mouse model of human T1D used in our study. Diabetes development in the NOD mouse provides insights into the functions of immune cells, showing many similarities to human autoimmune T1D. Chemically induced diabetic mice undergo initial β-cell destruction, but there is no autoimmune attack against the islet β-cells, so that β-cells do not suffer from a sustained and continued immune attack that would neutralize possible β-cell regeneration. Therefore, the therapeutic benefit of the GABA and sitagliptin combination in chemically-induced diabetic mice will not be reflective of the β-cell regeneration process in NOD mice and human T1D, where autoimmunity has to be countered to allow for possible β-cell regeneration.

The third component of the A+B+C combination, the PPI, omeprazole increases endogenous gastrin levels, which stimulates β-cell regeneration and improves glucose tolerance (59). Here, we investigated the addition of a PPI in form of

omeprazole because it was previously reported to improve glycemic control by increasing gastrin levels in the blood and pancreas and has been shown to act as an adjuvant with hypoglycemic drugs (60–62). Additionally, omeprazole was reported to simultaneously increase insulin secretion by inhibiting the V-ATPase proton pump in β-cell insulin granules (63).

The combination of a DPP-4i with a PPI was examined before and found to restore normoglycemia in db/db diabetic mice (64) and in NOD mice (35). In agreement with the latter study in NOD mice (35), we found that the dual combination of a DPP-4i and a PPI in the present study was able to significantly reduce the blood glucose elevation in the prevention arm of the study in comparison with the placebo. However, the DPP-4i and PPI combination in the present study was not able to significantly decrease severe hyperglycemia in the diabetes reversal arm of the study, whereas the triple therapy of GABA, a DPP-4i and a PPI did significantly decrease blood glucose levels in both the diabetes reversal and prevention arms of the study. The different results reported in these two studies probably derive from a difference in experimental design. In the earlier study in NOD mice (35), a blood glucose level above 10 mmol/L was used to define diabetes onset and the start of therapies, whereas NOD mice in our study were defined to be diabetic only when blood glucose levels exceeded 13.9 mmol/L. Moreover, the test therapies in our study started when the entire reversal arm cohort of mice became diabetic with a median blood glucose level of 27.4 mmol/L for the A+B+C group and 28.0 mmol/L for the sitagliptin and omeprazole group, which represent severe hyperglycemia. Therefore, it is apparent that the A+B+C triple combination was needed to successfully treat severe hyperglycemia, whereas a dual drug combination of sitagliptin and omeprazole would suffice when starting from a much lower level of hyperglycemia (35).

Conclusion

Our findings demonstrate superior efficacy of the combination of GABA, sitagliptin and omeprazole in prevention and reversal of diabetes in NOD mice when compared to double therapies. Additional glucose tolerance tests are recommended to assess the full extent of the triple combination therapy to improve stimulated β-cell function. GABA, DPP-4 inhibitors such as sitagliptin, and PPIs such as omeprazole can be taken orally, which is a significant clinical benefit. Toxicology studies on both DPP-4i and PPI have been extensively investigated, and clinical studies on GABA are currently underway. The promising findings in the present study warrant conducting clinical trials to examine the safety and effectiveness of the triple combination of GABA, sitagliptin and omeprazole as a novel pharmacological treatment for patients with insulin-dependent T1D and prevention for high-risk groups.

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 animal study was reviewed and approved by I. M. Sechenov First Moscow State Medical University.

Author contributions

Conceptualization: DK, LK, and SL; Data curation: FL-R, DK, LK, VT, AR, and SL; Formal analysis: FL-R, DK, LK, VT, AR, and SL; Funding acquisition: DK, LK, SL and HS; Investigation: FL-R, DK, AN, LK, VT, AR, HS, and SL; Methodology: FL-R, DK, AN, LK, and VT; Project administration: DK, LK, SL, and HS; Resources: DK, LK, VT, HS, and SL; Supervision: FL-R, DK, LK, VT, AR, HS, and SL; Validation: FL-R, DK, AN, LK, VT, AR, HS, and SL; Writing—original draft: FL-R, DK, LK, VT, AR, HS, and SL; Writing—review and editing: FL-R, DK, LK, VT, AR, HS, and SL. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Authors SL, DK, and LK are members of Levicure LTD and have patents related to the triple combination GABA, PPI, DPP4-i. VT was employed by Advanced Molecular Technology LLC.

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

References

1. DiMeglio LA, Evans-Molina C, Oram RA. Type 1 diabetes. *Lancet* (2018) 391:2449–62. doi: 10.1016/S0140-6736(18)31320-5

2. Atkinson MA, von Herrath M, Powers AC, Clare-Salzler M. Current concepts on the pathogenesis of type 1 diabetes–considerations for attempts to prevent and reverse the disease. *Diabetes Care* (2015) 38:979–88. doi: 10.2337/dc15-0144

3. Mayer-Davis EJ, Lawrence JM, Dabelea D, Divers J, Isom S, Dolan L, et al. Incidence trends of type 1 and type 2 diabetes among youths, 2002–2012. *N Engl J Med* (2017) 376:1419–29. doi: 10.1056/NEJMoa1610187

4. Primavera M, Giannini C, Chiarelli F. Prediction and prevention of type 1 diabetes. *Front Endocrinol (Lausanne)* (2020) 11:248. doi: 10.3389/fendo.2020.00248

5. Warshauer JT, Bluestone JA, Anderson MS. New frontiers in the treatment of type 1 diabetes. *Cell Metab* (2020) 31:46–61. doi: 10.1016/j.cmet.2019.11.017

6. Sperling MA, Laffel LM. Current management of glycemia in children with type 1 diabetes mellitus. *N Engl J Med* (2022) 386:1155–64. doi: 10.1056/NEJMcp2112175

7. Beck RW, Bergenstal RM, Laffel LM, Pickup JC. Advances in technology for management of type 1 diabetes. *Lancet* (2019) 394:1265–73. doi: 10.1016/S0140-6736(19)31142-0

8. Katsarou A, Gudbjörnsdottir S, Rawshani A, Dabelea D, Bonifacio E, Anderson BJ, et al. Type 1 diabetes mellitus. *Nat Rev Dis Prim* (2017) 3:17016. doi: 10.1038/nrdp.2017.16

9. Roep BO, Thomaidou S, van Tienhoven R, Zaldumbide A. Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?). *Nat Rev Endocrinol* (2021) 17:150–61. doi: 10.1038/s41574-020-00443-4

10. Leighton E, Sainsbury CA, Jones GC. A practical review of c-peptide testing in diabetes. *Diabetes Ther* (2017) 8:475–87. doi: 10.1007/s13300-017-0265-4

11. Sun W, Sanderson PE, Zheng W. Drug combination therapy increases successful drug repositioning. *Drug Discovery Today* (2016) 21:1189–95. doi: 10.1016/j.drudis.2016.05.015

12. Dahlén AD, Dashi G, Maslov I, Attwood MM, Jonsson J, Trukhan V, et al. Trends in antidiabetic drug discovery: FDA approved drugs, new drugs in clinical trials and global sales. *Front Pharmacol* (2022) 12:807548. doi: 10.3389/fphar.2021.807548

13. Forst T, Uhlig-Laske B, Ring A, Ritzhaupt A, Graefe-Mody U, Dugi KA. The oral DPP-4 inhibitor linagliptin significantly lowers HbA1c after 4 weeks of treatment in patients with type 2 diabetes mellitus. *Diabetes Obes Metab* (2011) 13:542–50. doi: 10.1111/j.1463-1326.2011.01386.x

14. Herman G, Stevens C, Vandyck K, Bergman A, Yi B, Desmet M, et al. Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: Results from two randomized, double-blind, placebo-controlled studies with single oral doses. *Clin Pharmacol Ther* (2005) 78:675–88. doi: 10.1016/j.clpt.2005.09.002

15. He Y-L, Serra D, Wang Y, Campestrini J, Riviere G-J, Deacon CF, et al. Pharmacokinetics and pharmacodynamics of vildagliptin in patients with type 2 diabetes mellitus. *Clin Pharmacokinet* (2007) 46:577–88. doi: 10.2165/00003088-200746070-00003

16. Mari A, Sallas WM, He YL, Watson C, Ligueros-Saylan M, Dunning BE, et al. Vildagliptin, a dipeptidyl peptidase-IV inhibitor, improves model-assessed β-cell function in patients with type 2 diabetes. *J Clin Endocrinol Metab* (2005) 90:4888–94. doi: 10.1210/jc.2004-2460

17. Yaribeygi H, Sathyapalan T, Sahebkar A. Molecular mechanisms by which GLP-1 RA and DPP-4i induce insulin sensitivity. *Life Sci* (2019) 234:116776. doi: 10.1016/j.lfs.2019.116776

18. Yang S-N, Shi Y, Yang G, Li Y, Yu J, Berggren P-O. Ionic mechanisms in pancreatic β cell signaling. *Cell Mol Life Sci* (2014) 71:4149–77. doi: 10.1007/s00018-014-1680-6

19. Shao S, Xu Q, Yu X, Pan R, Chen Y. Dipeptidyl peptidase 4 inhibitors and their potential immune modulatory functions. *Pharmacol Ther* (2020) 209:107503. doi: 10.1016/j.pharmthera.2020.107503

20. Conarello SL, Li Z, Ronan J, Roy RS, Zhu L, Jiang G, et al. Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance. *Proc Natl Acad Sci* (2003) 100:6825–30. doi: 10.1073/pnas.0631828100

21. Pospisilik JA, Martin J, Doty T, Ehses JA, Pamir N, Lynn FC, et al. Dipeptidyl peptidase IV inhibitor treatment stimulates β-cell survival and islet neogenesis in streptozotocin-induced diabetic rats. *Diabetes* (2003) 52:741–50. doi: 10.2337/diabetes.52.3.741

22. Takeda Y, Fujita Y, Honjo J, Yanagimachi T, Sakagami H, Takiyama Y, et al. Reduction of both beta cell death and alpha cell proliferation by dipeptidyl peptidase-4 inhibition in a streptozotocin-induced model of diabetes in mice. *Diabetologia* (2012) 55:404–12. doi: 10.1007/s00125-011-2365-4

23. Tian L, Gao J, Hao J, Zhang Y, Yi H, O'Brien TD, et al. Reversal of new-onset diabetes through modulating inflammation and stimulating β-cell replication in nonobese diabetic mice by a dipeptidyl peptidase IV inhibitor. *Endocrinology* (2010) 151:3049–60. doi: 10.1210/en.2010-0068

24. Jelsing J, Vrang N, van Witteloostuijn SB, Mark M, Klein T. The DPP4 inhibitor linagliptin delays the onset of diabetes and preserves β-cell mass in non-obese diabetic mice. *J Endocrinol* (2012) 214:381–7. doi: 10.1530/JOE-11-0479

25. Foley J, Ligueros-Saylan M, He Y-L, Holst J, Deacon C, Dunning B, et al. Effect of vildagliptin on glucagon concentration during meals in patients with type 1 diabetes. *Horm Metab Res* (2008) 40:727–30. doi: 10.1055/s-2008-1078754

26. Farngren J, Persson M, Schweizer A, Foley JE, Ahrén B. Vildagliptin reduces glucagon during hyperglycemia and sustains glucagon counterregulation during hypoglycemia in type 1 diabetes. *J Clin Endocrinol Metab* (2012) 97:3799–806. doi: 10.1210/jc.2012-2332

27. Zhao Y, Yang L, Xiang Y, Liu L, Huang G, Long Z, et al. Dipeptidyl peptidase 4 inhibitor sitagliptin maintains β-cell function in patients with recent-onset latent autoimmune diabetes in adults: One year prospective study. *J Clin Endocrinol Metab* (2014) 99:E876–80. doi: 10.1210/jc.2013-3633

28. Garg SK, Moser EG, Bode BW, Klaff LJ, Hiatt WR, Beatson C, et al. Effect of sitagliptin on post-prandial glucagon and GLP-1 levels in patients with type 1 diabetes: Investigator-initiated, double-blind, randomized, placebo-controlled trial. *Endocr Pract* (2013) 19:19–28. doi: 10.4158/EP12100.OR

29. Al-Kuraishy HM, Hussian NR, Al-Naimi MS, Al-Gareeb AI, Al-Mamorri F, Al-Buhadily AK. The potential role of pancreatic γ-aminobutyric acid (GABA) in diabetes mellitus: A critical reappraisal. *Int J Prev Med* (2021) 12:19. doi: 10.4103/ijpvm.IJPVM_278_19

30. Prud'homme GJ, Glinka Y, Wang Q. Immunological GABAergic interactions and therapeutic applications in autoimmune diseases. *Autoimmun Rev* (2015) 14:1048–56. doi: 10.1016/j.autrev.2015.07.011

31. Rachdi L, Maugein A, Pechberty S, Armanet M, Hamroune J, Ravassard P, et al. Regulated expression and function of the GABAB receptor in human pancreatic beta cell line and islets. *Sci Rep* (2020) 10:13469. doi: 10.1038/s41598-020-69758-6

32. Ben-Othman N, Vieira A, Courtney M, Record F, Gjernes E, Avolio F, et al. Long-term GABA administration induces alpha cell-mediated beta-like cell neogenesis. *Cell* (2017) 168:73–85.e11. doi: 10.1016/j.cell.2016.11.002

33. Liu W, Son DO, Lau HK, Zhou Y, Prud'homme GJ, Jin T, et al. Combined oral administration of GABA and DPP-4 inhibitor prevents beta cell damage and promotes beta cell regeneration in mice. *Front Pharmacol* (2017) 8:362. doi: 10.3389/fphar.2017.00362

34. Pozzilli P, Maddaloni E, Buzzetti R. Combination immunotherapies for type 1 diabetes mellitus. *Nat Rev Endocrinol* (2015) 11:289–97. doi: 10.1038/nrendo.2015.8

35. Suarez-Pinzon WL, Cembrowski GS, Rabinovitch A. Combination therapy with a dipeptidyl peptidase-4 inhibitor and a proton pump inhibitor restores normoglycaemia in non-obese diabetic mice. *Diabetologia* (2009) 52:1680–2. doi: 10.1007/s00125-009-1390-z

36. Suarez-Pinzon WL, Rabinovitch A. Combination therapy with a dipeptidyl peptidase-4 inhibitor and a proton pump inhibitor induces β-cell neogenesis from

adult human pancreatic duct cells implanted in immunodeficient mice. *Cell Transplant* (2011) 20:1343–50. doi: 10.3727/096368910X557263

37. Magami Y, Azuma T, Inokuchi H, Moriyasu F, Kawai K, Hattori T. Heterogeneous cell renewal of pancreas in mice: [3H]-thymidine autoradiographic investigation. *Pancreas* (2002) 24:153–60. doi: 10.1097/00006676-200203000-00006

38. Gvazava IG, Rogovaya OS, Borisov MA, Vorotelyak EA, Vasiliev AV. Pathogenesis of type 1 diabetes mellitus and rodent experimental models. *Acta Naturae* (2018) 10:24–33. doi: 10.32607/20758251-2018-10-1-24-33

39. Chen Y-G, Mathews CE, Driver JP. The role of NOD mice in type 1 diabetes research: Lessons from the past and recommendations for the future. *Front Endocrinol (Lausanne)* (2018) 9:51. doi: 10.3389/fendo.2018.00051

40. von Herrath M, Nepom GT. Animal models of human type 1 diabetes. *Nat Immunol* (2009) 10:129–32. doi: 10.1038/ni0209-129

41. Todd JA, Wicker LS. Genetic protection from the inflammatory disease type 1 diabetes in humans and animal models. *Immunity* (2001) 15:387–95. doi: 10.1016/S1074-7613(01)00202-3

42. Mathews CE. Utility of murine models for the study of spontaneous autoimmune type 1 diabetes. *Pediatr Diabetes* (2005) 6:165–77. doi: 10.1111/j.1399-543X.2005.00123.x

43. American Diabetes Association. Standards of Medical Care in Diabetes—2019 Abridged for Primary Care Providers. *Clin Diabetes* (2019) 37:11–34. doi: 10.2337/cd18-0105

44. Carr ALJ, Perry DJ, Lynam AL, Chamala S, Flaxman CS, Sharp SA, et al. Histological validation of a type 1 diabetes clinical diagnostic model for classification of diabetes. *Diabetes Med* (2020) 37:2160–8. doi: 10.1111/dme.14361

45. Jamiołkowska-Sztabkowska M, Głowińska-Olszewska B, Bossowski A. C-peptide and residual β-cell function in pediatric diabetes – state of the art. *Pediatr Endocrinol Diabetes Metab* (2021) 27:123–33. doi: 10.5114/pedm.2021.107165

46. Pociot F. Capturing residual beta cell function in type 1 diabetes. *Diabetologia* (2019) 62:28–32. doi: 10.1007/s00125-018-4768-y

47. Jones AG, Hattersley AT. The clinical utility of c-peptide measurement in the care of patients with diabetes. *Diabetes Med* (2013) 30:803–17. doi: 10.1111/dme.12159

48. Greenbaum CJ, Harrison LC. Guidelines for intervention trials in subjects with newly diagnosed type 1 diabetes. *Diabetes* (2003) 52:1059–65. doi: 10.2337/diabetes.52.5.1059

49. Prud'homme GJ, Glinka Y, Udovyk O, Hasilo C, Paraskevas S, Wang Q. GABA protects pancreatic beta cells against apoptosis by increasing SIRT1 expression and activity. *Biochem Biophys Res Commun* (2014) 452:649–54. doi: 10.1016/j.bbrc.2014.08.135

50. Soltani N, Qiu H, Aleksic M, Glinka Y, Zhao F, Liu R, et al. GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes. *Proc Natl Acad Sci* (2011) 108:11692–7. doi: 10.1073/pnas.1102715108

51. Dalle S, Burcelin R, Gourdy P. Specific actions of GLP-1 receptor agonists and DPP4 inhibitors for the treatment of pancreatic β-cell impairments in type 2 diabetes. *Cell Signal* (2013) 25:570–9. doi: 10.1016/j.cellsig.2012.11.009

52. Drucker DJ. Incretin action in the pancreas: Potential promise, possible perils, and pathological pitfalls. *Diabetes* (2013) 62:3316–23. doi: 10.2337/db13-0822

53. Lee Y-S, Jun H-S. Anti-diabetic actions of glucagon-like peptide-1 on pancreatic beta-cells. *Metabolism* (2014) 63:9–19. doi: 10.1016/j.metabol.2013.09.010

54. Lee SA, Kim YR, Yang EJ, Kwon E-J, Kim SH, Kang SH, et al. CD26/DPP4 levels in peripheral blood and T cells in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* (2013) 98:2553–61. doi: 10.1210/jc.2012-4288

55. Shao W, Liu W, Liang P, Song Z, Israel O, Prud'homme GJ, et al. GABA requires GLP-1R to exert its pancreatic function during STZ challenge. *J Endocrinol* (2020) 246:207–22. doi: 10.1530/JOE-20-0109

56. He X, Li W, Xie Y, Zhao Y. Long-term inhibition of dipeptidyl-peptidase 4 reduces islet infiltration and downregulates IL-1β and IL-12 in NOD mice. *Int Immunopharmacol* (2020) 88:106945. doi: 10.1016/j.intimp.2020.106945

57. Liu W, Lau HK, Son DO, Jin T, Yang Y, Zhang Z, et al. Combined use of GABA and sitagliptin promotes human β-cell proliferation and reduces apoptosis. *J Endocrinol* (2021) 248:133–43. doi: 10.1530/JOE-20-0315

58. Son DO, Liu W, Li X, Prud'homme GJ, Wang Q. Combined effect of GABA and glucagon-like peptide-1 receptor agonist on cytokine-induced apoptosis in pancreatic β-cell line and isolated human islets. *J Diabetes* (2019) 11:563–72. doi: 10.1111/1753-0407.12881

59. Téllez N, Joanny G, Escoriza J, Vilaseca M, Montanya E. Gastrin treatment stimulates β-cell regeneration and improves glucose tolerance in 95% pancreatectomized rats. *Endocrinology* (2011) 152:2580–8. doi: 10.1210/en.2011-0066

60. Castillo GM, Nishimoto-Ashfield A, Banerjee AA, Landolfi JA, Lyubimov AV, Bolotin EM. Omeprazole and PGC-formulated heparin binding epidermal growth factor normalizes fasting blood glucose and suppresses insulitis in multiple low dose streptozotocin diabetes model. *Pharm Res* (2013) 30:2843–54. doi: 10.1007/s11095-013-1112-6

61. Rajput MA, Ali F, Zehra T, Zafar S, Kumar G. The effect of proton pump inhibitors on glycaemic control in diabetic patients. *J Taibah Univ Med Sci* (2020) 15:218–23. doi: 10.1016/j.jtumed.2020.03.003

62. Phadatare PD, Chandrashekhar VM. Influence of esomeprazole on hypoglycemic activity of oral antidiabetic agents in rats and rabbits. *Mol Cell Biochem* (2011) 354:135–40. doi: 10.1007/s11010-011-0812-7

63. Hirao J, Tojo A, Hatakeyama S, Satonaka H, Ishimitsu T. V-ATPase blockade reduces renal gluconeogenesis and improves insulin secretion in type 2 diabetic rats. *Hypertens Res* (2020) 43:1079–88. doi: 10.1038/s41440-020-0450-0

64. Moritoh Y, Takeuchi K, Asakawa T, Kataoka O, Odaka H. Combining a dipeptidyl peptidase-4 inhibitor, alogliptin, with pioglitazone improves glycaemic control, lipid profiles and β-cell function in db/db mice. *Br J Pharmacol* (2009) 157:415–26. doi: 10.1111/j.1476-5381.2009.00145.x

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A model to design financially sustainable algorithm-enabled remote patient monitoring for pediatric type 1 diabetes care

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Introduction: Population-level algorithm-enabled remote patient monitoring (RPM) based on continuous glucose monitor (CGM) data review has been shown to improve clinical outcomes in diabetes patients, especially children. However, existing reimbursement models are geared towards the direct provision of clinic care, not population health management. We developed a financial model to assist pediatric type 1 diabetes (T1D) clinics design financially sustainable RPM programs based on algorithm-enabled review of CGM data.

Methods: Data were gathered from a weekly RPM program for 302 pediatric patients with T1D at Lucile Packard Children's Hospital. We created a customizable financial model to calculate the yearly marginal costs and revenues of providing diabetes education. We consider a baseline or status quo scenario and compare it to two different care delivery scenarios, in which routine appointments are supplemented with algorithm-enabled, flexible, message-based contacts delivered according to patient need. We use the model to estimate the minimum reimbursement rate needed for telemedicine contacts to maintain revenue-neutrality and not suffer an adverse impact to the bottom line.

Results: The financial model estimates that in both scenarios, an average reimbursement rate of roughly $10.00 USD per telehealth interaction would be sufficient to maintain revenue-neutrality. Algorithm-enabled RPM could potentially be billed for using existing RPM CPT codes and lead to margin expansion.

Conclusion: We designed a model which evaluates the financial impact of adopting algorithm-enabled RPM in a pediatric endocrinology clinic serving T1D patients. This model establishes a clear threshold reimbursement value for

maintaining revenue-neutrality, as well as an estimate of potential RPM reimbursement revenue which could be billed for. It may serve as a useful financial-planning tool for a pediatric T1D clinic seeking to leverage algorithm-enabled RPM to provide flexible, more timely interventions to its patients.

KEYWORDS

type 1 diabetes (T1D), continuous glucose monitoring (CGM), remote patient monitoring (RPM), algorithm-enabled telemedicine, hemoglobin A1c (HbA1c), pediatrics, health economics, population health

Background and aims

The use of continuous glucose monitoring (CGM) is recommended as standard of care by the American Diabetes Association (ADA) for individuals with type 1 diabetes (T1D) and is associated with improved glycemic outcomes and quality of life (1–3). Analyzing and interpreting CGM data for a large number of patients in the clinical setting is challenging in part because of the complexities of data aggregation and a lack of Electronic Medical Record (EMR) integration. In a previous analysis (4–6), we examined a new, telemedicine-based T1D care model based on the use of a remote patient monitoring (RPM) tool that analyzes CGM data and identifies patients likely to benefit from contact from the diabetes care team. This algorithm-enabled tool, known as Timely Interventions for Diabetes Excellence (TIDE), facilitates personalized care for the entire population cared for by the clinic as part of the Teamwork, Targets, Technology, and Tight Control (4T) Study, in which CGM was initiated in the first month for youth with new-onset T1D. In this context, the program – combined with the use of TIDE – was associated with a 0.5% reduction in hemoglobin A1c (HbA1c) and an 86% reduction (4–10) in provider review time per patient. This algorithm-enabled tool, which drastically reduces the per-patient time required for review and ranks patients by who may benefit most from review, may help patients achieve better glycemia while also increasing a clinic's per-patient capacity. Moreover, algorithm-enabled CGM data review presents an opportunity to expand access to a larger population base, other clinics, and underserved populations, especially those in rural areas which do not have local access to pediatric endocrinologists.

Currently, TIDE is deployed in the context of the 4T Study, meaning that the process of CGM data review is not being billed for. An additional focus of the 4T Study is articulating a sustainable payment model which would allow remote patient monitoring, facilitated by a tool such as TIDE, to be billed for as a component of routine patient care. While the technology is promising (11–13), current provider payment models are based on the direct provision of clinical care, not population health management. Developing robust analytics to serve a T1D population requires investment in data collection, curation, and analysis, and benefits from scale in terms of the amount of data available to clinical staff. While there is recognition that investment is required to develop technology to support high-performing clinical services and Medicare has reimbursement models for RPM, there is not yet an analogous reimbursement model in place for T1D with RPM. Ironically, the benefits of using algorithm-enabled RPM may be the very same factors which also preclude it from being financially sustainable in a traditional Fee-For-Service (FFS) setting. For example, the fact that algorithm-enabled RPM requires significantly less provider time per patient may result in unsustainably low rates of reimbursement or fail to meet the minimum threshold for any reimbursement.

We created a model to design financially sustainable population-level algorithm-enabled RPM based on CGM data review for a diabetes clinic seeking to serve pediatric T1D patients under a hybrid care model where routine appointments delivered by Certified Diabetes Care and Education Specialists (CDCES) are supplemented with flexible, timelier message-based contacts delivered according to patient need. Though these messages are not like-for-like replacements for in-depth education, they can provide quick, actionable feedback to "nudge" patients in a positive direction (14). This model explores a capacity-neutral scenario in which a clinic incorporates algorithm-enabled telemedicine into T1D care and a scenario requiring additional capacity. While maintaining clinic capacity, we examine the feasibility of decreasing the frequency of routine diabetes education visits and repurposing the existing capacity for algorithm-enabled telemedicine. When augmenting clinic capacity, we consider the feasibility of maintaining the regular cadence of routine visits and

Abbreviations: Remote patient monitoring (RPM); Type 1 diabetes (T1D); Continuous glucose monitoring (CGM); Certified diabetes care and education specialist (CDCES); Lucile Packard Children's Hospital Stanford (LPCH); Teamwork, Targets, Technology and Tight Control (4T); Timely Interventions for Diabetes Excellence (TIDE); Fee-for-Service (FFS).

delivering algorithm-enabled telemedicine services as a supplement to routine care. In both scenarios, our aim is to quantify the financial impact of transitioning to algorithm-enabled telemedicine on a clinic's bottom line and estimate the minimum reimbursement rate necessary from telemedicine interactions to maintain cost-neutrality while reproducing previously observed improvements to A1c.

The one-time fixed costs of developing and deploying the necessary hardware and software vary significantly. For institutions with an established telemedicine program, the costs would primarily stem from the provider time for operational planning, training, and patient recruitment. These costs also apply to institutions interested in using free, online, open-source tools, as well as those with Tableau Server already deployed interested in using the free Tableau-based tool. For institutions interested in deploying with enterprise data visualization software (e.g., Tableau or PowerBI) with automatic feeds from the electronic medical record, costs can range from tens of thousands to hundreds of thousands of dollars per year (depending on the server type and the number of users). Of course, most institutions would likely use such enterprise software for a variety of projects and initiatives. For a full integration with the electronic medical record, the costs are difficult to estimate, due to the difficulty of working with modern EMRs, but could be on the order of hundreds of thousands of dollars and require months of effort. In this work we focus primarily on the marginal costs of deploying an algorithm-enabled tool, since an estimate of these costs would be a central component in deciding how much to invest in launching the program.

Although this analysis is grounded in our experience with TIDE in the context of the 4T research study at Lucile Packard Children's Hospital Stanford (LPCH), it is broadly applicable to any clinic currently using their own version of a tool like TIDE, or seeking to build an algorithm-enabled tool which allocates capacity based on patient need. Moreover, though this analysis primarily applies to the US medical system, concepts of scalability are generalizable to other health care systems.

Methods

Setting and population

The protocol for the 4T Program (8), as well as a summary timeline showcasing the major milestones of the program (15), have been previously described. Briefly, all youth with newly diagnosed T1D between July 2018 and June 2020 were offered the opportunity to start on CGM (Dexcom G6, Dexcom Inc., San Diego, CA) in the first month of diabetes diagnosis following the clinic's standard of care. Those who chose to start on CGM had a follow-up visit (either in-person or telemedicine) with a CDCES to initiate CGM. At this visit, participants were provided with CGM supplies (i.e., a transmitter, three sensors, and a receiver) by the 4T Study team. The diabetes care team applied for ongoing insurance approval for CGM coverage and if it was not covered by insurance, the research study provided CGM supplies. One week after initiating CGM, the youth and family were encouraged to meet with a nurse practitioner *via* telemedicine for a follow-up visit for additional CGM education and continued with routine care.

Youth diagnosed in March 2019 or later were additionally offered the opportunity to participate in remote patient monitoring (clinical trial No. NCT03968055). Data were shared from the patient's device to the Dexcom Clarity cloud-based platform. To facilitate CGM data sharing, youth who did not have their own iOS device were provided with an iPod touch (Apple Inc., Cupertino, CA) for the duration of the study. Each week, CGM data were reviewed by a CDCES and when necessary, insulin dose adjustments were recommended using secure messaging within the EMR system. Initially, CDCES manually reviewed the CGM glucose data of every participant, but by January 2020, they relied on the TIDE population health management tool to facilitate 4T Study scaling to a larger patient population (4–6). The health management tool was based on CGM consensus guidelines for percent time in range (TIR; 70-180 mg/dL), time in hypoglycemia (<70 mg/dL), and time in clinically significant hypoglycemia (<54 mg/dL) (16). The modified tool prioritized patients for CDCES review by identifying participants with TIR of less than 65%, or percentage of time CGM was worn less than 50% over a one week period to increase scalability with constrained CDCES time, but the flags for hypoglycemia were unchanged (8). This protocol was approved by the Stanford Institutional Review Board (IRB) and informed consent (and assent for participants aged 7-18 years) was obtained for all participants.

Study population

TIDE is currently in use for youth in four institutional review board–approved studies: 4T pilot, 4T phase 1, 4T phase 2 (15), and CGM Time in Range Program at Stanford (CGM TIPS). Among the four, TIDE is now used to support scaling RPM to 299 youth with T1D (Table 1). All of those enrolled gave informed consent for the care team to review the data collected by their CGM every week and to send them a message with suggestions for glucose management, when appropriate.

Overview

We created a financial model to calculate the yearly marginal costs and revenues of providing diabetes education from the point of view of a diabetes clinic serving pediatric T1D patients. We evaluated two different care delivery scenarios and compared them to a status quo baseline scenario.

TABLE 1 Participants demographics for the 4T Pilot Study, 4T Study 1, 4T Study 2, and CGM TIPS Study.

	Study			
	Pilot	**4T Study 1**	**4T Study 2**	**CGM TIPs**
Participants, N	135	133	31	94
Age at T1D diagnosis, median (Q1-Q3), y	9.7 (6.8-12.7)	11 (7–14)	11 (9.5-14.0)	9.0 (5.2 - 11.6)
Sex				
Female, n (%)	64 (47.4)	60 (45)	20 (64.5)	48 (51.1)
Male, n (%)	71 (52.6)	73 (55)	11 (35.5)	46 (48.9)
Race/Ethnicity, n (%)				
Non-Hispanic White	50 (37.0)	43 (32.3)	7 (22.6)	18 (19.1)
Non-Hispanic Black	0 (0)	0 (0)	0 (0)	5 (5.3)
Hispanic	25 (18.5)	38 (28.6)	3 (9.7%)	40 (42.6)
Asian or Pacific Islander	17 (12.6)	13 (9.8)	3 (9.7%)	2 (2.1)
American Indian or Alaska Native	0 (0)	0 (0)	0 (0)	0 (0)
Other	11 (8.1)	13 (9.8)	12 (38.7)	7 (7.4)
Unknown	32 (23.7)	26 (19.5)	6 (19.4)	22 (23.4)
Insurance Type, n (%)				
Private	104 (77.0)	80 (60.2)	28 (90.3)	6 (6.4)
Public	31 (23.0)	47 (35.3)	3 (9.7)	82 (87.2)
Both	0 (0)	2 (1.5)	0 (0)	4 (4.3)
Unknown or No Insurance	0 (0)	4 (3.0)	0 (0)	2 (2.1)

In the baseline scenario, diabetes education is provided by CDCES at a fixed, regular cadence and reimbursed under a traditional FFS model. The incentives under this system are to ensure the maximum utilization of each resource, resulting in scheduled appointments with no allocation to flexible capacity. In this model, services are not optimized for patients requiring more assistance. Rather, all patients receive the same number of appointments, which are regularly scheduled throughout the year.

In a capacity-neutral scenario, we assumed that the clinic shifted to population-level algorithm-enabled RPM based on CGM data review. Some proportion of existing CDCES capacity allocated for regularly scheduled visits is re-allocated to flexible telemedicine visits reserved for patients who are most likely to benefit from contact. Rather than augmenting the clinic capacity with additional resources, this telemedicine capacity is drawn from existing resources, and the capacity devoted to routine care is reduced accordingly. As a result, the overall effect on clinic capacity is neutral, as is the effect on clinic costs (Figure 1).

In an augmented-capacity scenario, we assumed that the clinic maintained all routine appointments, but increased its overall capacity to deliver algorithm-enabled telemedicine over and above its existing care model. Moreover, the clinic incurs additional marginal labor costs to meet the increased CDCES capacity requirements (Figure 2).

In both population health scenarios, telemedicine capacity is flexible and allocated to patients with the highest need in each time period (4–6). In both cases, we then estimated the minimum reimbursement rate needed for telemedicine contacts to offset this revenue gap. If this payment threshold is met, the effect on the overall clinic bottom line is neutral. In addition, we evaluated the marginal revenue which could potentially be billed for using RPM billing codes and compared this to the break-even threshold value.

Base case

We modelled a cohort of 100 pediatric patients served by a T1D clinic. We assumed that patients received one diabetes education session every quarter (four visits per year), which is scheduled alongside concurrent routine appointments with other members of the clinical staff (e.g., endocrinologists, registered dieticians, psychologists). We assumed that diabetes education was provided entirely by the CDCES team at a fixed, regular cadence. We assumed an average per appointment reimbursement rate of $56.00 USD, which is reflective of the average reimbursement rate as per the 2022 Medicare National Fee Schedule for Diabetes outpatient self-management training services delivered by a CDCDS in an ADA-recognized program (per individual, per 30 minutes - CPT code G0108)[1]. This reimbursement rate is varied in sensitivity analysis and can be modified to reflect differences in state-specific rates, as well as payer environment and composition (e.g., to account for a mix

1 https://www.cms.gov/medicare/physician-fee-schedule/search?Y=0&T=4&HT=0&CT=0&H1=G0108&M=5

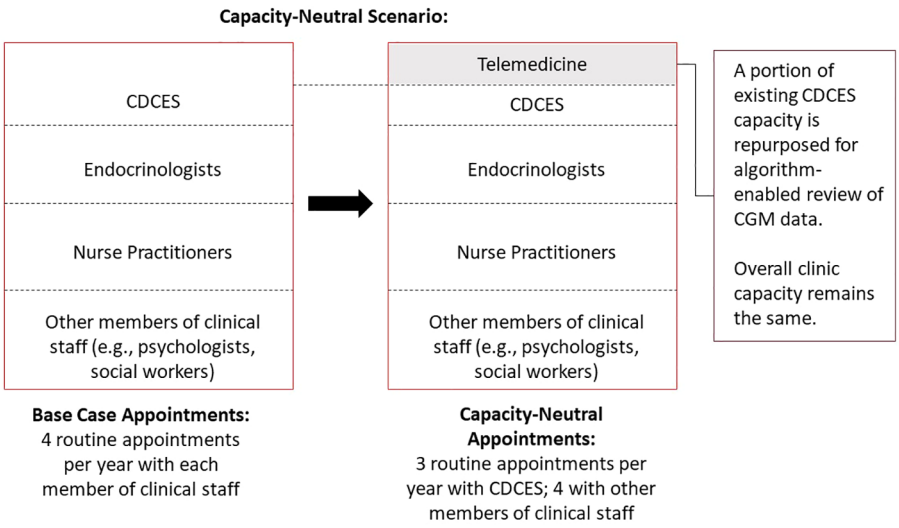

FIGURE 1
Schematic diagram of capacity-neutral scenario, whereby a clinic decreases the frequency of routine diabetes education visits and repurposes the existing capacity for algorithm-enabled telemedicine.

of private and public insurance plans with differential rates of reimbursement). Our model estimates the total marginal yearly reimbursement revenue from patients receiving diabetes education sessions at this fixed cadence. Given that our model only considers modifications to CDCES capacity and appointments, we did not estimate marginal revenue stemming from appointments with other members of the clinical staff, as these remain constant throughout our modeling scenarios.

We assumed that on average, a CDCES conducts five education sessions per day, five days per week, for a total of 25 education sessions per week. Over the course of 52 weeks per year, this corresponds to a total yearly capacity of 1,300 education sessions. Using this estimate of capacity, we

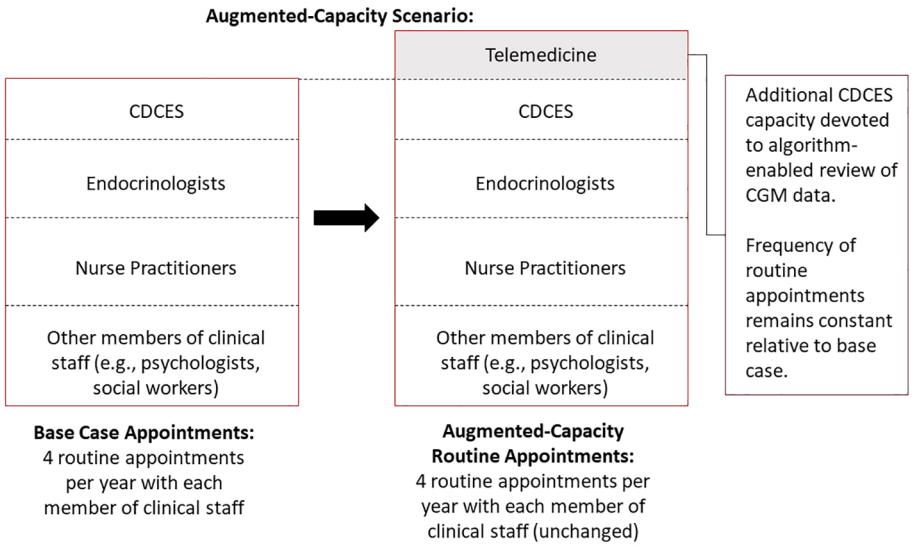

FIGURE 2
Schematic diagram of augmented-capacity scenario, whereby a clinic maintains all routine appointments but increases its overall capacity to deliver supplemental, algorithm-enabled telemedicine interactions.

TABLE 2 Key input parameters for base case under status quo, in which diabetes education is provided by CDCES at a fixed, regular cadence and reimbursed under a traditional FFS model.

Base Case	Value
Number of patients in practice	100
Routine visits per patient per year	4
Reimbursement rate per routine visit	$56
Marginal Reimbursement Revenue	$22,400
Annual CDCES salary	$85,000
CDCES capacity (appointments per year)	1,300
Total appointments needed (per year)	400
FTE needed	0.31
Total CDCES labor costs	$26,154

determined the number of full-time equivalent hours (FTE) necessary to provide coverage for our patient population. By pairing this with the average US national yearly salary of a CDCES reported by a commercial compensation database[2], we then calculated the total marginal CDCES labor costs associated with delivering care to the clinic population (Table 2). These base case parameters are defined as modifiable in our model.

Capacity-neutral scenario

In the capacity-neutral scenario, our assumptions for the size of the patient population, the reimbursement rate, and the CDCES labor costs and capacity remain constant relative to the base case scenario. However, we assumed that some of the existing CDCES capacity reserved for routine appointments is reallocated for flexible telemedicine contacts (i.e., for reviewing patients identified by algorithm-enabled CGM data review, and – if necessary – sending them a message through the EMR to suggest a dose change (4–6)). We assumed that one out of the four quarterly routine CDCES appointments was repurposed for telemedicine, meaning that all patients received one less routine education session per year (other appointments with other members of the clinical staff remain unchanged). However, given that algorithm-enabled data review takes up significantly less provider time than a routine diabetes education appointment, repurposing routine appointments frees up capacity for multiple flexible, telemedicine contacts. Assuming that an average routine appointment lasts 30 minutes, compared to 5 minutes for a message-based contact, then repurposing one routine appointment per patient frees up sufficient capacity for up to six message-based contacts.

TABLE 3 Key input parameters for capacity-neutral scenario, in which a proportion of existing CDCES capacity for routine appointments is repurposed for dynamically allocated, algorithm-enabled contacts.

Capacity-neutral scenario	Value
In person visits per patient per year	3
Marginal Reimbursement Revenue	$ 16,800
Revenue Gap	$ 5,600
Appointments repurposed for telehealth	100
Duration of in-person visit (mins)	30
Duration of telemedicine appointment (mins)	5
Total telemedicine appointments freed up	600
Minimum reimbursement rate per telemedicine appointment	$9.33
RPM reimbursement rate	$35
Potential marginal RPM reimbursement revenue	$ 21,000
Margin	$ 15,400

Given a population of 100 patients, this flexible telemedicine capacity amounts to a total of 600 message-based contacts per year, or 50 per month, which provides telemedicine coverage for 50% of the total patient population in every monthly review period. We assumed that all of this capacity is utilized by the 50% of patients who meet the criteria for intervention. Note that this capacity is flexible, and not earmarked for any patients in particular. Given that algorithm-enabled RPM ranks and identifies patients who are most likely to benefit from contact, some patients may receive more contacts than others in a given year, while some may not receive any.

We then calculated the marginal revenue lost from repurposing routine education sessions. Based on the number of message-based contacts delivered, we estimated the minimum reimbursement rate per telemedicine contact necessary to offset this revenue loss and maintain strict revenue-neutrality. In addition, we calculated the potential marginal revenue which could be billed for using existing RPM CPT codes and compared this to the break-even threshold value. We assumed an average reimbursement rate of $35.00 USD per telemedicine-based contact, which reflects the average reimbursement rate as per the 2022 Medicare National Fee Schedule for analysis and interpretation of CGM data (CPT code 95251)[3] (Table 3).

Augmented-capacity scenario

In the augmented-capacity scenario, our assumptions for the size of the patient population, reimbursement rate, and the frequency of routine visits remained constant relative to the base case. However, we assumed that the clinic increased its

2 https://www.salary.com/research/salary/benchmark/diabetes-educator-salary

3 https://www.cms.gov/medicare/physician-fee-schedule/search?Y=0&T=4&HT=0&CT=0&H1=95251&M=5

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CDCES capacity in a two-step approach. In the first year, we assumed a capacity increase sufficient to review data for up to 75% of patients in any given review period (which is in line with the proportion of patients failing to meet the target HbA1c observed in our baseline population and the US pediatric T1D population) (9, 17). We assumed that more intensive management of patients who did not reach HbA1c targets in the first year improved overall outcomes on the long-term, and that in the second year, the clinic would reduce this extra capacity to provide algorithm-enabled telemedicine coverage for 50% of its patient population. This mirrors the improved, long-term outcomes observed over the course of the 4T Study (9). Moreover, these parameters are modifiable, and can be adjusted to reflect the makeup of a clinic's patient population.

Given a population of 100 patients, these additional capacity requirements translate to 75 telemedicine interactions needed per month in the first year (i.e. 900 total), and 50 needed per month in the second year (i.e. 600 total). Based on the yearly capacity of a CDCES, we estimated the additional FTE needed to deliver these appointments. We then calculated the additional marginal labor costs incurred from increasing CDCES capacity. Based on the number of message-based contacts delivered, we then estimated the minimum telemedicine reimbursement rate per telemedicine contact necessary to offset these additional marginal costs and maintain strict revenue-neutrality. In addition, we calculated the potential marginal revenue which could be billed for using existing RPM CPT codes, assuming an average reimbursement rate of $35.00 USD per telemedicine-based contact as in the capacity-neutral scenario[4] (Table 4).

The input parameters for both scenarios are comparable to the ones observed in our patient population and are defined as modifiable in our financial model.

TABLE 4 Key input parameters for augmented-capacity scenario, in which clinic maintains all routine appointments, but increases overall capacity to deliver algorithm-enabled telemedicine over and above its existing care model.

Augmented-Capacity Scenario	Value
Year 1	
Percentage of patients receiving telemedicine	75%
Telemedicine appointments needed per year	900
CDCES capacity (appointments per year)	1300
Additional FTE needed	0.12
Additional marginal CDCES labor costs	$ 9,808
Minimum reimbursement rate per telemedicine appointment	$10.90
RPM reimbursement rate	$35
Potential marginal RPM reimbursement revenue	$ 31,500
Margin	$ 21,692
Year 2	
Percentage of patients receiving telehealth	50%
Telehealth appointments needed	600
CDCES capacity (appointments per year)	1300
Additional FTE needed	0.08
Additional marginal CDCES labor costs	$ 6,538
Minimum reimbursement rate per telemedicine appointment	$10.90
RPM reimbursement rate	$35
Potential marginal RPM reimbursement revenue	$ 21,000
Margin	$ 14,462

Projecting impact of improved outcomes

Given that the 4T program and the use of the TIDE tool to identify participants who would benefit from dose adjustments was associated with a 0.5% reduction in HbA1c (9), we examine scenarios in which personalized, timely, telemedicine-based interventions may lead to long-term improved patient outcomes (18), and therefore reduced healthcare costs in the long-term (e.g., by reducing the incidence of diabetic ketoacidosis and chronic vascular complications, or by driving down the demand for CGM data review). Accordingly, as an additional input for the model, a user may indicate how much a 0.5% improvement in HbA1c would be worth to their clinic, on average per patient. The value stemming from improved patient outcomes is then subtracted from the revenue gap to calculate the resulting, reduced minimum reimbursement rate to ensure strict revenue-neutrality.

Sensitivity analysis

We varied the number of routine appointments delivered per year, the corresponding reimbursement rate, the CDCES capacity, the CDCES salary, the number of routine appointments shifted to telemedicine, the average time per appointment, and the value of a 0.5% improvement in HbA1c in one-way sensitivity analyses, and evaluated the impact on the minimum reimbursement rate.

Results

Financial impact of capacity-neutral scenario

For an initial cohort of 100 pediatric patients, shifting one out of four yearly routine diabetes education appointments to telemedicine translates to repurposing 100 diabetes education sessions. Given these are reimbursed at a rate of $56.00 USD per

4 https://www.cms.gov/medicare/physician-fee-schedule/search?Y=0&T=4&HT=0&CT=0&H1=95251&M=5

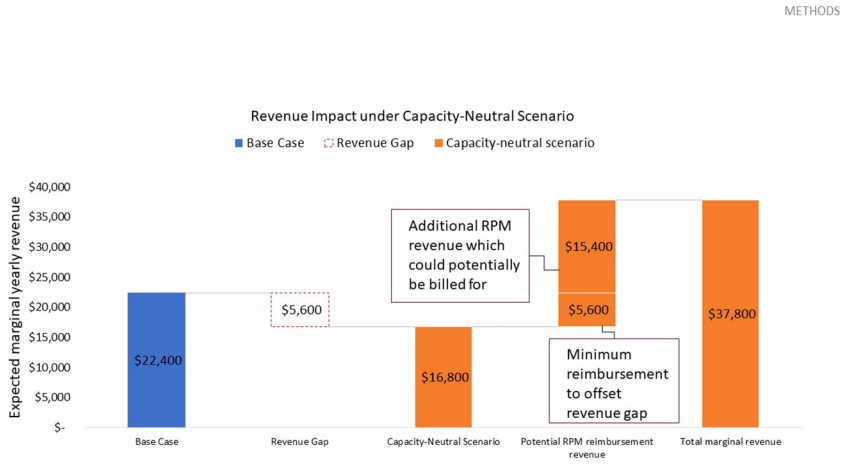

FIGURE 3
Expected yearly marginal revenue under capacity-neutral scenario, assuming 600 total telehealth appointments delivered, a $9.33 USD reimbursement rate to offset the revenue gap, and a potential RPM reimbursement rate of $35.00 USD based on existing CPT codes.

session[5], this results in a marginal revenue loss of $5,600.00 USD per year. As telemedicine interactions only take 5 minutes (vs 30 minutes for an education session), sufficient capacity is freed up for 600 messaged-based contacts per year. Assuming these are flexibly booked out in their entirety in each review period, these telemedicine interactions would need to be reimbursed at a minimum rate of roughly $9.33 USD per contact to bridge the revenue gap. In this capacity-neutral view, no additional investment in clinic resources would be necessary, and CDCES labor costs remain constant. Given that remote CGM analysis and interpretation is currently reimbursed at a rate of $35.00 USD[6], the potential marginal RPM revenue which could be billed for is sufficient to offset this revenue loss. In fact, this marginal RPM revenue totals $21,000.00 USD and results in a margin increase of $15,400.00 USD, relative to the base case (Table 3 and Figure 3).

Financial impact of augmented-capacity scenario

For an initial cohort of 100 pediatric patients, adding sufficient telemedicine capacity to cover 75% of patients in each review period translates to 900 telemedicine interactions in year 1. Given that a CDCES, on average, can conduct 1,300 education sessions per year – or 7,800 telemedicine contacts per year – this translates to an additional 0.12 FTE. Given a yearly salary of $85,000.00 USD for a CDCES[7], the clinic incurs an additional $9,808.00 USD in marginal CDCES labor costs in year 1. In year 2, assuming sufficient telemedicine capacity to provide coverage for 50% of patients (which translates to 600 telemedicine interactions), the clinic requires an additional 0.08 FTE and incurs an additional $6,538.00 USD in marginal labor costs. If flexibly booked out in their entirety in each review period, these telemedicine interactions would need to be reimbursed at a minimum rate of roughly $10.90 USD per contact to bridge the revenue gap, in both year 1 and year 2. In this augmented-capacity view, the frequency of routine appointments remains unchanged, and the corresponding marginal revenue remains constant.

Given that remote CGM analysis and interpretation is currently reimbursed at a rate of $35.00 USD[8], the potential marginal RPM revenue which could be billed for is sufficient to offset this revenue loss. In fact, in year 1, this marginal RPM revenue totals $31,500.00 USD and results in a margin increase of $21,692.00 USD, relative to the base case. In year 2, this marginal RPM revenue totals $21,000.00 USD and results in a margin increase of $14,462.00 USD, relative to the base case (Table 4 and Figure 4).

A side-by-side summary of the key inputs and outputs of the model for the base case, capacity-neutral, and augmented-capacity scenarios is included in Table 5.

5 https://www.cms.gov/medicare/physician-fee-schedule/search?Y=0&T=4&HT=0&CT=0&H1=G0108&M=5

6 https://www.cms.gov/medicare/physician-fee-schedule/search?Y=0&T=4&HT=0&CT=0&H1=95251&M=5

7 https://www.salary.com/research/salary/benchmark/diabetes-educator-salary

8 https://www.cms.gov/medicare/physician-fee-schedule/search?Y=0&T=4&HT=0&CT=0&H1=95251&M=5

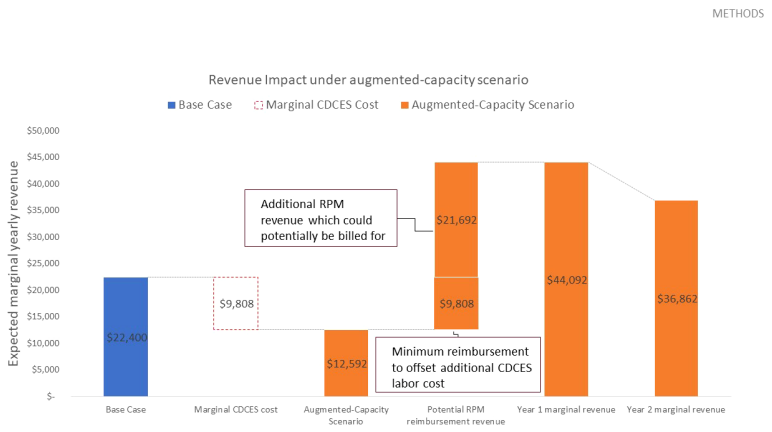

FIGURE 4
Expected yearly marginal revenue under augmented-capacity scenario, assuming 900 total telehealth appointments delivered in year 1 and 600 in year 2, a $10.90 USD reimbursement rate to offset the additional CDCES labor costs, and a potential RPM reimbursement rate of $35.00 USD based on existing CPT codes.

Sensitivity analysis

In the capacity-neutral scenario, the minimum telemedicine reimbursement rate was sensitive only to the routine appointment reimbursement rate, the average time per visit, and the value of a 0.5% improvement in HbA1c. The routine reimbursement rate determines the base case marginal reimbursement revenue received by the clinic, and therefore the magnitude of the revenue loss when transitioning to telemedicine. The average time per visit determines the number of message-based contacts that can be freed up by repurposing a routine appointment, and therefore the fraction of lost marginal revenue that each message-based contact must recoup. If repurposing one routine visit per patient frees up sufficient capacity for six telemedicine visits per patient, then the minimum telemedicine reimbursement rate is one sixth of the routine reimbursement rate to maintain revenue-neutrality (Table 6).

In the augmented-capacity scenario, the minimum telemedicine reimbursement rate was sensitive only to the salary of a CDCES, the capacity of a CDCES, and the value of a 0.5% improvement in HbA1c. Given that the additional marginal labor costs are proportional to the number of appointments delivered, it stands to reason that the capacity and the salary of a CDCES would have a linear effect on the marginal costs incurred (Table 6).

In both cases, as the value derived by the clinic from a 0.5% improvement in HbA1c increases, the magnitude of the revenue gap shrinks. Beyond a certain threshold value, the benefit of achieving improved outcomes may be sufficient in and of itself to recoup the lost reimbursement revenue. In a healthcare system

TABLE 5 Side-by-side comparison of key input and output parameters of the financial model for base case, capacity-neutral scenario, and augmented-capacity scenario.

Parameters	Base case	Capacity-Neutral scenario	Augmented-Capacity Scenario
Routine visits per patient per year	4	3	4
Reimbursement rate per routine visit	$56	$56	$56
CDCES capacity (appointments per year)	1,300	1,300	1,300
Annual CDCES salary	$85,000	$85,000	$85,000
Duration of in-person visit (mins)	30	30	30
Duration of telemedicine appointment (mins)	5	5	5
Routine appointments repurposed for telehealth	N/A	100	0
Lost revenue from routine appointments	N/A	$5,600	0
Additional FTE needed	N/A	0	0.12
Additional marginal CDCES labor costs	N/A	0	$9,808
Minimum reimbursement rate per telemedicine appointment	N/A	$9.33	$10.90

N/A means not applicable for the base case.

TABLE 6 Selected results of one-way sensitivity analyses, showing key input parameters which have an impact on the minimum reimbursement rate per telemedicine appointment needed to maintain revenue-neutrality.

Sensitivity Analysis	Minimum reimbursement rate per telemedicine appointment
Capacity-Neutral Scenario	$ 9.33
50% increase in routine appointment reimbursement rate	$ 14.00
50% decrease in routine appointment reimbursement rate	$ 4.67
50% increase in average time per routine appointment	$ 6.22
50% decrease in average time per routine appointment	$ 18.67
Augmented-Capacity Scenario	$ 10.90
50% increase in salary of CDCES	$ 16.35
50% decrease in salary of CDCES	$ 5.45
50% increase in capacity of CDCES	$ 7.26
50% decrease in capacity of CDCES	$ 21.79

in which patients are cared for long-term, reductions in long-term complications which are predicted by HbA1c may lead to cost savings.

Discussion

We created a model to design financially sustainable algorithm-enabled CGM data review for a clinic that serves pediatric T1D patients. This model is aligned with the 4T Study and the TIDE tool, which has been deployed for population health management of our patients but has not yet been billed for as a routine component of diabetes care. While this model closely tracks the characteristics of our environment, it has broad applicability beyond our clinic, and can serve as a capacity- and financial-planning tool for a pediatric T1D clinic seeking to leverage algorithm-enabled RPM to provide flexible, more timely interventions to its patients. Given the rapid transition to remote care spurred by the COVID-19 pandemic (19), T1D clinics expanding their telemedicine offerings may be especially interested in population-level algorithm-enabled RPM based on CGM data review, whose unique value proposition includes reduced administrative burden, reduced provider review time per patient, more intensive, targeted management of patients failing to meet HbA1c targets, improved patient and parent satisfaction, and improved patient quality of life and glycemic outcomes. Financial planning can be facilitated by and should be done in complement with operational planning. To that end, this financial model can be used in combination with the capacity planning dashboard developed by our team to contribute to care delivery that is both operationally efficient and financially sustainable (20).

However, T1D clinics may be reticent to invest in deploying this tool out of a concern that it may unduly disrupt clinic operations and lead to increased costs. To address these concerns, we have explored two possible pathways to adopting algorithm-enabled RPM. While some institutions may wish to convert their existing capacity, others may have a financially viable path to making an upfront investment in expanding their CDCES capacity. In both cases, our analysis reveals that despite a substantially lower reimbursement rate for remote analysis and interpretation of CGM data compared to a routine diabetes education session ($35.00 vs $56.00 USD), algorithm-enabled CGM data review is financially sustainable due to its scalability. By expediting data analysis and interpretation and cutting down on provider review time, algorithm-enabled RPM allows monitoring multiple patients in the same time that it would take to provide a diabetes education session to a single patient. As a result, the lower reimbursement rate is offset by the sheer volume of telemedicine-based contacts that can be provided in a comparable timeframe. Moreover, it is important to note that our estimates of potential reimbursement are conservative. For example, we have only considered the potential revenue stemming from CGM interpretation and analysis, however other CPT codes (e.g., 95250 for initial training and set-up of CGM) could also be billed for. Moreover, we have assumed an average reimbursement rate in line with the 2022 Medicare National Fee Schedule. However, in practice reimbursement rates for privately insured patients is typically higher.

Even if this level of RPM reimbursement is not achievable, there are other reasons to believe that the long-term benefits of using algorithm-enabled RPM may justify its initial investment. A population health model of care which dynamically allocates capacity to patients who need it the most is likely to lead to additional sources of long-term cost savings stemming from improved patient outcomes. Moreover, reducing the provider review time per patient increases the number of patients that can be seen per provider, which translates to reduced FTE (and its attendant cost savings), an increased patient population, additional visits per patient beyond the recommended four per year, or some combination of these.

We recognize that there are limitations in our model. Though our algorithm-enabled tool has been made available as open-source software, additional ancillary costs may need to be

factored into the cost of deploying the tool (e.g., hosting, technical support, customization costs), and may therefore increase the minimum reimbursement rate. In particular, our model does not account for the one-time fixed costs of developing and deploying the necessary hardware and software, can be used as a financial tool to assist stakeholders in deciding how much to spend on fixed costs. In the capacity-neutral view, we assumed that one out of the four quarterly routine appointments is repurposed for telemedicine, however in practice a clinic may not wish to reduce routine appointments unilaterally for all patients (e.g., new-onset patients typically require additional appointments in the first year after diagnosis). We assumed that all flexible, telemedicine capacity could be fully booked out in any given monthly review period, and that none of this capacity would sit idle. In the most extreme case, our augmented-capacity model assumed that in year 1, 75% of patients would meet criteria for review. Although it is possible that fewer patients would be eligible for a telemedicine-based contact, it is important to note that the ADA HbA1c goal of <58 mmol/mol for youth was achieved by only 17% of youth between 2016 and 2018 (17). In our patient population, nearly half of patients still did not reach HbA1c targets after one year and continued to benefit from support from a 4T treatment model (9). Given that a majority of patients still do not meet HbA1c targets in the US, it would appear reasonable to assume that the entirety of this flexible capacity could be safely booked out in any given review period, and that no slack capacity would go to waste.

Though this analysis is grounded in the characteristics of TIDE and our patient population, the parameters of this financial model can be customized to address the factors specific to another care provider's clinic, population, and payer environment. However, it is important to note that the 4T model and TIDE tool were used in study populations and may not be generalizable to the wider patient population at this time. Future directions include scaling this beyond new onset T1D and adapting these concepts for adult T1D patients and patients with type 2 diabetes (T2D). Of course, this model is not directly applicable to patients without access to a CGM, which underscores the need for increased coverage in under- or un-insured families who would otherwise not benefit from algorithm-enabled CGM data review.

Conclusion

We created a model to design financially sustainable CGM-based algorithm-enabled RPM based on data from 4 studies at a single site. Our model establishes a clear threshold reimbursement value for maintaining revenue-neutrality, as well as an estimate of potential marginal RPM reimbursement revenue which could be billed for. The minimum reimbursement rate for telemedicine interactions will ultimately depend on a given clinic's pathway to adopting algorithm-enabled RPM (i.e., repurposing vs. expanding capacity), as well as the value they derive from improving patient outcomes on the long-term. Existing reimbursement levels for RPM suggest that algorithm-enabled CGM data review may in fact increase marginal revenues for T1D clinics. Our analysis may inform operational and financial planning at the clinic level as well as reimbursement policy design to facilitate the transition to remote diabetes care.

Author's note

A complete list of members of the 4T Study Research Team appears in the Acknowledgments.

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

PD performed the research and designed the model. PD prepared the manuscript with input from all authors. AC and MG designed the capacity planning dashboard. DZ, DM, PP, and KS provided clinical guidance and input on the manuscript. RJ and DS supervised the project and provided input on the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

PP is affiliated with the Stanford Diabetes Research Center. DZ has received speaker's honoraria from Medtronic Diabetes, Ascensia Diabetes, and Insulet Canada; and research support from the Helmsley Charitable Trust and ISPADJDRF research Fellowship. She is also on the Dexcom Advisory board. DM has had research support from the National Kidney Foundation, Juvenile Diabetes Research Foundation, NSF, and the Helmsley Charitable Trust and his institution has had research support from Medtronic, Dexcom, Insulet, Bigfoot Biomedical, Tandem, and Roche. DM has consulted for Abbott, Aditxt, the Helmsley Charitable Trust, Lifescan, Mannkind, Sanofi, Novo Nordisk, Eli Lilly, Medtronic, Insulet, Dompe, and Biospex. DM is affiliated with the Stanford Diabetes Research Center. DS is advisor to Carta Healthcare.

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

References

1. Committee ADAPP. Diabetes technology: Standards of medical care in diabetes–2022. *Diabetes care* (2022) 45(Suppl 1):S97–112. doi: 10.2337/dc22-S007

2. Laffel LM, Kanapka LG, Beck RW, Bergamo K, Clements MA, Criego A, et al. Effect of continuous glucose monitoring on glycemic control in adolescents and young adults with type 1 diabetes: A randomized clinical trial. *Jama* (2020) 323 (23):2388–96. doi: 10.1001/jama.2020.6940

3. Sanderson EE, Abraham MB, Smith GJ, Mountain JA, Jones TW, Davis EA. Continuous glucose monitoring improves glycemic outcomes in children with type 1 diabetes: Real-world data from a population-based clinic. *Diabetes Care* (2021) 44 (9):e171–e2. doi: 10.2337/dc21-0304

4. Ferstad JO, Vallon JJ, Jun D, Gu A, Vitko A, Morales DP, et al. Population-level management of type 1 diabetes *via* continuous glucose monitoring and algorithm-enabled patient prioritization: Precision health meets population health. *Pediatr diabetes* (2021) 22(7):982–91. doi: 10.1111/pedi.13256

5. Scheinker D, Gu A, Grossman J, Ward A, Ayerdi O, Miller D, et al. Algorithm-enabled, personalized glucose management for type 1 diabetes at the population scale: Prospective evaluation in clinical practice. *JMIR diabetes* (2022) 7 (2):e27284. doi: 10.2196/27284

6. Scheinker D, Prahalad P, Johari R, Maahs DM, Majzun R. A new technology-enabled care model for pediatric type 1 diabetes. *NEJM Catalyst* (2022) 3(5): CAT.21.0438. doi: 10.1056/CAT.21.0438

7. Addala A, Maahs DM, Scheinker D, Chertow S, Leverenz B, Prahalad P. Uninterrupted continuous glucose monitoring access is associated with a decrease in HbA1c in youth with type 1 diabetes and public insurance. *Pediatr diabetes* (2020) 21(7):1301–9. doi: 10.1111/pedi.13082

8. Prahalad P, Addala A, Scheinker D, Hood KK, Maahs DM. CGM initiation soon after type 1 diabetes diagnosis results in sustained CGM use and Wear time. *Diabetes Care* (2020) 43(1):e3–4. doi: 10.2337/dc19-1205

9. Prahalad P, Ding VY, Zaharieva DP, Addala A, Johari R, Scheinker D, et al. Teamwork, targets, technology, and tight control in newly diagnosed type 1 diabetes: The pilot 4T study. *J Clin Endocrinol Metab* (2022) 107(4):998–1008. doi: 10.1210/clinem/dgab859

10. Prahalad P, Zaharieva DP, Addala A, New C, Scheinker D, Desai M, et al. Improving clinical outcomes in newly diagnosed pediatric type 1 diabetes: Teamwork, targets, technology, and tight control-the 4T study. *Front endocrinol* (2020) 11:360. doi: 10.3389/fendo.2020.00360

11. Eberle C, Stichling S. Telemetric interventions offer new opportunities for managing type 1 diabetes mellitus: Systematic meta-review. *JMIR diabetes* (2021) 6 (1):e20270. doi: 10.2196/20270

12. Carlson AL, Martens TW, Johnson L, Criego AB. Continuous glucose monitoring integration for remote diabetes management: Virtual diabetes care with case studies. *Diabetes Technol Ther* (2021) 23(S3):S–56-S-65. doi: 10.1089/dia.2021.0241

13. Garg SK. Emerging landscape of continuous glucose monitoring. *Diabetes Technol Ther* (2021) 23(S3):S–1-S-4. doi: 10.1089/dia.2021.0271

14. Thaler R, Sunstein C. *Nudge : Improving decisions about health, wealth, and happiness.* (Penguin Books: Revised & Expanded edition) (2009).

15. Zaharieva DP, Bishop FK, Maahs DM. Advancements and future directions in the teamwork, targets, technology, and tight control-the 4T study: Improving clinical outcomes in newly diagnosed pediatric type 1 diabetes. *Curr Opin pediatrics* (2022) 34(4):423–9. doi: 10.1097/MOP.0000000000001140

16. Battelino T, Danne T, Bergenstal RM, Amiel SA, Beck R, Biester T, et al. Clinical targets for continuous glucose monitoring data interpretation: Recommendations from the international consensus on time in range. *Diabetes Care* (2019) 42(8):1593–603. doi: 10.2337/dci19-0028

17. Foster NC, Beck RW, Miller KM, Clements MA, Rickels MR, DiMeglio LA, et al. State of type 1 diabetes management and outcomes from the T1D exchange in 2016-2018. *Diabetes Technol Ther* (2019) 21(2):66–72. doi: 10.1089/dia.2018.0384

18. Nirantharakumar K, Mohammed N, Toulis KA, Thomas GN, Narendran P. Clinically meaningful and lasting HbA(1c) improvement rarely occurs after 5 years of type 1 diabetes: an argument for early, targeted and aggressive intervention following diagnosis. *Diabetologia* (2018) 61(5):1064–70. doi: 10.1007/s00125-018-4574-6

19. Danne T, Limbert C. COVID-19, type 1 diabetes, and technology: Why paediatric patients are leading the way. *Lancet Diabetes Endocrinol* (2020) 8 (6):465–7. doi: 10.1016/S2213-8587(20)30155-8

20. Chang A, Gao MZ, Ferstad J, Maahs DM, Prahalad P, Johari R, et al. 1033-p: An interactive capacity planning dashboard for algorithm-enabled telemedicine-based diabetes care. *Diabetes* (2022) 71(Supplement_1). doi: 10.2337/db22-1033-P

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Sustained high glucose intake accelerates type 1 diabetes in NOD mice

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Introduction: Epidemiological studies have suggested that dietary factors, especially high consumption of high glycaemic index carbohydrates and sugars, may trigger or exacerbate the progression of type 1 diabetes. We aimed to provide experimental evidence to confirm this relevance and to explore the underlying mechanisms.

Methods: NOD mice were given sustained high-glucose drinking or glucose-free water and observed for the incidence of type 1 diabetes and islet inflammation. RNAseq was performed to detect the transcriptome changes of the NOD islet beta cell line NIT-1 after high glucose treatment, and mass spectrometry was performed to detect the proteome changes of NIT-1-cells-derived sEVs.

Results: Sustained high glucose drinking significantly aggravates islet inflammation and accelerates the onset of type 1 diabetes in NOD mice. Mechanistically, high glucose treatment induces aberrant ER stress and up-regulates the expression of autoantigens in islet beta cell. Moreover, high glucose treatment alters the proteome of beta-cells-derived sEVs, and significantly enhances the ability of sEVs to promote DC maturation and stimulate immune inflammatory response.

Discussion: This study provides evidence for negative effect of high glucose intake as a dietary factor on the pathogenesis of type 1 diabetes in genetically predisposed individuals. Therefore, avoiding high sugar intake may be an effective disease prevention strategy for children or adults susceptible to type 1 diabetes.

KEYWORDS

type 1 diabetes, high glucose, proteomics, RNAseq, endoplasmic reticulum stress, dendritic cells

Introduction

Type 1 diabetes (T1D) is an organ-specific autoimmune disease characterized by insulin deficiency, hyperglycemia, and complications due to inappropriate treatment (1). Worldwide, the incidence of T1D increased 3–4% annually over the past 30 years, and T1D develops more in children and adolescents than adults, resulting in a heavy social and medical burden (2–4). The rise in the incidence of T1D cannot be attributed to genetic factors alone; environmental factors may also contribute (5, 6). So far, the exact pathogenesis of T1D is not completely clear. Increasing evidence indicates that endoplasmic reticulum stress (ERS) in beta cells may lead to the generation and exposure of autoantigens and the activation of autoreactive T cells, and eventually result in the destruction of beta cells (7–9). Drugs that inhibit ERS can alleviate the incidence of T1D (10). Small extracellular vesicles (sEVs, 30-150 nm) are thought to be autoantigen carriers with potent adjuvant activity and may act as autoimmune triggers in T1D (11).

Adverse circumstances such as viral infection, inflammation, diet, stress, and toxins can lead to ERS in beta cells and initiate T1D development. Viral infection, as potential triggers for the pathogenesis of T1D, has been well studied (12, 13). Diet is another possible risk factor involved in the development of T1D, which has received increasing attention in recent years (14, 15). The consumption of sugar-sweetened beverages (SSBs) is increasing every year around the world, especially among children, which leads to a series of diseases, including obesity, dental caries and diabetes (16). In the mid-1990s, children's intake of sugared beverages passed that of milk (17). Some studies have reported that consumption of sugar, specifically SSBs is widely considered the cause of the global rise in type 2 diabetes (T2D) (18, 19). Although an epidemiological survey showed that the possibility of islet autoimmunity progression to T1D in children was significantly correlated with total sugar intake (20), there is still lack of laboratory evidence that high-glucose intake promotes the pathogenesis of T1D. And it is not clear whether beta cells, in responding to high glucose-induced ERS, may stimulate an enhanced autoimmune response *via* altered sEVs.

In this study, we demonstrate that sustained high-glucose drinking significantly accelerates the onset of T1D in NOD mice. We found that high-glucose treatment can significantly enhance ERS and autoantigens expression in NIT-1 cell, a NOD mouse pancreatic beta-cell line. Additionally, high-glucose treatment can significantly enhance the immunostimulatory ability of beta-cell-derived sEVs. Mass spectrometry analysis shows that high-glucose treatment can significantly change cargo of NIT-1-cells-derived sEVs, and increase their ability to promote maturation of dendritic cells (DCs). We propose that a high-glucose diet is an important dietary factor in the development of T1D in genetically predisposed individuals.

Materials and methods

Mice treatment

NOD/Ltj mice were purchased from Beijing Huafukang Biotechnology Co, Ltd. All mice were bred and maintained in specific pathogen-free facilities and handled according to "Principles of Laboratory Animal Care and Use in Research" (Ministry of Health, China). NOD/Ltj mice were allowed to freely intake 1.1M glucose water starting at 4 weeks of age until onset of T1D. Control NOD/Ltj mice were given normal water. The intake of food and water in each cage of mice were recorded every week. Each NOD mouse given high-glucose drinking consumed about 1g glucose per day from water, and thus consumed nearly 50% less food than NOD mice given normal water. Random blood glucose was monitored using a glucometer (OneTouch Ultral, LifeScan). Once random blood glucose

Abbreviations: ER, Endoplasmic reticulum; SSBs, Sugar-sweetened beverages; DEGs, Differentially expressed genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; ELISPOT, Enzyme-linked Immunospot; IA-2, Insulinoma-associated protein 2; DC, Dendritic cell; APC, Antigen-presenting cell; sEVs, small Extracellular Vesicles; eIF-2α, eukaryotic initiation factor-2α; The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium *via* the PRIDE partner repository with the dataset identifier PXD032768.; RNAseq data of NIT-1 cells from control and high glucose group have been uploaded to Sequence Read Archive (SRA) with BioProject ID: PRJNA816263.

exceeded 13.9 mM for two consecutive days, NOD mice were diagnosed as T1D and euthanized immediately. All experimental protocols were approved by the Animal Ethics Committee of the Army Medical University (Third Military Medical University).

Isolation and identification of sEVs

We isolated sEVs from cell culture supernatants according to MISEV guidelines (PMID: 30637094). NIT-1 cells were cultured within passages 20 (the cell culture time < one month) to ensure the stability of the cells. To prepare control sEVs, NIT-1 cells were cultured in 75-cm^2 culture flasks using Ham's F-12 Nutrient Mixture containing 7mM glucose (Thermo Fisher Scientific, USA), supplied with 10% exosomes-free FCS (icell, China) for 48 hours, and the cell viability was strictly guaranteed to be above 95% during cell culture. For high-glucose (HG) treatment, NIT-1 cells were pretreated with 20mM glucose for 24 hours. After the supernatants of cell culture were collected, the sEVs were isolated by ultracentrifugation. Briefly, the supernatants were centrifuged at 300g for 5 min to discard dead cells, then 2000g for 30 min, and 12,000g for 45 min at 4°C, followed by filtration using 450-nm pore-size membrane to remove cell debris and large particles. The sEVs were collected by spinning the final supernatants in a himac ultracentrifuge (Koki Holding Co. Ltd, Japan) at 110,000g for 70 min with P40ST-2690 rotor. Braking degree is set to 3 of 9. After washing once with an equal volume of PBS, the sEVs pellet was resuspended in PBS (1:500 concentrated from the original volume of culture supernatant). Protein concentration was determined by BCA Protein Assay Kit (Beyotime, China) and record the volume and particle concentration of the supernatant every time. Then the sEVs were identified by particle size detection, electron microscopy and western blotting. The size and concentration of particles were detected by NTA (nanoparticle tracking analysis).

Isolation of bone marrow-derived dendritic cells and co-culture with sEVs

Bone marrow cells were obtained from the femurs and tibias of NOD/Ltj mice, and red blood cells were eliminated using ACK Lysis Buffer (Beyotime, China). Then bone marrow cells were washed and cultured in six-well tissue culture plates at 1×10^6 cells/mL in complete RPMI culture medium supplemented with 10% fetal bovine serum and GM-CSF (20 ng/ml, Abcam) at 37°C, 5% CO2. The culture medium was changed on culture day 2 and 4. Fresh medium and GM-CSF were added after flushing out non-adherent cells. On day 6, loosely adherent clustered cells were harvested as immature BMDCs. To detect the ability of sEVs to promote DC maturation, immature BMDCs, cultured in complete medium supplemented with GM-CSF (10ng/ml) and IL-4 (5ng/ml), were treated with HG-sEV and C-sEV (9×10^9 particles/ml) respectively for 24 h. LPS (1μg/ml) was used as a positive stimulant to promote DC maturation.

Isolation of splenocytes and $CD11c^+$ cells depletion

The spleen tissues were taken out from NOD/Ltj mice, and mashed on a sterile nylon mesh to prepare splenocytes suspension. Then splenocytes were re-suspended with ACK Lysis Buffer (Beyotime, China) for 2 min after centrifugation, and finally washed twice with PBS buffer. $CD11c^+$ cells were depleted from splenocytes using the EasySep™ Mouse CD11c Positive Selection Kit II (#18780) according to the manufacturer's instruction. Briefly, 0.5 ml splenocytes suspension at 1×10^8 cells/ml was added into 5 ml (12×75 mm) polystyrene round-bottom tube, and 50 μl/ml rat serum was added. Then 25 μl selection cocktail (12.5 μl of Component A + 12.5 μl of Component B) was added into it and incubated for 5 min at room temperature. After incubating the cell sample with 20 μl RapidSpheres™ for 3 min, the tube was filled with about 2 ml medium and placed into the magnet. Finally, supernatant which contained splenocytes without $CD11c^+$ cells was poured out. The purity of $CD11c^-$ splenocytes was detected by flow cytometry.

Proteome bioinformatics analysis

The three batches of beta-cell-derived sEVs from two groups were prepared. This project used the high-resolution mass spectrometer to analysis samples. Quantitative analysis was performed based on information such as peak intensity, peak area, and LC retention time of peptides related to first-order mass spectrometry. Briefly, proteins were extracted and digested into peptides by Trypsin enzyme, then peptide liquid was freeze-dried after salt removal. The dried peptide samples were reconstituted with mobile phase A (2% ACN, 0.1% FA), centrifuged at 20,000g for 10 minutes, and the supernatant was taken for injection. Separation was performed by Thermo UltiMate 3000 UHPLC. The nanoliter liquid phase separation end was directly connected to the mass spectrometer. The peptides separated by liquid phase chromatography were ionized by a nano-ESI source and then passed to a tandem mass spectrometer Orbitrap Fusion Lumos (Thermo Fisher Scientific, San Jose, CA) for DDA (Data Dependent Acquisition) mode detection. The main parameters were set: ion source voltage was set to 2kV, MS1 mass spectrometer scanning range was 350~1,500m/z; resolution was set to 60,000; MS2 starting m/z was fixed at 100; resolution was 15,000. The ion screening conditions for MS2 fragmentation: charge 2+ to 6+, and the top 30 parent ions with the peak intensity exceeding 20,000. The ion fragmentation mode was HCD, and the fragment ions were detected in Orbitrap. The

dynamic exclusion time was set to 30 seconds. The AGC was set to: MS1 1E5, MS2 2E4.

The MS data were identified using the MaxQuant's integrated Andromeda engine. UniProtKB/Swiss-Prot subset were used for protein identification. At the spectrum level, filtering was performed with PSM-level FDR ≤ 1%, and at the protein level, further filtering was performed with Protein-level FDR ≤ 1% to obtain significant identification result.

RNA-seq and analysis of differentially expressed genes

NIT-1 cells were cultured in 75-cm^2 culture flasks using Ham's F-12 Nutrient Mixture containing 7 mM glucose. For high-glucose (HG) treatment, NIT-1 cells were pretreated with 20mM glucose for 24 hours. Cells from two groups were collected for RNA-Seq. After enriching mRNA with Oligo (dT) magnetic beads, mRNA was fragmented by breaking reagent, and the first and second strands of cDNA were synthesized by PCR instrument. After the end repair and joint connection, the PCR reaction and product recovery were carried out. Finally, the final library was obtained by cyclization of the product. Agilent 2100 Bioanalyzer was used to detect the fragment size and concentration of the library. Finally, the sequence reading length of 50bp/100bp/150bp was obtained by combined probe anchored polymerization.

The sequenced raw reads were filtered by SOAPnuke software (21) to remove joint contamination, reads with unknown base N content greater than 5% and low-quality reads. HISAT (22) was used to compare the clean reads obtained by quality control with the reference genome sequence of mouse genome, and the gene quantitative analysis was carried out. The screening of differential genes was based on DEseq2 method (23), which was negative binomial distribution, and the differentially expressed genes (DEGs) between HG group and C group were screened. FDR< 0.05 and |fold change, FC| ≥ 1.5 were set as filter thresholds for DEGs identification. Pearson correlation analysis of RNA-seq data indicated that one of the three biological replicates in the control group deviated greatly from the other two, so we removed the one with lower correlation when analyzing the data. The Dr. Tom platform independently developed by BGI was used to carry out more in-depth analysis of DEGs, such as cluster analysis, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis.

Mouse IFN-ϒ ELISPOT Assays

The ability of NIT-1 cells or their sEVs to stimulate IFN-γ secretion by splenocytes of NOD mice was evaluated using an ELISPOT assay. Briefly, 10^5 splenocytes or $CD11c^+$ depleted splenocytes from 10-week-old NOD mice were inoculated in each well of anti-mouse IFN-γ antibody-precoated ELISPOT plates, and co-cultured with 10^4 high glucose-treated or untreated NIT-1 cells or their sEVs (9×10^9 particles/ml) for 24 hours, respectively. Splenocytes cultured alone were used for negative control, and splenocytes stimulated with PMA and ionomycin were used for positive control. After incubation, the cells were removed and plates were processed according to the instructions of mouse IFN-γ precoated ELISPOT kit (Cat#: DKW22-2000-096). The spots were counted using a spot reader system (Saizhi, Beijing, China).

Real-time quantitative PCR

Total RNA was isolated with RNAiso Plus (Takara, Japan) from NIT-1 cells of two groups and cDNA was synthesized with PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara, Japan). Quantification of cDNA was performed by qRT-PCR (iCycler, BioRad, USA) with the TB Green Premix Ex Taq™ ll (Tli Rnaseh Plus) (Takara, Japan). Cycling parameters were 95°Cfor 3 min and 39 cycles of 95°C for 10 s, 58 °C for 10 s, and 72 °C for 20 s. The sequences used were as follows: mouse Atf6, Sense 5'-TCGCCTTTTAGTCCGGTTCTT-3', Anti-sense 5'-GGCTCCATAGGTCTGACTCC-3'; mouse eIF-2α, Sense 5'-ATGCCGGGGCTAAGTTGTAGA-3'; Anti-sense 5'-AACGGATACGTCGTCTGGATA-3'; mouse Chga, Sense 5'-CCAAGGTGATGAAGTGCGTC-3'; Anti-sense 5'-GGTGTCGCAGGATAGAGAGGA-3'; mouse Txnip, Sense 5'-GGCCGGACGGGTAATAGTG-3'; Anti-sense 5'-AGCGCA AGTAGTCCAAAGTCT-3'; mouse Xbp-1s Sense 5'-CTGAG TCCGAATCAGGTGCAG-3'; Anti-sense 5'-GTCCATGGG AAGATGTTCTGG-3';

Gene expression was normalized using the $\triangle\triangle C_t$ method, where the amount of target, normalized to an endogenous reference and relative to a calibrator, is given by $2^{-\triangle\triangle Ct}$, where C_t is the cycle number of the detection threshold.

Flow cytometry

Single-cell suspensions were made from spleens and pancreas infiltrating immune cells of 12-15-week-old NOD mice. Fluorochrome-conjugated antibodies specific for surface markers used in this study were anti-CD3-PE (145-2C11), anti-CD4-PE-Cy™7 (RM4-5), anti-CD8-BV510 (53-6.7), anti-CD11c-FITC (N418), anti-(I-A/I-E)-PE-Cyanine7 (M5/114.15.2), Anti-CD86-APC (GL-1) (eBiosciences, USA); and anti-IFN-γ-APC (XMG1.2) (eBiosciences, USA) for Intracellular staining. All samples were stained with Fixable Viability Dye eFluor™ 780 (Invitrogen™, USA), to facilitate live-cell gating before cell surface and intracellular staining. Doublets were excluded with forward scatter height against forward scatter area and subsequently side scatter height

against side scatter area. All data were acquired on an BD FACSCanto™ II Flow Cytometer (Biosciences, USA) and analyzed with FlowJo software (Tree Star, Inc, USA).

IA-2 antibody ELISA

The mouse IA-2 antibody (IgG) ELISA was performed according to the manufacturer's suggested protocol. Briefly, serums from mice fed with high-glucose water and normal water were run in duplicate to determine IA-2 antibody titers. Optical densities were determined with a microplate reader (800 TS, Biotek) set to 450 nm, with the correction wavelength set to 540 nm.

Histopathology

At the end of the experimental procedure, mice were dissected by cervical dislocation and mice pancreas and spleen quickly removed. Mice pancreas after fixation with 4% formaldehyde, were processed for histopathological examinations using hematoxylin and eosin staining due to instructions of a standard protocol. Each mouse included at least 10 islets. Insulitis was scored as below: 0, no insulitis; 1, peri-insulitis; 2, mild insulitis (<25% of infiltrated islets); 3, severe insulitis (25–75% of infiltrated islets).

SDS-PAGE and western blotting analysis

The sEVs lysates were prepared by resuspending sEVs pellets in equal volume sEV-lysing buffer (Umibio, China), and then incubated on ice for 10 min, followed by centrifugation at 12,000 rpm for 5 min at 4˚C. To perform SDS-PAGE, sEVs lysates were denatured by incubation with sample buffer at 95°C for 10 min. SDS-PAGE-separated protein samples were electro-transferred to polyvinylidene fluoride membrane (Beyotime, China) using wet transfer (BG-blotMINI, China). The membrane was immunoblotted with 1 mg/ml primary Abs, followed by a respective secondary HRP-labeled anti-IgG (Beyotime, China). The protein bands were visualized with an ECL detection system (Amersham, USA). The antibodies for eIF2α and phospho-eIF2α West blotting were purchased from Cell Signaling technology, Inc., USA. The clone EPR7130 (B) (abcam, UK) were used for detecting TSG101. The clone EPR23105-125 (abcam, UK) were used for detecting CD9.

Statistical analyses

Data are expressed as means ± SD. Student's t test was used only when the two sets of data conformed to normal distribution and had the same SD, regardless of sample size. Otherwise, nonparametric tests were used. Insulitis score was analyzed by Chi square test. Statistical analyses were performed in Prism 8 (GraphPad software Inc, USA). P <0.05 was considered statistically significant.

Results

Sustained high-glucose intake aggravates islet inflammation and accelerates the onset of T1D in NOD mice

We first determined whether high-glucose intake accelerates the onset of T1D in NOD mice. It was observed that dynamic blood glucose levels of NOD mice fed with high-glucose water for 1 weeks fluctuated in a relatively high range at different time points throughout the day, compared with control NOD mice fed with normal water (Figure 1A). Continuous blood glucose monitoring indicated that the level of blood glucose in NOD mice treated with high-glucose water were well controlled in a relatively safe range before 12 weeks of age (Supplementary Figure 1). As expected, control NOD mice started to develop diabetes at about 16 weeks of age, and 70% became diabetic by the age of 30 weeks. However, NOD mice fed with high-glucose drinking began to develop diabetes much earlier, and 90% became diabetic by the age of 30 weeks (Figure 1B). Consistently, histopathological analysis revealed that sustained high-glucose drinking aggravates the development of insulitis in NOD mice at 12 weeks of age (Figure 1C). We then examined the levels of autoantibodies and proinflammatory T cell responses in NOD mice fed with different drinking at 12-14 weeks of age. When compared with control NOD mice, serum levels of autoantibodies against Insulinoma associated protein 2 (IA-2), one of major T1D autoantigens, were significantly increased in the NOD mice fed with glucose drinking for 3 weeks (Figure 1D). As expected, the absolute number of pancreas-infiltrating $CD4^+$ and $CD8^+$ T cells was markedly increased in NOD mice fed with high-glucose water compared to control NOD mice (Figure 1E). The frequency of IFN-γ-producing $CD4^+$ T cells in the spleen and pancreas of NOD mice given high-glucose drinking was significantly higher than that of NOD mice given normal drinking (Figure 1F). The frequency of IFN-γ-producing $CD8^+$ T cells and Th17 cells in the spleen and pancreas were no significant difference between the two groups (Supplementary Figure 2). Together, these data indicate that sustained high-glucose drinking aggravates islet inflammation, enhances autoimmune response, and accelerates the onset of T1D in NOD mice.

High-glucose induces up-regulation of islet beta-cell autoantigens and ERS-related genes in NIT-1 cells

Since islet beta cells are extremely sensitive to high-glucose exposure, then we questioned whether high-glucose treatment

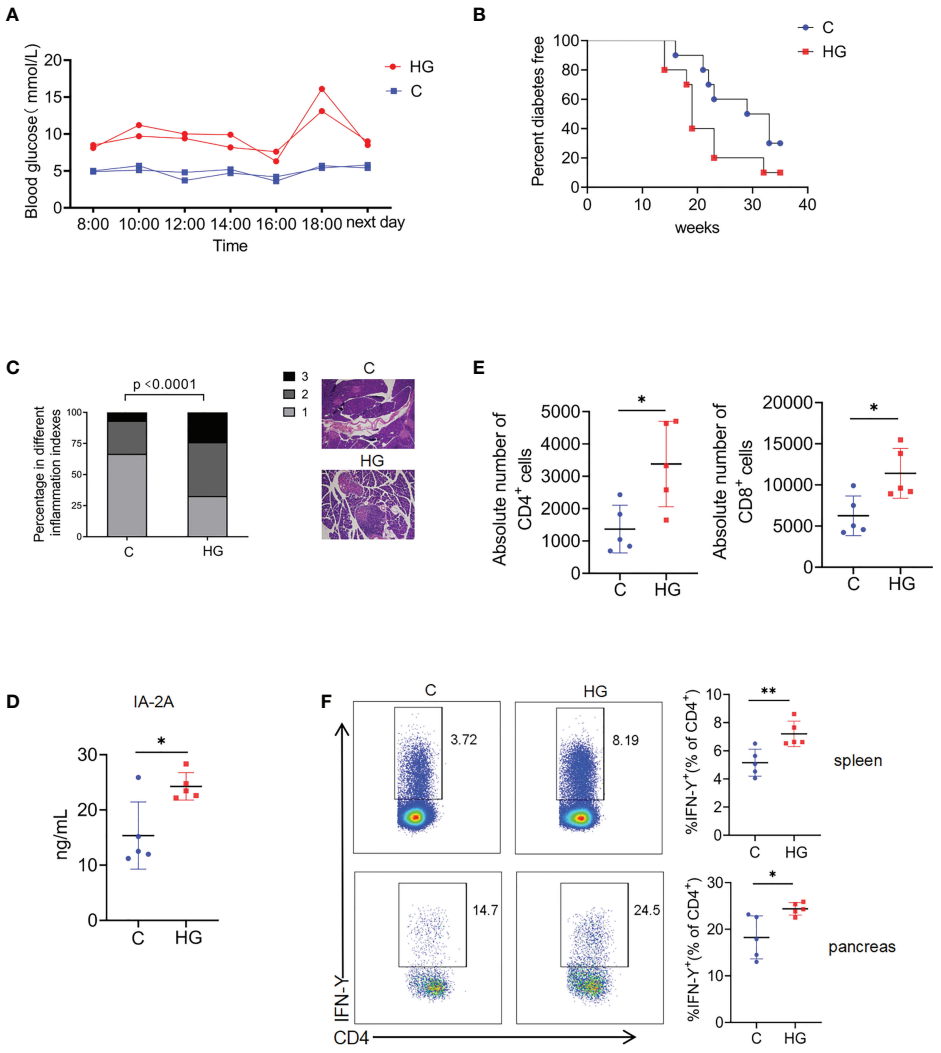

FIGURE 1
Sustained high-glucose intake aggravates islet inflammation and accelerates the onset of T1D in NOD mice **(A)** Blood glucose at different time points in a day in NOD mice given normal water **(C)** or high-glucose water (HG) (n=2). **(B)** The diabetes-free percentage in NOD mice given normal water **(C)** or high-glucose water (HG) over time, significance was determined by Gehan-Breslow-Wilcoxon test, P<0.05 (n=10). **(C)** Representative images of HE staining of pancreas (magnification 20×) and insulitis scores in NOD mice given normal water **(C)** or high-glucose water (HG) (n=5). Statistical significance was analyzed by Chi square test, p<0.0001. **(D)** Serum concentrations of Insulinoma Associated-2 Autoantibodies (IA-2A) in NOD mice given normal water **(C)** or high-glucose water (HG) (n=5). **(E)**The absolute number of pancreas-infiltrating $CD4^+$ and $CD8^+$ T cells of NOD mice given normal water **(C)** or high-glucose water (HG) (n=5). **(F)** The secretion of IFN-γ by $CD4^+$ T cells in the spleen (up) and pancreas (down) of NOD mice given normal water **(C)** or high-glucose water (HG) (n=5). Data are representative of two independent experiments. Summary data are presented as mean ± SD. *p < 0.05, and **p < 0.01, unpaired two-tailed Student's t tests.

increased the visibility of beta cells to the immune system. IFN-γ ELISPOT analysis showed that high-glucose-treated NIT-1 cells stimulated significantly increased IFN-γ secretion in splenocytes from NOD mice compared with untreated NIT-1 cells (Figure 2A). To further explore the possible mechanisms, we performed global RNA-seq analysis of NIT-1 cells treated with or without high-glucose (Supplementary Table 1). Upon high-glucose treatment, 684 unique genes were significantly upregulated and 425 unique genes were significantly downregulated (FDR<0.05 and fold change [FC]≥1.5) in NIT-1 cells (Figure 2B; Supplementary Table 2). KEGG pathway analysis depicted significant enrichment of DEGs belonging to pathways of autophagy, insulin, mTOR, MAPK signaling and protein processing in ER, etc. (Figure 2C). Moreover, ERS-related genes such as *Atf6, eIF2α* and *Txnip* and islet beta-cell autoantigen genes such as *Chga, Ins2, Hsp60* and *Gad65* were significantly up-regulated in high-glucose-treated NIT-1 cells (Figure 2D). Consistently, real-time quantitative PCR confirmed that mRNA levels of *Atf6, eIF2α, Txnip*, *Chga*, *Ins* and *Xbp-1s* were significantly up-regulated in NIT-1 cells treated with high-

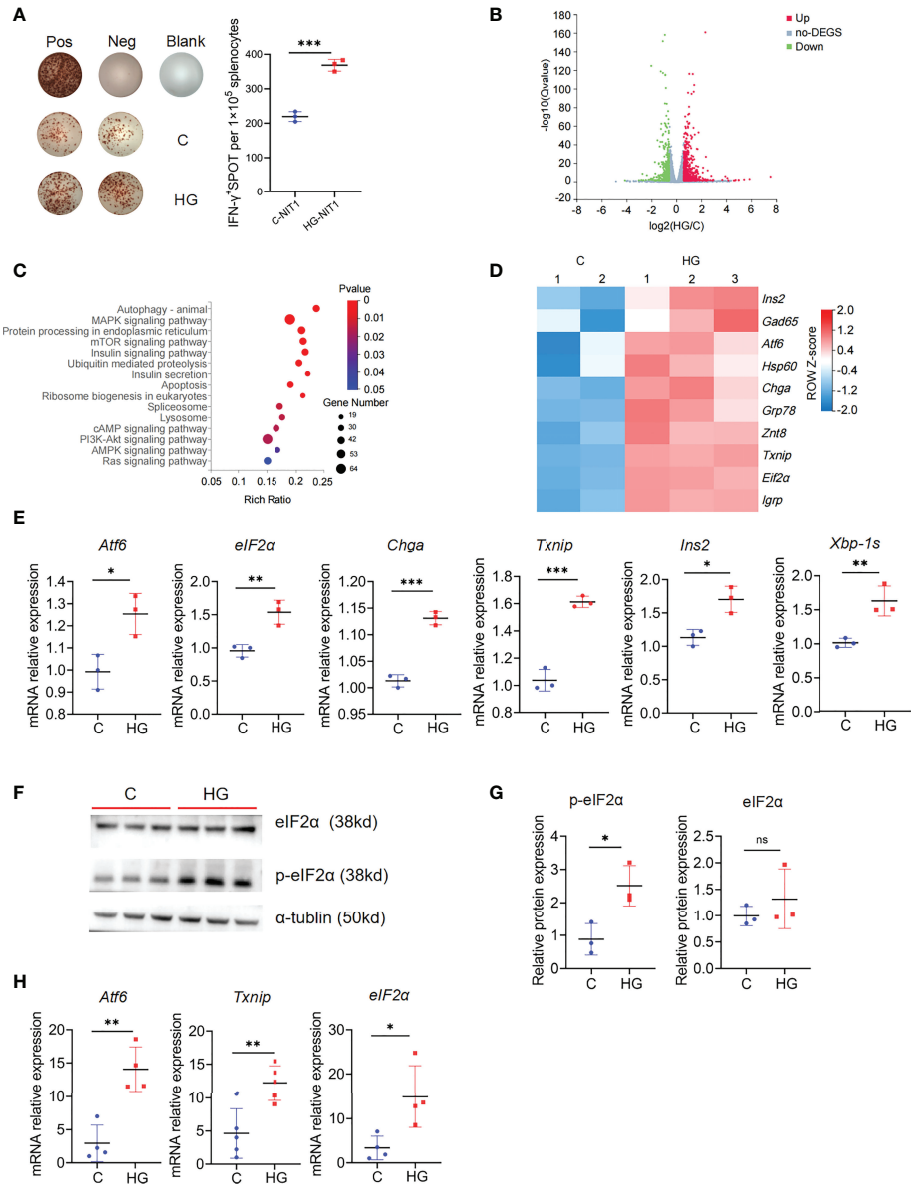

FIGURE 2
High-glucose treatment enhance the immunogenicity of NIT-1 cells and up-regulate the autoantigen and ERS related genes expression
(A) Representative plots of IFN-γ production by NOD mouse splenocyte alone (Neg), or incubated with untreated (C-NIT1) or high-glucose treated NIT-1 cells (HG-NIT1), or PMA plus ionomycin (Pos). Summary data are presented as the mean IFN-γ spots number per $1x10^5$ splenocytes ± SD in triplicate for one representative result of at least two independent experiments. **(B)** The volcano plot of NIT-1 cell transcriptome after high-glucose treatment, 684 genes were up-regulated (red dot), and 425 genes were down-regulated (blue dot) (|FC| ≥1.5, fold change, FDR< 0.05). **(C)** KEGG pathway analysis of 684 up-regulated genes in high-glucose treated NIT-1 cell transcriptome (|FC|≥1.5, FDR<0.05). **(D)** The T1D autoantigens and ERS-related genes expression in the transcriptome of NIT-1 cell with or without high-glucose treatment, red means up-regulated and blue means down-regulated, each column depicts a biological duplication. **(E)** The mRNA levels of ERS-related genes *Atf6, eIF2α, Txnip, Chga, Xbp-1s* and *Ins* in NIT-1 cells with or without high-glucose treatment, which were detected by qPCR. **(F)** The protein level of p-eIF2α and eIF2α in untreated NIT-1 cell **(C)** and high-glucose treated NIT-1 cell (HG) detected by western blot. **(G)** The ratio of p-eIF2α/tublin and eIF2α/tublin were quantified. **(H)** The mRNA levels of ERS-related genes *Atf6, eIF2α* and *Txnip* in pancreatic tail tissues of NOD mouse, which were detected by qPCR (n=5). Three independent biological samples were used for quantitative PCR and WB analysis. Summary data are presented as mean ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001, unpaired two-tailed Student's t tests.

glucose (Figure 2E). Meanwhile, the protein level of phosphorylated-eIF2α, but not total eIF2α, was also up-regulated in NIT-1 cells treated by high-glucose (Figures 2F, G). Moreover, the mRNA expression levels of the ERS marker *Atf6, eIF2α* and *Txnip* were significantly increased in the pancreatic tail tissues of NOD mice drinking high-glucose water (Figure 2H). Collectively, these data suggest that NIT-1 cells used in our study exhibit the characteristics of primary beta cells in response to high-glucose stimulation to some extent, including upregulation of ERS and autoantigen expression, which may be related to the enhanced visibility of islet beta-cells to the immune system.

High-glucose treatment enhances the immunostimulatory activity of NIT-1 cell-derived sEVs

Since sEVs are considered as extracellular messengers with potent immune-modulatory activities linking islet beta-cells to immune cells, we next explored whether high-glucose treatment affects the immunostimulatory activity of sEVs released by ER stressed islet beta-cells. The sEVs were collected from the culture supernatants of NIT-1 cells treated with or without high-glucose through sequential ultracentrifugation. The purified sEVs particles were round vesicles about 100 nm in size, as demonstrated by the electron micrograph (Figure 3A). Consistently, the particle size detection results showed that most vesicles had a particle size of 30-150nm (Figure 3B). Meanwhile, we observed that high-glucose treatment seemed to induce more formation and release of sEVs from NIT-1 cells (Supplementary Figure 3). The enrichment of sEVs proteins such as tumor susceptibility gene 101 protein (TSG101) and CD9 antigen (CD9) in sEVs samples was identified by western blot analysis (Figure 3C). To examine the immunostimulatory activity of sEVs released by NIT-1 cells under high-glucose or normal culture condition, splenocytes from 6- to 8-week-old NOD mice were incubated with sEV samples (9×10^9 particles/ml) for 24 h, and IFN-γ secretion were measured by ELISPOT assay. Compared with the sEVs released by NIT-1 cells under normal culture condition (C-sEV), the same dose of sEVs derived from high-glucose treated-NIT-1 cells (HG-sEV) stimulated significantly increased IFN-γ secretion in splenocytes from NOD mice (Figure 3D). These results suggest that high glucose-induced ERS conditions significantly enhances the immunostimulatory activity of NIT-1 cells-derived sEVs.

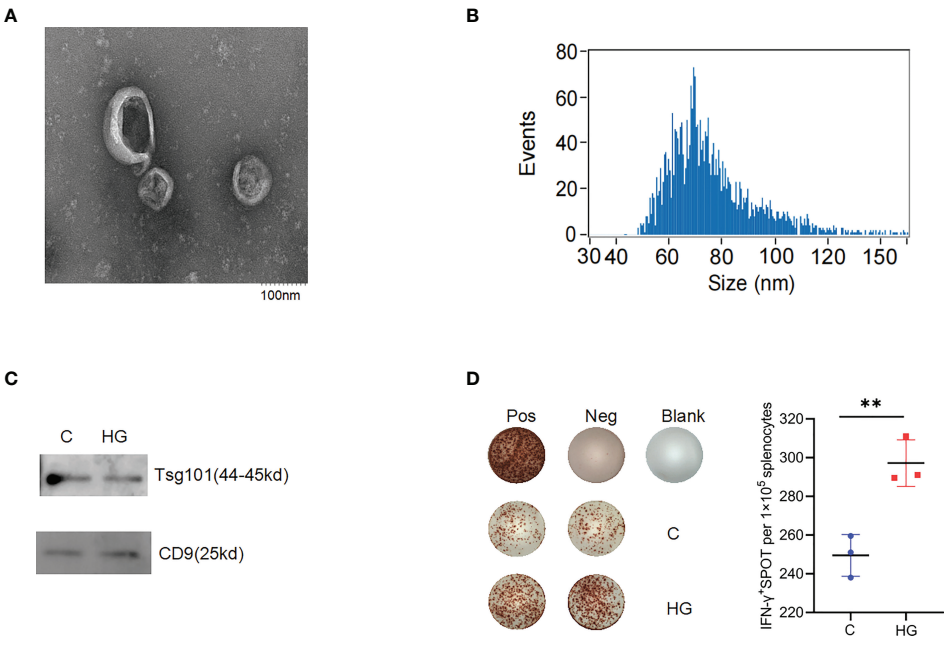

FIGURE 3
High-glucose treatment enhance the immunogenicity of sEVs derived from NIT-1 cell **(A)** The electron microscopic image of purified sEVs derived from NIT-1 cells. **(B)** The particle size distribution of sEVs derived from NIT-1 cells by Nanoparticle Tracking Analysis. **(C)** Identification of sEVs markers TSG101 and CD9 in sEVs derived from untreated NIT-1 cells **(C)** and high-glucose treated NIT-1 cells (HG) by western blot. **(D)** Representative plots of IFN-γ production by NOD mouse splenocytes alone (Neg), or incubated with PMA plus ionomycin (Pos), or sEVs derived from untreated **(C)**, or high-glucose treated (HG) NIT-1 cells. Summary data are presented as the mean IFN-γ spots number per $1x10^5$ splenocytes ± SD in triplicate for one representative result of two independent experiments. **p < 0.01, unpaired two-tailed Student's t tests.

High-glucose treatment alters the proteome of NIT-1 cells-derived sEVs

Then we were encouraged to study whether high glucose-induced ERS state can affect the composition of protein cargo of sEVs released by stressed islet beta-cells. Three independent sEVs samples purified from the culture supernatant of NIT-1 cells treated with or without high glucose were analyzed by LC-MS/MS. The MS data were identified using the MaxQuant's integrated Andromeda engine (Supplementary Table 3 for analysis parameters). A total of 1381 proteins and 6250 peptides were identified in all samples (Supplementary Tables 4, 5), including several sEVs markers, such as FLOT-1, CD81, CD63, syntenin-1 and TSG101. A few of T1D-associated autoantigens, such as Chromogranin-A (ChgA), 60 kDa heat shock protein (HSP60) and the beta-cell stress marker heat shock protein 90 (HSP90), were identified in these NIT-1-released sEVs proteins. However, some major autoantigens in T1D such as Insulin, Proinsulin, GAD65, IA-2, IGRP, ZnT8 and ICA69 were not found in these sEVs proteins. A comparative analysis of proteomic data between HG-sEV and C-sEV showed that 1087 proteins were shared in common, 210 proteins were unique to HG-sEV (Supplementary Table 6), and 84 proteins were unique to C-sEV (Figure 4A). Among the shared proteins, only five proteins (ELAV1, PGBM, SL7A1, PRIO, IF2B) were significantly upregulated in HG-sEV compared to C-sEV, while three proteins (SYDC, MIF, PSB2) were significantly down-regulated, probably due to reproducibility limitations in both biological samples and mass spectrometry (Supplementary Table 7). So far, little is known about the direct relationship between these proteins and the pathogenesis of T1D.

We then mapped 210 HG-sEV exclusive proteins to the GO enrichment analysis and found that the significantly enriched biological process terms included toll-like receptor (TLR) signaling pathway, and pathway of DC activation and differentiation (Figure 4B). By analyzing the protein-protein interaction network of 210 HG-sEV proteins, we noticed that these proteins have close interactions (Figure 4C). These results show that high glucose-induced ERS state alters the proteome of NIT-1 cell-derived sEVs, and we presume that some HG-sEV

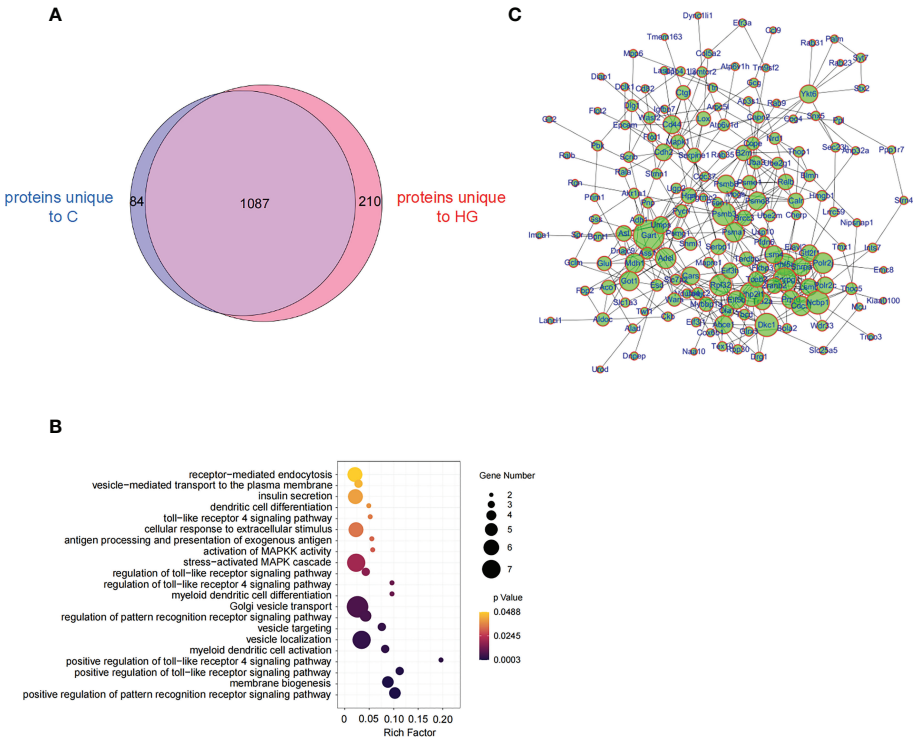

FIGURE 4
High-glucose treatment reshape the proteome of NIT-1 cell-derived sEVs **(A)** Venn diagram of proteins identified from high-glucose treated (HG) or untreated **(C)** NIT-1 cell. **(B)** GO analysis of 210 unique proteins in high-glucose treated NIT-1 cells. **(C)** A protein-protein interaction (PPI) network of unique proteins derived from high glucose treated NIT-1 cells (HG).

exclusive components are probably associated with DC activation and function, which might be involved in the enhanced immunostimulatory activity of HG-sEV.

The enhanced immunostimulatory activity of sEVs derived from high glucose treated-NIT-1 cells is largely dependent on the enhanced DC function

In the pathogenesis of T1D, DCs play a key role in antigen capture and presentation to autoreactive T cells. We observed that the expression level of costimulatory molecule CD86 on the surface of DCs in spleen and pancreas of NOD mice with continuous high-glucose drinking water was significantly higher than that in NOD mice with normal drinking water, suggesting that high-glucose treatment may promote DC maturation *in vivo* (Figure 5A). Since sEVs are small particles of 30 to 150nm in diameter, DCs may be more efficient at capturing particles in this size range than other subpopulations, such as macrophages or B cells. Therefore, we asked whether HG-sEV has a stronger ability to stimulate DC maturation than C-sEV. FACS analysis demonstrated that HG-sEV stimulation significantly increased the expression levels of CD86 on the bone marrow-derived DCs cultured *in vitro* compared with C-sEV stimulation (Figure 5B).

Finally, we wished to determine whether the enhanced immunostimulatory activity of sEVs released by NIT-1 cells with high-glucose treatment could be mediated by DC function. CD11c positive cells in mouse spleen cells were effectively removed by magnetic sorting (Supplementary Figure 4). The same number of whole splenocytes or $CD11c^+$ cells-removed splenocytes from 6- to 8-wk-old NOD mice were incubated with C-sEV or HG-sEV ($9*10^9$ particles/ml) for 24h, and IFN-γ secretion were measured by ELISPOT assay. Although HG-sEV consistently displayed a significantly enhanced ability to stimulate IFN-γ secretion by whole splenocytes compared with C-sEV, this effect was almost eliminated after the removal of $CD11c^+$ cells (Figure 5C). Collectively, these data suggest that the enhanced immunostimulatory activity of sEVs derived from NIT-1 cells treated with high-glucose is largely dependent on enhanced DC function.

Discussion

High total sugar intakes (20) and higher glycaemic index of the diet (24) have been thought to be associated with increased risk of progression to T1D in children. Nevertheless, there is a lack of direct evidence available that illustrates the effects of high-glucose intake on T1D progression. In the present study, we provided laboratory evidence that sustained high-glucose drinking significantly promotes the onset of T1D in NOD mice. Mechanistically, high-glucose treatment induces aberrant ERS, up-regulates the expression of autoantigens in islet beta cell, and enhances the visibility of islet beta-cells to the immune system. Moreover, high-glucose treatment alters the proteome of beta-cells-derived sEVs, and significantly enhances the ability of sEVs to promote DC maturation and stimulate autoimmune response.

Dietary sugar consumption is a controversial public health subject. Children are most attracted to drinks and foods high in sugar. For example, Coca-Cola, the most popular soft-drink in the world, contains more than 10% sugar. High sugar intake has been associated to increase the risk for obesity, T2D, cardiovascular diseases, and cancer (25–28). The incidence of T1D and T2D showed a concomitant increase, suggesting that they may share a common environmental trigger. We hypothesized that excessive intake of sugar, from various high-sugar beverages and high-glycemic index foods, can temporarily expose the body's islet beta cells to high glucose influx and high intracellular glucose levels, which may lead to islet beta cells hyperactivity and ERS, thereby increasing the visibility of beta cells to the immune system and accelerating autoimmune destruction of beta cells in individuals genetically susceptible to T1D. To test this hypothesis, NOD mice were allowed to freely intake 1.1M (20%) high-glucose water, which is expected to maximally and safely simulate individual over-consumption of various high sugar beverages and high-glycemic index foods, ultimately leading to rapid absorption of a large amount of glucose into the blood. Other studies have used such high concentration sugar drinking water as the only source of water for mice. For example, EAE model mouse was treated with 20% high-glucose water to study the role of high-glucose intake in promoting autoimmunity and inflammation (29). In another study on how fructose triggers hepatosteatosis and NASH, mice were allowed free access to 30% fructose in water solution for more than 10 months (30). The published studies have shown that long-term intake of high sugar drinking water has no obvious toxic effect. It was shown that high fructose and sucrose water intake for 10 weeks don't cause significant change of serum uric acid that is one of indexes of kidney injury (31). A study about the long-term effects of mannose (C-2 epimer of glucose) supplementation indicated that intake of 20% mannose in the drinking water for 5 months does not cause bloating, diarrhea, abnormal behavior, weight change, increase in hemoglobin glycation, or histology changes of organs (32).

Our study proved that NOD mice given high-glucose water exhibited significantly earlier onset of T1D and more severe autoimmunity-mediated islet inflammation. We found that free intake of high-glucose water kept the daily dynamic random blood glucose of NOD mice at a relatively and temporarily high level, but has not yet met the diagnostic criteria for diabetes, that is, the random blood glucose value greater than 13.9 mM for 2 consecutive days. In response to this irregular but transient significant increase in blood glucose, beta cells need to produce more insulin to control blood glucose in a relatively safe range, resulting in an imbalance in

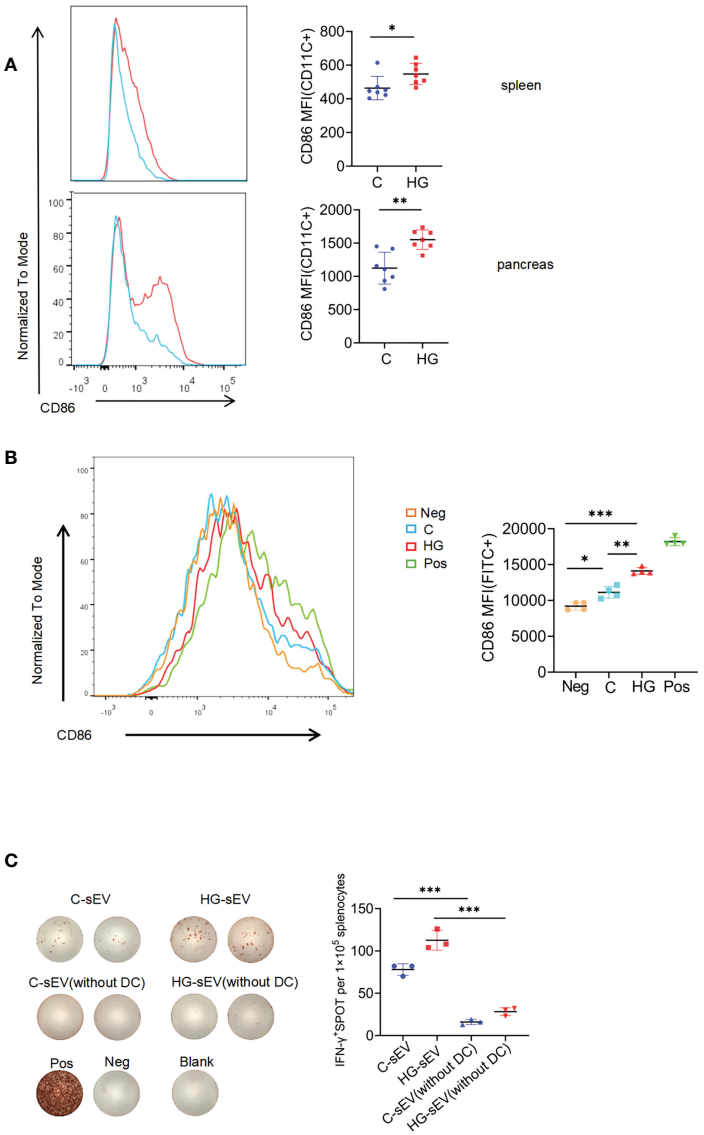

FIGURE 5
sEVs communicate with immune system *via* dendritic cells **(A)** The expression of costimulatory molecule CD86 on $CD11c^+$ subset in spleen and pancreatic infiltrating cells of NOD mice given normal water **(C)** and high-glucose water (HG) (n=7). **(B)** The expression of CD86 on bone marrow-derived dendritic cells alone (Neg), or co-cultured with sEVs released from untreated **(C)**, or high-glucose treated NIT-1 cells (HG), or LPS (Pos). **(C)** Representative plots of IFN-γ production by NOD mouse splenocytes (with or without dendritic cells) alone (Neg), or incubated with untreated NIT-1 cells-derived sEVs (C-sEV) or high-glucose treated NIT-1 cells-derived sEVs (HG-sEV), or PMA plus ionomycin (Pos). Summary data are presented as mean ± SD for one representative result of two independent experiments. *p < 0.05, **p < 0.01, and ***p < 0.001, unpaired two-tailed Student's t tests.

ER protein load and ER folding capacity, known as ERS. With the gradual destruction of islet beta cells, NOD mice could no longer maintain normal blood glucose level and then developed T1D. In T2D, dysregulated ERS occurs in beta cells exposed to high blood glucose, leading to beta cell dysfunction and apoptosis (28). As a possible mechanism for the induction of autoimmunity in T1D, abnormal ERS may result in the generation of autoantigens by post-translational modification of proteins in beta cells (8, 33–35). Our study confirmed that the expression levels of genes related to ERS and autoantigens in NIT-1 beta cells were significantly increased under high-glucose treatment, and more importantly, the ability of NIT-1 beta cells to stimulate IFN-γ secretion by splenocytes from NOD mice was significantly enhanced by high-glucose treatment. This is consistent with previous studies showing that the ability of beta cells to produce autoantigens and activate autoreactive T cells was enhanced under high-glucose treatment (36, 37). In fact, it has been suggested that NIT-1 beta cell line is a useful tool to understand changes in primary islet beta cells in the pathogenesis

of T1D (38, 39). We also confirmed that free intake of high-glucose water can induce the up-regulation of ERS markers, such as *Txnip, eIF2α, Atf6* in the pancreatic tissues of NOD mice, accompanied by enhanced levels of autoantibodies and IFN-γ producing T cell responses. Increasing levels of IFN-γ have been shown to correlate with diabetes progression in NOD mice as well as being necessary for virus-related diabetes (40, 41). Although it has been shown that high-glucose intake exacerbates autoimmunity in experimental colitis and experimental autoimmune encephalomyelitis by promoting Th17 cell differentiation (30), we did not observe a significant increase in Th17 cells in the spleen and pancreas of NOD mice fed with high-glucose water.

DCs have been shown to be essential in T cell-mediated destruction of beta cells in T1D by making beta cells visible to the immune system through uptake, processing and presentation of beta cells-derived autoantigens (42–44). Recently, sEVs from beta cells or mesenchymal stem cells have emerged as novel autoimmune targets, carrying autoantigens and other molecules that can stimulate both innate and adaptive immune responses in T1D (11, 45–51). Researchers found that beta cell autoantigens are taken up, processed and presented by DCs *via* sEVs, thereby initiating autoreactive T cell responses and promoting T1D development (52). Since it's very difficult to get enough sEVs from primary islet beta cells, several insulinoma cells such as NIT-1, MIN-6, INS-1, etc. have been used to isolate sEVs and to study the role of sEVs in the pathogenesis of T1D due to their low experimental cost and better availability (46, 47, 53). A proteomics study of sEVs derived from MIN-6 cells suggested that intercellular communication mediated by sEVs transfer among tissues may account for the major reason of beta cell destruction (54). Our data showed that high-glucose treated NIT-1 cells tend to release more sEVs than untreated NIT-1 cells. Consistently, high glucose-stimulated human mesangial cells released a higher number of sEVs compared to unstimulated cells (55). Similar effects were also observed in trophoblastic cells (56). Thus, the effect of high-glucose on sEVs secretion deserves more attention. Notably, purified sEVs secreted from NIT-1 cells effectively stimulates splenocytes from NOD mice to secrete inflammatory cytokines, and high-glucose treatment significantly enhances the immunostimulatory activity of NIT-1 cells-derived sEVs. And removal of CD11c-positive cells from splenocytes of NOD mice dramatically reduced the ability of sEVs to stimulate IFN-γ secretion by splenocytes. Considering that CD11c-positive cell subset represents the majority of antigen-presenting cells (APC), since CD11c is expressed on DCs, monocytes, macrophages and a subset of B cells, and that CD11c-positive classical DCs can capture sEVs more efficiently than macrophages and B cells (57), we hypothesized that this effect might be attributed to enhanced DC antigen presentation function mediated by sEVs.

Given that different types of cellular stress affect the proteome composition of sEVs (58), and stress-induced exosomal release of intracellular autoantigens and immunostimulatory chaperones may play a role in the initiation of autoimmune responses in T1D (11), we speculate that high glucose-induced ERS might be related to change in sEVs profile that enhances autoantigenicity in T1D. Our proteomics study identified much more proteins in NIT-1 cell-derived sEVs than a previous study by Lee et al. (53), and most proteins (181 in 269) identified in Lee's study were also found by our study. We found that high-glucose treatment greatly altered the proteome of NIT-1 cell-derived sEVs. However, only limited autoantigens, such as ChgA and HSP60, were identified in these NIT-1-released sEVs proteins, but their content in sEVs were not changed by high-glucose treatment. Several common beta cell autoantigens, such as Insulin, GAD65, IA-2, ZnT8, were not found in NIT-1-released sEVs proteins. Consistently, Lee et al. also failed to identify these known autoantigens in the NIT-1-derived sEVs proteome (53). There are several possible explanations for this: 1. the abundance of these known autoantigens contained in NIT-1-derived sEVs may be lower than the detection range of mass spectrometry; 2. Some known autoantigens contained in NIT-1-derived sEVs may exist as peptides segments rather than complete proteins. If these peptides are further degraded by trypsin during sample preparation into short peptides less than 6 amino acids in length, they will be directly filtered out during data analysis; 3. The sEVs may contain certain unconventional autoantigens with strong immunogenicity, which are encoded by noncoding RNA or unconventional open reading frame and cannot be identified by traditional proteomics. More studies are needed to confirm these speculations.

There is another possibility that high-glucose treatment may further enhance the ability of sEVs to promote $CD11c^{+}$DC maturation, providing a stronger costimulatory signal for the activation of autoreactive T cells, which may ultimately induce stronger autoreactive T cell activation. Our MS data preliminarily revealed that a series of proteins exclusively present in HG-sEV are enriched in DC differentiation, activation, and function-related pathways.Consistent with this finding, HG-sEV promoted the expression of the costimulatory molecule CD86 on the surface of bone-marrow derived DCs compared with C-sEV. Thus, we speculate that some components (e.g., proteins) in HG-sEV might enhance the immunostimulatory activity of HG-sEV by modulating the activation and function of DC. However, the molecular mechanisms underlying this process needs to be further studied.

There are several limitations in this study. First, this study lacked a 'low glucose' control group, which could have served as control for not only the total amount of consumption but the actual concentration of sugar. Second, there is a bit of a leap between using the NIT-1 beta cell line and presuming the role of primary NOD islet beta cells *in vivo* during T1D, since there are some differences between this transformed cell line and primary NOD beta cells. Third, this study did not investigate the specific components and underlying mechanisms of high-glucose treated-NIT-1 beta cell-derived sEVs that enhance DC function.

Taken together, we provide evidence for negative effect of high-glucose intake as a dietary factor on the pathogenesis of

T1D. Our findings suggest that sustained high-glucose intake leads to abnormal ERS in islet beta cells of NOD mice, which enhances the visibility of islet beta cells to the immune system. Meanwhile, high-glucose treatment may increase the ability of islet beta cells-derived sEVs to promote $CD11c^+$ DC maturation and antigen presentation, ultimately leading to an enhanced T-cell response to beta cells in NOD mice. Therefore, avoiding high sugar intake may be an effective disease prevention strategy for children or adults susceptible to T1D.

Data availability statement

The data presented in the study are deposited in the ProteomeXchange repository, accession number PXD032768 and Sequence Read Archive (SRA) repository, accession number PRJNA816263.

Ethics statement

The animal study was reviewed and approved by Animal Ethics Committee of the Army Medical University (Third Military Medical University).

Author contributions

XL and LNW performed main experiments and data analysis, and draft the manuscript. GM, XC prepared the biological samples and performed histopathological analysis. SY, MZ, ZZ and JZ isolated and identified sEVs. ZL analyzed MS data. YW interpreted the results and evaluated the data. LW supervised and managed the research process, completed the manuscript and provided research funds. 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.2022.1037822/full#supplementary-material

References

1. DiMeglio LA, Evans-Molina C, Oram RA. Type 1 diabetes. *Lancet* (2018) 391:2449–62. doi: 10.1016/S0140-6736(18)31320-5

2. Weng J, Zhou Z, Guo L, Zhu D, Ji L, Luo X, et al, et al. Incidence of type 1 diabetes in China, 2010-13: population based study. *BMJ* (2018) 360:j5295. doi: 10.1136/bmj.j5295

3. Dabelea D, Mayer-Davis EJ, Saydah S, Imperatore G, Linder B, Divers J, et al. Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. *JAMA* (2014) 311:1778–86. doi: 10.1001/jama.2014.3201

4. Patterson CC, Harjutsalo V, Rosenbauer J, Neu A, Cinek O, Skrivarhaug T, et al. Trends and cyclical variation in the incidence of childhood type 1 diabetes in 26 European centres in the 25 year period 1989-2013: a multicentre prospective registration study. *Diabetologia* (2019) 62:408–17. doi: 10.1007/s00125-018-4763-3

5. Pociot F, Lernmark Å. Genetic risk factors for type 1 diabetes. *Lancet* (2016) 387:2331–9. doi: 10.1016/S0140-6736(16)30582-7

6. Esposito S, Toni G, Tascini G, Santi E, Berioli MG, Principi N. Environmental factors associated with type 1 diabetes. *Front Endocrinol (Lausanne)* (2019) 10:592. doi: 10.3389/fendo.2019.00592

7. Marré ML, Profozich JL, Coneybeer JT, Geng X, Bertera S, Ford MJ, et al. Inherent ER stress in pancreatic islet beta cells causes self-recognition by autoreactive T cells in type 1 diabetes. *J Autoimmun* (2016) 72:33–46. doi: 10.1016/j.jaut.2016.04.009

8. Marre ML, McGinty JW, Chow IT, DeNicola ME, Beck NW, Kent SC, et al. Modifying enzymes are elicited by ER stress, generating epitopes that are selectively recognized by CD4(+) T cells in patients with type 1 diabetes. *Diabetes* (2018) 67:1356–68. doi: 10.2337/db17-1166

9. Marré ML, James EA, Piganelli JD. Beta cell ER stress and the implications for immunogenicity in type 1 diabetes. *Front Cell Dev Biol* (2015) 3:67. doi: 10.3389/fcell.2015.00067

10. Clark AL, Urano F. Endoplasmic reticulum stress in beta cells and autoimmune diabetes. *Curr Opin Immunol* (2016) 43:60–6. doi: 10.1016/j.coi.2016.09.006

11. Cianciaruso C, Phelps EA, Pasquier M, Hamelin R, Demurtas D, Alibashe AM, et al. Primary human and rat beta-cells release the intracellular autoantigens GAD65, IA-2, and proinsulin in exosomes together with cytokine-induced enhancers of immunity. *Diabetes* (2017) 66:460–73. doi: 10.2337/db16-0671

12. Conrad B, Weissmahr RN, Böni J, Arcari R, Schüpbach J, Mach B. A human endogenous retroviral superantigen as candidate autoimmune gene in type I diabetes. *Cell* (1997) 90:303–13. doi: 10.1016/s0092-8674(00)80338-4

13. Jun HS, Yoon JW. A new look at viruses in type 1 diabetes. *Diabetes Metab Res Rev* (2003) 19:8–31. doi: 10.1002/dmrr.337

14. Funda DP, Kaas A, Bock T, Tlaskalová-Hogenová H, Buschard K. Gluten-free diet prevents diabetes in NOD mice. *Diabetes Metab Res Rev* (1999) 15:323–7. doi: 10.1002/(sici)1520-7560(199909/10)15:5<323::aid-dmrr53>3.0.co;2-p

15. Borch-Johnsen K, Joner G, Mandrup-Poulsen T, Christy M, Zachau-Christiansen B, Kastrup K, et al. Relation between breast-feeding and incidence rates of insulin-dependent diabetes mellitus. a hypothesis. *Lancet* (1984) 2:1083–6. doi: 10.1016/s0140-6736(84)91517-4

16. Scharf RJ, DeBoer MD. Sugar-sweetened beverages and children's health. *Annu Rev Public Health* (2016) 37:273–93. doi: 10.1146/annurev-publhealth-032315-021528

17. Brownell KD, Farley T, Willett WC, Popkin BM, Chaloupka FJ, Thompson JW, et al. The public health and economic benefits of taxing sugar-sweetened beverages. *N Engl J Med* (2009) 361:1599–605. doi: 10.1056/NEJMhpr0905723

18. Imamura F, O'Connor L, Ye Z, Mursu J, Hayashino Y, Bhupathiraju SN, et al. Consumption of sugar sweetened beverages, artificially sweetened beverages, and fruit juice and incidence of type 2 diabetes: systematic review, meta-analysis, and estimation of population attributable fraction. *Br J Sports Med* (2016) 50:496–504. doi: 10.1136/bjsports-2016-h3576rep

19. Lean ME, Te ML. Sugar and type 2 diabetes. *Br Med Bull* (2016) 120:43–53. doi: 10.1093/bmb/ldw037

20. Lamb MM, Frederiksen B, Seifert JA, Kroehl M, Rewers M, Norris JM. Sugar intake is associated with progression from islet autoimmunity to type 1 diabetes: the diabetes autoimmunity study in the young. *Diabetologia* (2015) 58:2027–34. doi: 10.1007/s00125-015-3657-x

21. Cock PJ, Fields CJ, Goto N, Heuer ML, Rice PM. The Sanger FASTQ file format for sequences with quality scores, and the Solexa/Illumina FASTQ variants. *Nucleic Acids Res* (2010) 38:1767–71. doi: 10.1093/nar/gkp1137

22. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. *Nat Methods* (2015) 12:357–60. doi: 10.1038/nmeth.3317

23. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* (2014) 15:550. doi: 10.1186/s13059-014-0550-8

24. Lamb MM, Yin X, Barriga K, Hoffman MR, Barón AE, Eisenbarth GS, et al. Dietary glycemic index, development of islet autoimmunity, and subsequent progression to type 1 diabetes in young children. *J Clin Endocrinol Metab* (2008) 93:3936–42. doi: 10.1210/jc.2008-0886

25. Goncalves MD, Lu C, Tutnauer J, Hartman TE, Hwang SK, Murphy CJ, et al. High-fructose corn syrup enhances intestinal tumor growth in mice. *Science* (2019) 363:1345–9. doi: 10.1126/science.aat8515

26. Qi Q, Chu AY, Kang JH, Jensen MK, Curhan GC, Pasquale LR, et al. Sugar-sweetened beverages and genetic risk of obesity. *N Engl J Med* (2012) 367:1387–96. doi: 10.1056/NEJMoa1203039

27. Yang Q, Zhang Z, Gregg EW, Flanders WD, Merritt R, Hu FB. Added sugar intake and cardiovascular diseases mortality among US adults. *JAMA Intern Med* (2014) 174:516–24. doi: 10.1001/jamainternmed.2013.13563

28. Jonas JC, Bensellam M, Duprez J, Elouil H, Guiot Y, Pascal SM. Glucose regulation of islet stress responses and beta-cell failure in type 2 diabetes. *Diabetes Obes Metab* (2009) 11 Suppl 4:65–81. doi: 10.1111/j.1463-1326.2009.01112.x

29. Zhang D, Jin W, Wu R, Li J, Park SA, Tu E, et al. High glucose intake exacerbates autoimmunity through reactive-Oxygen-Species-Mediated TGF-beta cytokine activation. *Immunity* (2019) 51:671–81. doi: 10.1016/j.immuni.2019.08.001

30. Todoric J, Di Caro G, Reibe S, Henstridge DC, Green CR, Vrbanac A, et al. Fructose stimulated *de novo* lipogenesis is promoted by inflammation. *Nat Metab* (2020) 2:1034–45. doi: 10.1038/s42255-020-0261-2

31. Yu Z, Lowndes J, Rippe J. High-fructose corn syrup and sucrose have equivalent effects on energy-regulating hormones at normal human consumption levels. *Nutr Res* (2013) 33:1043–52. doi: 10.1016/j.nutres.2013.07.020

32. Davis JA, Freeze HH. Studies of mannose metabolism and effects of long-term mannose ingestion in the mouse. *Biochim Biophys Acta* (2001) 1528:116–26. doi: 10.1016/S0304-4165(01)00183-0

33. McGinty JW, Chow IT, Greenbaum C, Odegard J, Kwok WW, James EA. Recognition of posttranslationally modified GAD65 epitopes in subjects with type 1 diabetes. *Diabetes* (2014) 63:3033–40. doi: 10.2337/db13-1952

34. Mannering SI, Harrison LC, Williamson NA, Morris JS, Thearle DJ, Jensen KP, et al. The insulin a-chain epitope recognized by human T cells is posttranslationally modified. *J Exp Med* (2005) 202:1191–7. doi: 10.1084/jem.20051251

35. McGinty JW, Marré ML, Bajzik V, Piganelli JD, James EA. T Cell epitopes and post-translationally modified epitopes in type 1 diabetes. *Curr Diabetes Rep* (2015) 15:90. doi: 10.1007/s11892-015-0657-7

36. Björk E, Kämpe O, Karlsson FA, Pipeleers DG, Andersson A, Hellerström C, et al. Glucose regulation of the autoantigen GAD65 in human pancreatic islets. *J Clin Endocrinol Metab* (1992) 75:1574–6. doi: 10.1210/jcem.75.6.1464667

37. Skowera A, Ellis RJ, Varela-Calviño R, Arif S, Huang GC, Van-Krinks C, et al. CTLs are targeted to kill beta cells in patients with type 1 diabetes through recognition of a glucose-regulated preproinsulin epitope. *J Clin Invest* (2008) 118:3390–402. doi: 10.1172/JCI35449

38. Dudek NL, Tan CT, Gorasia DG, Croft NP, Illing PT, Purcell AW. Constitutive and inflammatory immunopeptidome of pancreatic β-cells. *Diabetes* (2012) 61:3018–25. doi: 10.2337/db11-1333

39. Gorasia DG, Dudek NL, Veith PD, Shankar R, Safavi-Hemami H, Williamson NA, et al. Pancreatic beta cells are highly susceptible to oxidative and ER stresses during the development of diabetes. *J Proteome Res* (2015) 14:688–99. doi: 10.1021/pr500643h

40. Schloot NC, Hanifi-Moghaddam P, Goebel C, Shatavi SV, Flohé S, Kolb H, et al. Serum IFN-gamma and IL-10 levels are associated with disease progression in non-obese diabetic mice. *Diabetes Metab Res Rev* (2002) 18:64–70. doi: 10.1002/dmrr.256

41. Savinov AY, Wong FS, Chervonsky AV. IFN-gamma affects homing of diabetogenic T cells. *J Immunol* (2001) 167:6637–43. doi: 10.4049/jimmunol.167.11.6637

42. Vomund AN, Zinselmeyer BH, Hughes J, Calderon B, Valderrama C, Ferris ST, et al. Beta cells transfer vesicles containing insulin to phagocytes for presentation to T cells. *Proc Natl Acad Sci U.S.A.* (2015) 112:E5496–502. doi: 10.1073/pnas.1515954112

43. Ferris ST, Carrero JA, Unanue ER. Antigen presentation events during the initiation of autoimmune diabetes in the NOD mouse. *J Autoimmun* (2016) 71:19–25. doi: 10.1016/j.jaut.2016.03.007

44. Calderon B, Suri A, Miller MJ, Unanue ER. Dendritic cells in islets of langerhans constitutively present beta cell-derived peptides bound to their class II MHC molecules. *Proc Natl Acad Sci USA* (2008) 105:6121–6. doi: 10.1073/pnas.0801973105

45. Dai YD, Sheng H, Dias P, Jubayer RM, Bashratyan R, Regn D, et al. Autoimmune responses to exosomes and candidate antigens contribute to type 1 diabetes in non-obese diabetic mice. *Curr Diabetes Rep* (2017) 17:130. doi: 10.1007/s11892-017-0962-4

46. Bashratyan R, Sheng H, Regn D, Rahman MJ, Dai YD. Insulinoma-released exosomes activate autoreactive marginal zone-like b cells that expand endogenously in prediabetic NOD mice. *Eur J Immunol* (2013) 43:2588–97. doi: 10.1002/eji.201343376

47. Sheng H, Hassanali S, Nugent C, Rahman MJ, Dai YD. Insulinoma-released exosomes or microparticles are immunostimulatory and can activate autoreactive T cells spontaneously developed in nonobese diabetic mice. *J Immunol* (2011) 187:1591–600. doi: 10.4049/jimmunol.1100231

48. Rahman MJ, Regn D, Bashratyan R, Dai YD. Exosomes released by islet-derived mesenchymal stem cells trigger autoimmune responses in NOD mice. *Diabetes* (2014) 63:1008–20. doi: 10.2337/db13-0859

49. Wan X, Zinselmeyer BH, Zakharov PN, Vomund AN, Taniguchi R, Santambrogio L, et al. Pancreatic islets communicate with lymphoid tissues via exocytosis of insulin peptides. *Nature* (2018) 560:107–11. doi: 10.1038/s41586-018-0341-6

50. Rutman AK, Negi S, Gasparrini M, Hasilo CP, Tchervenkov J, Paraskevas S. Immune response to extracellular vesicles from human islets of langerhans in patients with type 1 diabetes. *Endocrinology* (2018) 159:3834–47. doi: 10.1210/en

51. Nojehdehi S, Soudi S, Hesampour A, Rasouli S, Soleimani M, Hashemi SM. Immunomodulatory effects of mesenchymal stem cell-derived exosomes on experimental type-1 autoimmune diabetes. *J Cell Biochem* (2018) 119:9433–43. doi: 10.1002/jcb.27260

52. Calderon B, Carrero JA, Unanue ER. The central role of antigen presentation in islets of langerhans in autoimmune diabetes. *Curr Opin Immunol* (2014) 26:32–40. doi: 10.1016/j.coi.2013.10.011

53. Lee HS, Jeong J, Lee KJ. Characterization of vesicles secreted from insulinoma NIT-1 cells. *J Proteome Res* (2009) 8:2851–62. doi: 10.1021/pr900009y

54. Li D, Zhang W, Chen X, Ling H, Xie P, Chen Z, et al. Proteomic profiling of MIN6 cell-derived exosomes. *J Proteomics* (2020) 224:103841. doi: 10.1016/j.jprot.2020.103841

55. Da SNA, Borges FT, Maquigussa E, Varela VA, Dias M, Boim MA. Influence of high glucose on mesangial cell-derived exosome composition, secretion and cell communication. *Sci Rep* (2019) 9:6270. doi: 10.1038/s41598-019-42746-1

56. Rice GE, Scholz-Romero K, Sweeney E, Peiris H, Kobayashi M, Duncombe G, et al. The effect of glucose on the release and bioactivity of exosomes from first trimester trophoblast cells. *J Clin Endocrinol Metab* (2015) 100:E1280–8. doi: 10.1210/jc.2015-2270

57. Ruhland MK, Roberts EW, Cai E, Mujal AM, Marchuk K, Beppler C, et al. Visualizing synaptic transfer of tumor antigens among dendritic cells. *Cancer Cell* (2020) 37:786–99. doi: 10.1016/j.ccell.2020.05.002

58. Abramowicz A, Widłak P, Pietrowska M. Different types of cellular stress affect the proteome composition of small extracellular vesicles: A mini review. *Proteomes* (2019) 7(2):23. doi: 10.3390/proteomes7020023

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Assessing temporal differences of baseline body mass index, waist circumference, and waist-height ratio in predicting future diabetes

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Objective: Obesity is the prominent modifiable risk factor known to influence the occurrence and progression of diabetes other than age, and the objective of this study was to evaluate and compare the predictive value of three simple baseline anthropometric indicators of obesity, body mass index (BMI), waist circumference (WC), and waist-height ratio (WHtR), for the occurrence of diabetes at different time points in the future.

Methods: The study subjects were 12,823 individuals with normoglycemic at baseline who underwent health screening and had measurements of BMI, WC, and WHtR. The outcome of interest was new-onset diabetes during follow-up. Time-dependent receiver operator characteristics (ROC) curves of baseline BMI, WC, and WHtR for predicting the risk of diabetes in the next 2 to 12 years were constructed and their area under the ROC curves (AUCs) and corresponding optimal threshold values were calculated for each time point, which were used to compare the accuracy and stability of the above three indicators for predicting the occurrence of diabetes in different future periods.

Results: During a median follow-up period of 7.02 years, with a maximum follow-up of 13 years, 320 new-onset diabetes were recorded. After adjusting for confounders and comparing standardized hazard ratios (HRs), WC was shown to be the best simple anthropometric indicator of obesity reflecting diabetes risk in all models, followed by WHtR. Time-dependent ROC analysis showed that WC had the highest AUC in predicting the occurrence of diabetes in the short term (2-5 years), and WHtR had the highest AUC in predicting the occurrence of diabetes in the medium to long term (6-12 years), while in any time point, both WC and WHtR had higher AUC than BMI in predicting future diabetes. In addition, we found relatively larger fluctuations in the thresholds of BMI and WC for predicting diabetes over time, while the thresholds of WHtR

consistently remained between 0.47-0.50; comparatively speaking, WHtR may have greater application value in predicting future diabetes.

Conclusions: Our analysis sustained that central obesity is a more important predictor of diabetes, and in clinical practice, we proposed measuring WHtR as a useful tool for predicting future diabetes.

KEYWORDS

waist circumference, BMI, central obesity, diabetes, time-dependent ROC, waist-height ratio

Introduction

Diabetes, one of the leading causes of death and disability, is now very prevalent worldwide, generating an economic burden of approximately 10% of global health expenditures (1, 2). There are many risk factors contributing to the development of diabetes, the most common of which are obesity, advanced age, family history of diabetes, race, lipid abnormalities, poor eating habits and lack of physical activity (3, 4). It is worth noting that age, race, and family history of diabetes cannot be changed, while obesity is generally considered to be an adverse consequence of poor eating habits and insufficient physical exercise, and dyslipidemia is a common metabolic abnormality in obese people (5, 6). Therefore, it is very important to effectively evaluate the relationship between obesity and diabetes to reduce the risk of diabetes.

BMI, WC, and WHtR are the simplest general anthropometric indicators of obesity used to assess diabetes risk (7–9), where BMI is calculated as weight(kg)/[height(m)]^2 and WHtR is calculated as WC(cm)/height(cm). Compared with BMI, evidence from recent observational studies has shown that measures of central obesity, WC and WHtR, are more strongly associated with diabetes and its associated cardiovascular disease risk because they take into account the distribution of fat (10–15). Furthermore, it is worth noting that in a recent meta-analysis of 216 cohort studies published in the BMJ, Jayedi et al. showed that in routine measurements, WHtR was more associated with diabetes than WC, waist-to-hip ratio and BMI (16). Although the correlation between simple obesity parameters and diabetes has been well unified, among which WHtR was considered to be the most appropriate index, there is no unified answer to the prediction of future diabetes by simple obesity parameters at present, and the viewpoints in several published meta-analyses were also inconsistent or even contradictory (9, 16–22). It is also important to note that although most of the published similar studies performed follow-up, these studies did not factor time into the ROC analysis (9, 18, 21), which may have some bias on the predictive results of the longitudinal analysis. To address this issue, in the current study, we constructed time-dependent ROC curves at multiple follow-up time points, based on a large longitudinal cohort of NAGALA, to evaluate the variations in predictive values of BMI, WC, and WHtR for future diabetes.

Methods

Study population and data sources

The population for this study was drawn from a longitudinal cohort study, the NAGALA study, in which we used data from 1994-2016 (Containing 20,944 participants) for an investigation into the predictive power of simple anthropometric indicators of obesity for future diabetes risk. This longitudinal cohort data has been uploaded to the Dryad database for public sharing by Okamura et al. (23), and according to the terms of service of the Dryad database, researchers can employ these data for secondary analysis based on different study hypotheses. In a previous study, Okamura et al. analyzed the association between obesity phenotype and diabetes, and the detailed study design has been published elsewhere (24). In short, the NAGALA cohort, initiated in 1994, continues to enroll the general population who have participated in a health check-up program at the Murakami Memorial Hospital Check-up Center to conduct an epidemiological study of diabetes and fatty liver disease. It should be mentioned that about 60% of the participants in the NAGALA cohort received one or two health check-ups per year, and the vast majority of participants underwent repeated check-ups (including blood glucose as well as abdominal ultrasound) at follow-up. During each physical examination, the participants were reviewed by trained investigators for diabetes diagnosis, including whether the participants had diabetes diagnosed by other medical staff during the follow-up period, and blood glucose parameters were assessed. In this study, based on the

new research hypothesis, we selected the target population of the current study according to the following exclusion criteria: (a) subjects with diabetes/impaired fasting glucose (6.1 mmol/L< baseline FPG <7.8 mmol/L)/liver disease (except fatty liver) at baseline; (b) subjects with alcohol abuse (>60 g per day for men and >40 g per day for women) (25); (c) subjects who were taking medication at baseline; (d) subjects with incomplete physical examination data; (e) subjects who withdrew from the survey for unknown reasons. To minimize the potential effect of reverse causality, we also excluded subjects with less than 2 years of follow-up data (26). Finally, we included 12,823 eligible subjects, and Figure 1 shows the process of inclusion and exclusion of the study population.

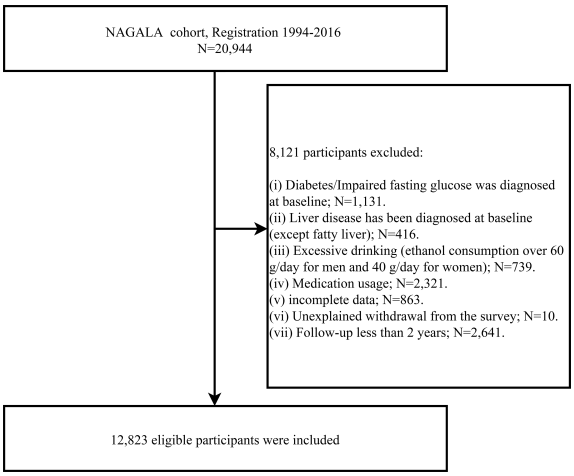

FIGURE 1
Study profile.

Ethical approval and consent to participate

The previous study was approved by the Ethics Committee of Murakami Memorial Hospital and authorized with informed consent from the subjects (24). In the present study, as it was a *post hoc* analysis of the NAGALA study, the Ethics Committee of Jiangxi Provincial People's Hospital waived repeated acquisition of informed consent from the subjects and approved the current study protocol (IRB2021-066), and all study procedures were performed in accordance with the Declaration of Helsinki. STROBE checklist (S1Text).

Data acquisition and measurement

As reported in the previous study (24), for demographic information, subjects reported factors such as gender, age, drinking/smoking status, exercise habits, medication use, and disease status through a standardized questionnaire. Anthropometric indicators including height, weight, WC, and blood pressure were measured by trained professionals using standard methods.

Venous blood samples were collected from the subjects after 8 hours of fasting by experienced medical personnel, and then biochemical markers such as liver enzymes [gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT)], lipid indicators [high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), total cholesterol (TC)], and blood glucose indicators [hemoglobin A1c (HbA1c), fasting plasma glucose (FPG)] were measured using an automated biochemical analyzer.

Fatty liver was determined by experienced gastroenterologists based on several sonograms of deep attenuation, hepatorenal echo contrast, vascular blurring, and liver brightness under abdominal ultrasound (27).

Definition

Exercise habit: Having more than 1 regular exercise session per week was defined as having an exercise habit, otherwise, it was considered as no exercise habit (28).

Drinking status: Professionals assessed alcohol intake by asking subjects about their weekly drinking in the month prior to the survey and classified subjects into four categories based on the following criteria: <40 g/week, no or little drinking; 40-140 g/week, light drinking; 140-280 g/week, moderate drinking; >280 g/week, heavy drinking (25).

Smoking status: Smokers were divided into three groups: non-smokers, past smokers, and current smokers based on the participants' smoking history before the baseline survey.

Diabetes determination: According to the American Diabetes Association criteria (29), subjects were defined as having diabetes if they tested FPG ≥ 7.0 mmol/L or HbA1c ≥ 6.5% during follow-up or if they were diagnosed with diabetes by other medical personnel.

Statistical analysis

The current research data were analyzed using R language version 4.2.0. The median, mean, or proportions of demographic and biochemical factors in the study population were summarized by grouping according to the presence or absence of diabetes during follow-up. Using the inverse probability of treatment weighting method to calculate the weighted standardized difference to estimate the magnitude of the difference between groups at baseline (the difference > 10%) was considered significant) (30, 31).

Cox proportional hazards regression models were used to assess the associations between baseline BMI, WC, WHtR and the risk of developing diabetes. Before modeling for data analysis, the possibility of multicollinearity between independent variables and covariates was tested using a variance inflation factor, where a variance inflation factor of less than 5 was considered desirable (32). In addition, the proportional hazards assumptions were assessed using Kaplan-Meier survival curves (33). Five multivariate Cox regression models were developed based on the epidemiological STROBE statement (34). The effects of age and gender on outcomes were considered in model 1. Model 2 was further adjusted for height and lifestyle-related factors such as exercise habits, smoking status, drinking status, and fatty liver. On this basis, model 3 additionally adjusted for liver enzyme parameters (ALT, AST, GGT). Model 4 adjusted all non-collinear covariates that were potentially associated with diabetes. Finally, in order to further consider the potential effects of insulin resistance (IR), we calculated the triglyceride-glucose index (35), an alternative indicator of IR, and adjusted this variable in model 5. Furthermore, we also used R-packet timeROC to construct ROC curves at 11 follow-up time points; then to calculate the AUC for each parameter from year 2 to year 12 and record the corresponding optimal thresholds for comparing the predictive ability and stability of BMI, WC and WHtR for predicting diabetes in different future periods, and finally compared the AUC of BMI, WC, WHtR at each time point. A 2-tailed value of $P<0.05$ was considered significant.

Results

Baseline characteristics of the study population

Data from 12,823 subjects were finally analyzed according to exclusion criteria, with a mean age of 43.54 (8.70) years. During a median follow-up period of 7.02 years (maximum 13 years), new-onset diabetes was recorded in 320 participants, with an incidence density of 34.93 per 10,000 person-years. We divided the population into diabetic and non-diabetic groups based on whether the subject developed diabetes during follow-up and descriptively compared the differences in clinical baseline characteristics between the two groups. As shown in Table 1, significant differences between those with and without future diabetes were already present at the time of the initial collection of baseline information (all standardized differences were >10%). Compared with the non-diabetic group, subjects in the diabetic group were older, more men, more alcohol consumption, and more of them had a history of smoking (including past and current smoking) and also a higher prevalence of fatty liver at baseline (105%). In terms of baseline glucose and lipid metabolism-related parameters, FPG, HbA1c, TG, and TC were significantly higher in the diabetic group than in the non-diabetic group; in terms of anthropometric parameters, weight, WC, BMI, and WHtR also differed significantly between the two groups (79%-96%).

Associations between BMI, WC, and WHtR and new-onset diabetes

Before building the Cox proportional hazards regression models, our data did not violate the proportional hazards assumptions based on the Kaplan-Meier graphical method for BMI quartiles, WC quartiles, and WHtR quartiles over time [see Additional File 1, Supplementary Figures 1-3]; moreover, covariates that were collinear with the independent variables will not be included in the subsequent multivariate Cox regression models [see Additional File 1, Supplementary Tables S1-S3].

Multivariate Cox regression models were developed to assess the associations between baseline BMI, WC, and WHtR and the occurrence of future diabetes. To standardize the hazard ratio (HR) of each independent variable affecting the outcome, we performed Z-transformation for BMI, WC, and WHtR before incorporating them into the Cox regression models. Based on epidemiology, we established five multivariate Cox regression models (Table 2), from model 1 to model 5 showing the dynamic changes in HR after stepwise adjustment. From the overall trend, the HR values of the three simple obesity indicators decreased gradually with the increase of covariates, and compared with WHtR and BMI, WC reflected a higher risk of developing diabetes in the future. In the final model (model 5), the risk of diabetes increased by 39% [95% confidence interval (CI): 1.23-1.56] for each standard deviation (SD) increase in BMI, 52% (95% CI: 1.33-1.75) for each SD increase in WC, and 47% (95% CI: 1.29-1.66) for each SD increase in WHtR.

Accuracy of BMI, WC, and WHtR in predicting the occurrence of diabetes in different future periods

Time-dependent ROC curves were plotted for assessing the accuracy of baseline BMI, WC, and WHtR in predicting the onset of diabetes at different times in the future, and the corresponding AUCs were shown in Table 3. Generally speaking, the simple obesity indicators, BMI, WC, and WHtR, were all highly stable in predicting future diabetes, and interestingly, the predictive accuracy of WHtR increased progressively with a longer follow-up time. It is worth mentioning that the AUC of WC was higher than that of BMI and WHtR in predicting the occurrence of diabetes in the year 2 to year 5 of follow-up [AUC:(2-years: WC 0.67 > WHtR 0.64 > BMI 0.63; 3-years: WC 0.70 > WHtR 0.68 > BMI 0.67; 4-years:

TABLE 1 Baseline demographic, lifestyle, and laboratory characteristics in subjects with and without diabetes.

	Non-diabetic	Diabetic	Standardized Difference (95% CI), %
Participants(n)	12503	320	
Age(years)	43.46 (8.69)	46.83 (8.38)	39 (28, 51)
Gender			48 (37, 59)
Women	5728 (45.81%)	75 (23.44%)	
Men	6775 (54.19%)	245 (76.56%)	
Height (m)	1.65 (0.08)	1.66 (0.09)	17 (5, 28)
Weight (kg)	60.38 (11.44)	70.24 (13.58)	79 (67, 90)
BMI (kg/m^2)	22.03 (3.05)	25.23 (3.90)	91 (80, 103)
WC (cm)	76.16 (8.98)	85.45 (10.38)	96 (85, 107)
WHtR	0.46 (0.05)	0.51 (0.06)	96 (85, 107)
ALT (IU/L)	17.00 (13.00-23.00)	25.00 (19.00-39.25)	69 (58, 80)
AST (IU/L)	17.00 (14.00-21.00)	20.00 (16.00-26.00)	42 (31, 53)
GGT (IU/L)	15.00 (11.00-22.00)	24.00 (17.00-36.00)	49 (38, 60)
HDL-C (mmol/L)	1.45 (0.39)	1.18 (0.31)	79 (67, 90)
TC (mmol/L)	5.11 (0.85)	5.44 (0.87)	38 (27, 49)
TG (mmol/L)	0.73 (0.50-1.11)	1.24 (0.88-1.96)	75 (64, 86)
HbA1c (%)	5.15 (0.32)	5.49 (0.36)	102 (91, 113)
FPG (mmol/L)	5.14 (0.41)	5.60 (0.36)	122 (110, 133)
SBP (mmHg)	114.24 (14.81)	122.45 (15.82)	54 (43, 65)
DBP (mmHg)	71.48 (10.33)	77.47 (10.23)	58 (47, 69)
Fatty liver	2036 (16.28%)	197 (61.56%)	105 (94, 116)
Exercise habits	2153 (17.22%)	41 (12.81%)	12 (1, 23)
Drinking status			20 (9, 31)
No/little	9581 (76.63%)	230 (71.88%)	
Light	1432 (11.45%)	34 (10.62%)	
Moderate	1075 (8.60%)	32 (10.00%)	
Heavy	415 (3.32%)	24 (7.50%)	
Smoking status			47 (36, 58)
Non	7378 (59.01%)	121 (37.81%)	
Past	2345 (18.76%)	66 (20.62%)	
Current	2780 (22.23%)	133 (41.56%)	

Values were expressed as mean (SD) or medians (quartile interval) or n (%). BMI, body mass index; WC, Waist circumference; WHtR, Waist-to-height ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; HbA1c, hemoglobin A1c; FPG, fasting plasma glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure.

WC 0.67 > WHtR 0.66 > BMI 0.64; 5-years: WC 0.70 > WHtR 0.69 > BMI 0.66)], and the AUC of WHtR was the highest in predicting the occurrence of diabetes in the year 6 to year 12 of follow-up [AUC:(6-years: WHtR 0.70 = WC 0.70 > BMI 0.68; 7-years: WHtR 0.72 = WC 0.72 > BMI 0.69; 8-years: WHtR 0.72 > WC 0.71 > BMI 0.70; 9-years: WHtR 0.71 > WC 0.70 > BMI 0.69; 10-years: WHtR 0.71 > WC 0.69 = BMI 0.69; 11-years: WHtR 0.71 > WC 0.69 = BMI 0.69; 12-years: WHtR 0.70 > WC 0.67 = BMI 0.67)]. In addition, the pairwise comparison results based on different time points showed that BMI and WC differed

TABLE 2 Cox regression analyses for the association between BMI, WC, WHtR and incident diabetes in different models.

	Hazard ratios (95% confidence interval)				
	Model 1	Model 2	Model 3	Model 4	Model 5
BMI (Per SD increase)	2.15 (1.98, 2.33)	1.67 (1.51, 1.85)	1.61 (1.45, 1.79)	1.38 (1.22, 1.55)	1.39 (1.23, 1.56)
WC (Per SD increase)	2.37 (2.15, 2.62)	1.85 (1.64, 2.09)	1.78 (1.57, 2.01)	1.51 (1.32, 1.73)	1.52 (1.33, 1.75)
WHtR (Per SD increase)	2.32 (2.12, 2.54)	1.76 (1.58, 1.96)	1.69 (1.51, 1.89)	1.46 (1.29, 1.65)	1.47 (1.29, 1.66)

BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio.
Model 1 adjusted for gender, age.
Model 2 adjusted for gender, age, height, fatty liver, exercise habits, drinking status and smoking status.
Model 3 adjusted for gender, age, height, fatty liver, exercise habits, drinking status. smoking status, ALT, AST and GGT.
Model 4 adjusted for gender, age, height, fatty liver, exercise habits, drinking status, smoking status, ALT, AST, GGT, HDL-C, TC, TG, FPG, HbA1c and SBP.

significantly only at the second- and fifth-year time points, BMI and WHtR differed from year 7 to year 12, and WC and WHtR only differed at year 11 and year 12. Although most of the results of the statistical pairwise comparison showed that these differences were not significant, it is undeniable that at all time points, the AUC of WC and WHtR for predicting future diabetes was higher than that of BMI.

Threshold analysis of BMI, WC, and WHtR for predicting the onset of diabetes in different future periods

Using time-dependent ROC curves, we also calculated the optimal thresholds and the corresponding sensitivities and specificities of baseline BMI, WC, and WHtR for predicting diabetes at different times in the future (Figure 2). We found large fluctuations in the optimal thresholds of BMI and WC for predicting diabetes in different future periods (BMI optimal thresholds range: 20.99-24.57; WC optimal thresholds range: 79.3-83.6), while the thresholds of WHtR remained relatively stable (WHtR optimal thresholds range: 0.47-0.5). In addition, it is worth mentioning that the sensitivity of BMI for predicting future diabetes gradually increased and the specificity gradually decreased over time; while the sensitivity and specificity of WC, and WHtR for predicting future diabetes fluctuated relatively little at multiple time points.

Discussion

This longitudinal cohort study explored the relationship between simple baseline anthropometric indicators of obesity, BMI, WC, and WHtR, and the risk of developing diabetes in different future periods. The study found that WC and WHtR may have better application value than BMI in assessing the risk of diabetes and predicting future diabetes.

The correlation between simple measurement of obesity parameters and diabetes has been widely studied before. WC and WHtR, which represent central obesity, have been recognized by most researchers as better indicators than BMI (10–16). In the current study, we employed a similar approach to that adopted in several previous meta-analyses, and our findings also supported a more important role of central obesity in the development of diabetes, where baseline WC may be the most useful simple obesity indicator to assess the risk of future diabetes, a finding that was consistent with the results of Prof. Kodama's as well as Prof. Hartwig's meta-analysis (19, 21). These findings collectively suggested that screening for central obesity should be given greater attention in diabetes risk assessment.

The diagnostic/predictive value of BMI, WC, and WHtR for diabetes had been summarized in several previous meta-analyses. Generally speaking, WHtR and WC were equivalent and both were superior to BMI (9, 18, 21); further distinguishing meta-analyses of cohort studies showed that WHtR was the best predictor (21). It is important to note that although most of the studies included in several published meta-analyses have performed follow-up, they did not factor time into the ROC analysis in assessing the predictive power of simple anthropometric indicators of obesity for diabetes (9, 18, 21), and only one study specially assessed the accuracy of these simple indicators for predicting the occurrence of diabetes at 5 years (22). In a meta-analysis of 21 cohort studies by Lee et al., they used Harrell's-index to test the predictive accuracy of simple obesity parameters for the risk of diabetes; and the results showed that there was no significant difference between WHtR, WC, and BMI in terms of the predictive accuracy of 5-year diabetes (22). In the current study, we used time-dependent ROC curves, in a cohort of 12,823 nondiabetic subjects, to evaluate the accuracy of baseline BMI, WC, and WHtR in predicting new-onset diabetes at different time points in the future, and the results showed that in terms of predicting diabetes at five years, both WC and WHtR had AUCs greater than BMI, which was different from the conclusion of Lee et al. Based on the results of the above analysis, we further analyzed the accuracy of using baseline BMI, WC, and WHtR for predicting diabetes at each time point from year 2 to year 12, and the results showed that WC had the highest AUC in predicting the onset of diabetes in the short term (2-5 years),

TABLE 3 Best threshold, sensitivities, specificities and areas under the time-dependent receiver operating characteristic curves for BMI, WC and WHtR predicting future diabetes risk.

		BMI				WC				WHtR			
	Diabetes events, n	Optimal threshold	AUC	Sensitivity	Specificity	Optimal threshold	AUC	Sensitivity	Specificity	Optimal threshold	AUC	Sensitivity	Specificity
2-years	38	20.99	0.63*	0.85	0.39	79.5	0.67	0.63	0.64	0.48	0.64	0.56	0.68
3-years	29	24.57	0.67	0.44	0.81	82.1	0.70	0.76	0.75	0.48	0.68	0.63	0.68
4-years	34	23.89	0.64	0.45	0.75	82.1	0.67	0.50	0.75	0.48	0.66	0.63	0.63
5-years	31	23.51	0.66*	0.54	0.71	83.6	0.70	0.54	0.79	0.50	0.69	0.54	0.76
6-years	39	23.41	0.68	0.57	0.70	79.7	0.70	0.65	0.65	0.48	0.70	0.63	0.68
7-years	32	22.26	0.69	0.74	0.57	80.1	0.72	0.66	0.68	0.49	0.72**	0.58	0.75
8-years	20	22.01	0.70	0.78	0.53	80.5	0.71	0.63	0.69	0.49	0.72**	0.63	0.71
9-years	34	22.00	0.69	0.76	0.53	79.3	0.70	0.67	0.64	0.49	0.71**	0.57	0.73
10-years	28	22.03	0.69	0.75	0.54	79.5	0.69	0.65	0.65	0.47	0.71**	0.73	0.59
11-years	22	22.00	0.69	0.75	0.54	79.6	0.69***	0.64	0.66	0.47	0.71**	0.72	0.60
12-years	13	21.11	0.67	0.84	0.42	79.7	0.67***	0.61	0.66	0.47	0.70**	0.68	0.62

AUC, area under the curve; other abbreviations as in Table 1.
*P < 0.05 BMI vs WC; **P < 0.05 BMI vs WHtR; ***P < 0.05 WC vs WHtR; Other variables with no special mark on the upper right corner had P values > 0.05 after pair-wise comparison.

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and WHtR had the highest AUC in predicting the onset of diabetes in the medium and long term (6-12 years), while in any time point, both WC and WHtR had higher AUC than BMI in predicting future diabetes. In general, an early assessment of central obesity status may be more beneficial for the primary prevention of diabetes than an assessment of general obesity.

We further evaluated the predictive thresholds of these simple obesity parameters for the occurrence of diabetes at each time point in the next 2-12 years. The results showed that in the same population the threshold of WHtR for predicting future diabetes was more stable over time than BMI/WC and may have better application value. Threshold analyses regarding using simple anthropometric indicators of obesity for the diagnosis/prediction of diabetes have also been reported in several previous studies (18, 20, 36–39), and in general, there were large differences in BMI and WC thresholds among different ethnic groups, while the threshold of WHtR was relatively stable. However, it is worth noting that according to

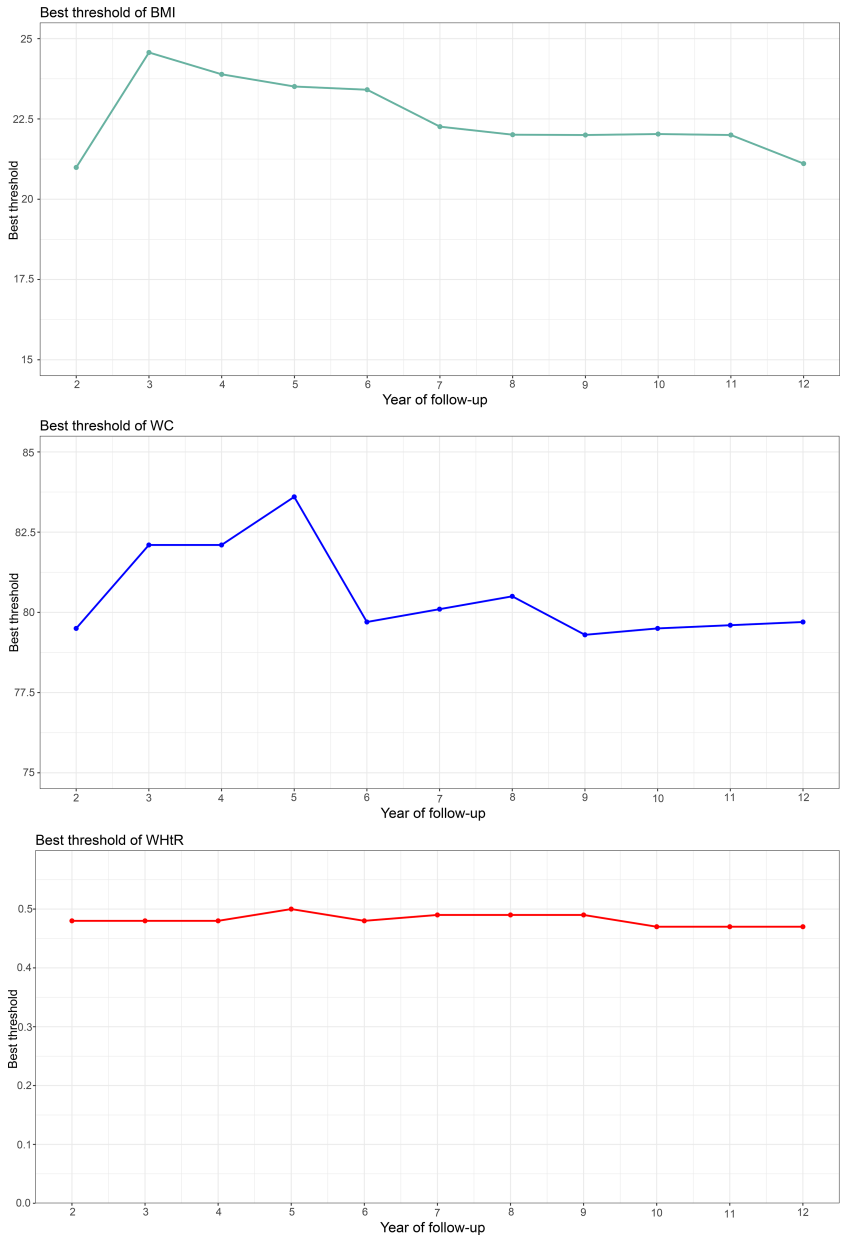

FIGURE 2
Threshold fluctuation of BMI, WC and WHtR used to predict future diabetes risk. BMI, body mass index; WC, waist circumference; WHtR, waist-height ratio.

the results of the meta-analysis by Savva et al. the median threshold of WHtR for identifying/predicting diabetes in Asian populations was 0.51 (20), a result similar to that of the current study by time-dependent ROC analysis; moreover, in the study by Savva et al., they further distinguished a WHtR threshold of 0.56 for identifying/predicting diabetes in non-Asian populations. These findings suggested that there may be small racial differences in WHtR for diagnosing/predicting diabetes risk, and further evaluation of the threshold of WHtR for predicting diabetes using time-dependent ROC in other races is needed to verify the stability of this finding.

As a chronic disease, early detection of potential risk factors and maintaining a healthy lifestyle are key to preventing diabetes (40), and screening for diabetes using non-invasive and simple anthropometric indicators is of greater importance compared to the drawing of venous blood for glucose measurement. Our results in the current study supported the idea that central obesity can provide additional information in terms of diabetes risk. In clinical practice, we suggested that more attention needs to be paid to those simple obesity indicators WC and WHtR which represent central obesity and have the most direct relevance for diabetes prevention. Furthermore, considering the performance of these simple obesity indicators for predicting diabetes at different time points, that is, the stability of predictive values and threshold fluctuations, we believed that WHtR was the best predictive indicator for diabetes. Our findings supported the public health initiative 'to keep WC at less than half of your height' (41) and demonstrated that this initiative is applicable at any time point. Referring to Professor Ashwell's vivid description, we only need a rope in the prediction of diabetes: use the rope to mark the height, fold it in half, and then wrap it around the waist. Also, according to the available literature, we have learned that WHtR is not only a good surrogate indicator of visceral fat compared to WC, but also has a great practical advantage in eliminating differences in body size among different ethnic groups, and using 0.5 as a threshold is generally suitable for health risk screening of the whole population, including children and adults (18, 42).

The main limitations of this study are found in the following (1): Waist-to-hip ratio is also useful in the assessment of central obesity among the simple anthropometric indicators of obesity (43, 44), however, hip circumference was not assessed in the current study, so it was not possible to further compare the predictive power of waist-to-hip ratio with BMI, WC, and WHtR for future diabetes. It is worth mentioning that several published meta-analyses generally support the superiority of WHtR over waist-to-hip ratio both in assessing diabetes risk and in identifying/predicting diabetes (16, 19, 21, 22). In addition, the influence of the history of gestational diabetes has not been taken into account in the current study, which may have an impact on the results of some women (2). The current study was a single-center cohort study, so the applicability of the findings to other races needs to be further validated. From another perspective, however, the single-center cohort ensured a good homogeneity of the study population (45) and yielded relatively reliable findings. Combined with the results of the meta-analysis by Savva et al. and the current analysis (20), the findings of the current study were applicable at least in Asian populations (3). The study population in the current study did not measure 2-hour postprandial glucose, therefore, we may have missed some of the diabetic patients (4). The survival status of the subjects was not recorded in the current study, thus some death cases during the follow-up period may pose some competing risks to the current study results (5). Because repeated-measures data of baseline indicators in the follow-up period were not included in the current study, the impact of dynamic changes in simple measures of obesity on diabetes cannot be further assessed, and further research is needed.

Conclusion

Altogether, our findings confirmed that central obesity was a more important risk/predictive factor for the assessment of diabetes than general obesity and identified WHtR as the most practical simple anthropometric indicator of obesity for predicting future diabetes, with 0.5 serving as a threshold value for initial diabetes risk screening.

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 Jiangxi Provincial People's Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

All authors conceived and designed the study. YZ, MK, GS, SZ and JQ conducted statistical analyses, and all authors interpreted the findings. YZ, GS, MK and JQ drafted the manuscript. GX, SZ, YC and NP critically reviewed the manuscript for key intellectual content. All authors approved the final manuscript. YZ and GS are the guarantors and, as such, had full access to the data and take responsibility for its integrity and accuracy. 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.2022.1020253/full#supplementary-material

References

1. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. *Lancet* (2017) 390(10113):2627–42. doi: 10.1016/S0140-6736(17)32129-3

2. Dal Canto E, Ceriello A, Rydén L, Ferrini M, Hansen TB, Schnell O, et al. Diabetes as a cardiovascular risk factor: An overview of global trends of macro and micro vascular complications. *Eur J Prev Cardiol* (2019) 26(2_suppl):25–32. doi: 10.1177/2047487319878371

3. American Diabetes Association. Standards of medical care in diabetes–2009. *Diabetes Care* (2009) 32 Suppl 1(Suppl 1):S13–61. doi: 10.2337/dc09-S013

4. Jørgensen JT, Bräuner EV, Backalarz C, Laursen JE, Pedersen TH, Jensen SS, et al. Long-term exposure to road traffic noise and incidence of diabetes in the Danish nurse cohort. *Environ Health Perspect* (2019) 127(5):57006. doi: 10.1289/EHP4389

5. Wadden TA, Brownell KD, Foster GD. Obesity: responding to the global epidemic. *J Consult Clin Psychol* (2002) 70(3):510–25. doi: 10.1037//0022-006x.70.3.510

6. Howard BV, Ruotolo G, Robbins DC. Obesity and dyslipidemia. *Endocrinol Metab Clin North Am* (2003) 32(4):855–67. doi: 10.1016/s0889-8529(03)00073-2

7. Merlotti C, Morabito A, Ceriani V, Pontiroli AE. Prevention of type 2 diabetes in obese at-risk subjects: A systematic review and meta-analysis. *Acta Diabetol* (2014) 51(5):853–63. doi: 10.1007/s00592-014-0624-9

8. Wang Y, Rimm EB, Stampfer MJ, Willett WC, Hu FB. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. *Am J Clin Nutr* (2005) 81(3):555–63. doi: 10.1093/ajcn/81.3.555

9. Ashwell M, Gunn P, Gibson S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: Systematic review and meta-analysis. *Obes Rev* (2012) 13(3):275–86. doi: 10.1111/j.1467-789X.2011.00952.x

10. Janssen I, Katzmarzyk PT, Ross R. Waist circumference and not body mass index explains obesity-related health risk. *Am J Clin Nutr* (2004) 79(3):379–84. doi: 10.1093/ajcn/79.3.379

11. Ashwell M, Gibson S. Waist-to-Height ratio as an indicator of 'Early health risk': Simpler and more predictive than using a 'Matrix' based on bmi and waist circumference. *BMJ Open* (2016) 14:e010159. doi: 10.1136/bmjopen-2015-010159

12. Mi SQ, Yin P, Hu N, Li JH, Chen XR, Chen B, et al. Bmi, wc, whtr, vfi and bfi: Which indictor is the most efficient screening index on type 2 diabetes in Chinese community population. *BioMed Environ Sci* (2013) 26(6):485–91. doi: 10.3967/0895-3988.2013.06.009

13. Kapoor N, Lotfaliany M, Sathish T, Thankappan KR, Thomas N, Furler J, et al. Obesity indicators that best predict type 2 diabetes in an Indian population: Insights from the kerala diabetes prevention program. *J Nutr Sci* (2020) 9:e15. doi: 10.1017/jns.2020.8

14. Moosaie F, Fatemi Abhari SM, Deravi N, Karimi Behnagh A, Esteghamati S , Dehghani Firouzabadi F, et al. Waist-To-Height ratio is a more accurate tool for predicting hypertension than waist-To-Hip circumference and BMI in patients with type 2 diabetes: A prospective study. *Front Public Health* (2021) 9:726288. doi: 10.3389/fpubh.2021

15. Ke JF, Wang JW , Lu JX, Zhang ZH, Liu Y, Li LX. Waist-to-height ratio has a stronger association with cardiovascular risks than waist circumference, waist-hip ratio and body mass index in type 2 diabetes. *Diabetes Res Clin Pract* (2022) 183:109151. doi: 10.1016/j.diabres.2021.109151

16. Jayedi A, Soltani S, Motlagh SZ, Emadi A, Shahinfar H, Moosavi H, et al. Anthropometric and adiposity indicators and risk of type 2 diabetes: systematic review and dose-response meta-analysis of cohort studies. *BMJ* (2022) 376:e067516. doi: 10.1136/bmj-2021-067516

17. Vazquez G, Duval S, Jacobs DRJr, Silventoinen K. Comparison of body mass index, waist circumference, and waist/hip ratio in predicting incident diabetes: A meta-analysis. *Epidemiol Rev* (2007) 29:115–28. doi: 10.1093/epirev/mxm008

18. Browning LM, Hsieh SD, Ashwell M. A systematic review of waist-to-height ratio as a screening tool for the prediction of cardiovascular disease and diabetes: 0·5 could be a suitable global boundary value. *Nutr Res Rev* (2010) 23(2):247–69. doi: 10.1017/S0954422410000144

19. Kodama S, Horikawa C, Fujihara K, Heianza Y, Hirasawa R, Yachi Y, et al. Comparisons of the strength of associations with future type 2 diabetes risk among anthropometric obesity indicators, including waist-to-height ratio: A meta-analysis. *Am J Epidemiol* (2012) 176(11):959–69. doi: 10.1093/aje/kws172

20. Savva SC, Lamnisos D, Kafatos AG. Predicting cardiometabolic risk: waist-to-height ratio or BMI. a meta-analysis. *Diabetes Metab Syndr Obes* (2013) 6:403–19. doi: 10.2147/DMSO.S34220

21. Hartwig S, Kluttig A, Tiller D, Fricke J, Müller G, Schipf S, et al. Anthropometric markers and their association with incident type 2 diabetes mellitus: which marker is best for prediction? Pooled analysis of four German population-based cohort studies and comparison with a nationwide cohort study. *BMJ Open* (2016) 6(1):e009266. doi: 10.1136/bmjopen-2015-009266

22. Lee CMY, Woodward M, Pandeya N, Adams R, Barrett-Connor E, Boyko EJ, et al. Comparison of relationships between four common anthropometric measures and incident diabetes. *Diabetes Res Clin Pract* (2017) 132:36–44. doi: 10.1016/j.diabres.2017.07.022

23. Okamura T, Hashimoto Y, Hamaguchi M, Obora A, Kojima T, Fukui M. Data from: Ectopic fat obesity presents the greatest risk for incident type 2 diabetes: A population-based longitudinal study. *Dryad Dataset* (2019). doi: 10.5061/dryad.8q0p192

24. Okamura T, Hashimoto Y, Hamaguchi M, Obora A, Kojima T, Fukui M. Ectopic fat obesity presents the greatest risk for incident type 2 diabetes: a population-based longitudinal study. *Int J Obes (Lond)* (2019) 43(1):139–48. doi: 10.1038/s41366-018-0076-3

25. Hashimoto Y, Hamaguchi M, Kojima T, Ohshima Y, Ohbora A, Kato T, et al. Modest alcohol consumption reduces the incidence of fatty liver in men: a population-based large-scale cohort study. *J Gastroenterol Hepatol* (2015) 30 (3):546–52. doi: 10.1111/jgh.12786

26. Sheng G, Kuang M, Yang R, Zhong Y, Zhang S, Zou Y. Evaluation of the value of conventional and unconventional lipid parameters for predicting the risk of diabetes in a non-diabetic population. *J Transl Med* (2022) 20(1):266. doi: 10.1186/s12967-022-03470-z

27. Hamaguchi M, Kojima T, Itoh Y, Harano Y, Fujii K, Nakajima T, et al. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. *Am J Gastroenterol* (2007) 102 (12):2708–15. doi: 10.1111/j.1572-0241.2007.01526.x

28. Ryu S, Chang Y, Kim DI, Kim WS, Suh BS. Gamma-glutamyltransferase as a predictor of chronic kidney disease in nonhypertensive and nondiabetic Korean men. *Clin Chem* (2007) 53(1):71–7. doi: 10.1373/clinchem.2006.078980

29. American Diabetes Association. Standards of medical care in diabetes–2011. *Diabetes Care* (2011) 34 Suppl 1(Suppl 1):S11–61. doi: 10.2337/dc11-S011

30. Muanda FT, Weir MA, Bathini L, Blake PG, Chauvin K, Dixon SN, et al. Association of baclofen with encephalopathy in patients with chronic kidney disease. *JAMA* (2019) 322(20):1987–95. doi: 10.1001/jama.2019.17725

31. Sato T, Matsuyama Y. Marginal structural models as a tool for standardization. *Epidemiology* (2003) 14(6):680–6. doi: 10.1097/01

32. Kim JH. Multicollinearity and misleading statistical results. *Korean J Anesthesiol.* (2019) 72(6):558–69. doi: 10.4097/kja.19087

33. Wei WQ, Chen ZF, He YT, Feng H, Hou J, Lin DM, et al. Long-term follow-up of a community assignment, one-time endoscopic screening study of esophageal cancer in China. *J Clin Oncol* (2015) 33(17):1951–7. doi: 10.1200/JCO.2014.58.0423

34. Fitchett EJA, Seale AC, Vergnano S, Sharland M, Heath PT, Saha SK, et al. Strengthening the reporting of observational studies in epidemiology for newborn infection (STROBE-NI): An extension of the STROBE statement for neonatal infection research. *Lancet Infect Dis* (2016) 16(10):e202–13. doi: 10.1016/S1473-3099(16)30082-2

35. Guerrero-Romero F, Simental-Mendía LE, González-Ortiz M, Martínez-Abundis E, Ramos-Zavala MG, Hernández-González SO, et al. The product of triglycerides and glucose, a simple measure of insulin sensitivity. comparison with the euglycemic-hyperinsulinemic clamp. *J Clin Endocrinol Metab* (2010) 95 (7):3347–51. doi: 10.1210/jc.2010-0288

36. Taylor R, Holman RR. Normal weight individuals who develop type 2 diabetes: the personal fat threshold. *Clin Sci (Lond)* (2015) 128(7):405–10. doi: 10.1042/CS20140553

37. Friedl KE. Waist circumference threshold values for type 2 diabetes risk. *J Diabetes Sci Technol* (2009) 3(4):761–9. doi: 10.1177/193229680900300424

38. Zeng Q, He Y, Dong S, Zhao X, Chen Z, Song Z, et al. Optimal cut-off values of bmi, waist circumference and Waist:Height ratio for defining obesity in Chinese adults. *Br J Nutr* (2014) 112(10):1735–44. doi: 10.1017/S0007114514002657

39. Oguoma VM, Coffee NT, Alsharrah S, Abu-Farha M, Al-Refaei FH, Alkandari A, et al. Anthropometric cut-points for discriminating diabetes and the metabolic syndrome among arabs and asians: The Kuwait diabetes epidemiology program. *Br J Nutr* (2022) 127(1):92–102. doi: 10.1017/S0007114521000751

40. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for diabetes development. *Lancet* (2012) 379(9833):2279–90. doi: 10.1016/S0140-6736(12)60283-9

41. Ashwell M, Gibson S. A proposal for a primary screening tool: 'Keep your waist circumference to less than half your height'. *BMC Med* (2014) 12:207. doi: 10.1186/s12916-014-0207-1

42. Ashwell M, Hsieh SD. Six reasons why the waist-to-height ratio is a rapid and effective global indicator for health risks of obesity and how its use could simplify the international public health message on obesity. *Int J Food Sci Nutr* (2005) 56(5):303–7. doi: 10.1080/09637480500195066

43. Young TK, Gelskey DE. Is noncentral obesity metabolically benign? implications for prevention from a population survey. *JAMA* (1995) 274(24):1939–41.

44. Liu MM, Liu QJ, Wen J, Wang M, Wu LY, Qu ML, et al. Waist-to-hip ratio is the most relevant obesity index at each phase of insulin secretion among obese patients. *J Diabetes Complications* (2018) 32(7):670–6. doi: 10.1016/j.jdiacomp.2018.04.006

45. Meng T, Yin H, Li B, Li Z, Xu W, Zhou W, et al. Clinical features and prognostic factors of patients with chordoma in the spine: A retrospective analysis of 153 patients in a single center. *Neuro Oncol* (2015) 17(5):725–32. doi: 10.1093/neuonc/nou331

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Performance of a dual-hormone closed-loop system versus insulin-only closed-loop system in adolescents with type 1 diabetes. A single-blind, randomized, controlled, crossover trial

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Objective: To assess the efficacy and safety of a dual-hormone (DH [insulin and glucagon]) closed-loop system compared to a single-hormone (SH [insulin only]) closed-loop system in adolescents with type 1 diabetes.

Methods: This was a 26-hour, two-period, randomized, crossover, inpatient study involving 11 adolescents with type 1 diabetes (nine males [82%], mean ± SD age 14.8 ± 1.4 years, diabetes duration 5.7 ± 2.3 years). Except for the treatment configuration of the DiaCon Artificial Pancreas: DH or SH, experimental visits were identical consisting of: an overnight stay (10:00 pm until 7:30 am), several meals/snacks, and a 45-minute bout of moderate intensity continuous exercise. The primary endpoint was percentage of time spent with sensor glucose values below range (TBR [<3.9 mmol/L]) during closed-loop control over the 26-h period (5:00 pm, day 1 to 7:00 pm, day 2).

Results: Overall, there were no differences between DH and SH for the following glycemic outcomes (median [IQR]): TBR 1.6 [0.0, 2.4] vs. 1.28 [0.16, 3.19]%, p=1.00; time in range (TIR [3.9-10.0 mmol/L]) 68.4 [48.7, 76.8] vs. 75.7 [69.8, 87.1]%, p=0.08; and time above range (TAR [>10.0 mmol/L]) 28.1 [18.1, 49.8] vs. 23.3 [12.3, 27.2]%, p=0.10. Mean (± SD) glucose was higher during DH than SH (8.7 (± 3.2) vs. 8.1 (± 3.0) mmol/L, p<0.001) but coefficient of variation was similar (34.8 (± 6.8) vs. 37.3 (± 8.6)%, p=0.20). The average amount of rescue carbohydrates was similar between DH and SH (6.8 (± 12.3) vs. 9.5 (± 15.4) grams/participant/visit, p=0.78). Overnight, TIR was higher, TAR was lower during the SH visit compared to DH. During and after exercise (4:30 pm until 7 pm) the SH configuration produced higher TIR, but similar TAR and TBR compared to the DH configuration.

Conclusions: DH and SH performed similarly in adolescents with type 1 diabetes during a 26-hour inpatient monitoring period involving several metabolic challenges including feeding and exercise. However, during the night and around exercise, the SH configuration outperformed DH.

KEYWORDS

type 1 diabetes mellitus, adolescents, dual-hormone, advanced hybrid closed-loop, artificial pancreas, non-linear model predictive control, moderate intensity continuous exercise

1 Introduction

People with type 1 diabetes (T1D) are advised to aim for near-normal blood glucose levels to reduce the risk of diabetes late complications (1, 2). However, achieving optimal metabolic control is challenging and many fail to meet recommended guidelines – especially adolescents (3).

The most advanced commercially available technology is a single-hormone (SH) closed-loop system, also known as an artificial pancreas (AP). These systems automatically adjust insulin pump delivery based on real-time values from a continuous glucose monitor (CGM). Relative to insulin pump and CGM systems without automated insulin dosing, APs have been shown to improve glucose control (4–6). Despite these technological improvements, adolescents with T1D still frequently experience non-severe hypoglycemia (<3.9 mmol/L) (5, 7). Furthermore, around exercise the risk of hypoglycemia is higher due to increased insulin sensitivity, insulin absorption, glucose uptake in combination with an impaired glucagon secretion (8).

A potential means of reducing the risk of hypoglycemia using closed-loop systems is to add the glucose-elevating hormone glucagon. Such dual-hormone (DH) hybrid closed-loop systems are not currently commercially available but have generated interest in research trials investigating their performance relative to SH systems. A meta-analysis found that both SH and DH closed-loop systems resulted in more time spent in the target glucose range (TIR [3.9-10.0 mmol/L]) compared to non-automated delivery systems. Furthermore, DH was superior to SH in increasing TIR and decreasing time below range (TBR [< 3.9 mmol/L]) (9). Limited data exist comparing DH and SH closed-loop treatments during and after exercise, however, some studies have found DH to minimize TBR in such circumstances (10–13).

Our group has developed the DiaCon AP (14) which can run in two configurations; SH and DH. A previous version of the system was tested among adults with T1D showing improvements in TIR during exercise and a lesser need for hypoglycemic-CHO treatments when using the DH configuration (15). However, the updated system is yet to be tested in an adolescent T1D cohort.

Abbreviations: AP, Artificial Pancreas; CGM, Continuous Glucose Monitoring; CHO, Carbohydrate intake; DH, Dual-Hormone; PG, Plasma Glucose; SG, Sensor Glucose; SH, Single-Hormone; T1D, Type 1 diabetes; TAR, Time above range; TBR, Time below range; TIR, Time in range.

The aim of this study was therefore to test our hypothesis, that the updated DiaCon AP DH configuration would be safe and effective to use in individuals with T1D between 13-17 years old and that it would be *superior* in managing glycemia compared to the DiaCon AP SH configuration.

2 Materials and methods

2.1 Methods

This was a randomized, single-blind, two-period, crossover study in adolescents with T1D recruited from Herlev and Gentofte Hospital Pediatric Department Outpatient Clinics and the Steno Diabetes Center Copenhagen. Enrollment was conducted from September 1st, 2021 until March 7th, 2022. All study participants' parents or legal guardians provided written informed consent and participants ≥15-years provided written, informed assent before participation. The study was approved by the Regional Committee in Health Research Ethics (H-21000207), the Danish Data Protection Agency (P-2021-326), and the Danish Medicines Agency (2020-005836-31). The trial was registered with ClinicalTrials.gov (NCT04949867).

Participants were included if they were: 13-17 years old; diagnosed with T1D for ≥two years; used an insulin pump for ≥one year; used a real-time or intermittently scanned CGM, had an HbA1c ≤75 mmol/mol; and used carbohydrate counting as well as the pump bolus calculator for all meals. Main exclusion criteria were known allergy to glucagon or lactose, use of diabetes medication other than insulin, and hypoglycemia unawareness.

2.2 Study device and drugs

We used our DiaCon system consisting of two Dana Diabecare RS insulin pumps (one for insulin and one for glucagon/saline), a Dexcom G6 sensor (Dexcom, San Diego, CA) and a smartphone (Samsung Galaxy A5 2017 Android phone) containing the DiaCon algorithm (14) to adjust the pump deliveries based on the CGM values. One pump was filled with insulin aspart (Fiasp®, Novo Nordisk, Bagsværd, Denmark) and the other with either glucagon (GlucaGen®, Novo Nordisk, Bagsværd, Denmark) or isotonic saline (sodium chloride 9 mg/dL). The glucagon pump was refilled with fresh glucagon every 22 hours after the first pump filling. The

individual parameter estimates for the insulin algorithm were set up using insulin pump, CGM and carbohydrate intake (CHO) data from each participant. The algorithm computed the insulin and glucagon administration based on predictions obtained with a mathematical model of the blood glucose response to CHO, insulin, and glucagon, i.e., based on nonlinear model predictive control (NMPC). Safety constraints on bolus and basal insulin were based on participants' insulin pump settings and the glucagon algorithm was constrained to deliver maximally 300 µg glucagon over a two hour period (14). Meals and exercise were announced to the DiaCon AP. The NMPC utilized insulin-carbohydrate-ratio and the announced meal carbohydrates when dosing meal boluses. Exercise mode increased target glucose from 6 to 7 mmol/L and the limit for when glucagon could be administered was increased from 4.5 to 7.0 mmol/L. If SG was ≤7.0 mmol/L at exercise announcement 100 µg glucagon was administered (14).

2.3 Study design

Participants went through a screening visit and two 26-h in-clinic visits with a wash-out period of at least three days. During each in-clinic visit, participants wore the DiaCon system set-up to run in either the DH or SH configuration depending on the randomization order. Except for the DH and SH configurations, the study visits were identical (Figure 1).

At the screening visit, participants' medical history (i.e., allergies, medications, other illnesses, diabetes complications) as well as results of blood and urine analyses were reviewed. A clinical examination was performed to assess height, weight, and blood pressure. Finally, 14 days of insulin pump and CGM data were downloaded to register the mean values for basal rate delivery, insulin sensitivity factor, insulin-carbohydrate-ratio, CHO, TBR, TIR, time above range (TAR [>10.0 mmol/L]) and mean glucose.

Two days prior to each in-clinic visit, participants inserted the Dexcom G6 sensor which linked to the Dexcom receiver. On visit days, participants arrived at the research facility at 4 pm following a three hour fast. Upon arrival, the Dexcom sensor was linked to the study equipment, an intravenous canula was inserted for blood sampling, and participants were fitted with an activity tracker (ActiGraph GT9X Link, Pensacola, FL). Intervention with one of the two closed-loop configurations was initiated at 5:00 pm and continued for the following 26-h (Figure 2). Meals (7:00 pm, 8:00 am and 12:00 noon) and snacks (3:00 pm) were served throughout the inpatient period and their CHO contents were based on the participants' average daily CHO intake entered in their own pumps evaluated over a seven-day period. Participants eating <100 g daily received 30 g CHO per meal, 100-150 g daily received 50 g CHO per meal, 150-200 g daily received 60 g CHO per meal, and >200 g per day received 70 g CHO per meal. The snack consisted of 1/3 of the CHO given per meal, and the dinner consisted of 1.5 times the CHO content of the other regular meals. The dinner was from McDonalds and the CHO content was determined using their nutrition calculator by the study personnel (16), who also prepared the remaining meals incl. snack. The CHO contents of all meals were blinded from participants, who made estimations which were used as the value entered into the DiaCon AP at the start of each meal. Though meals were kept identical between visits, participants' estimations could differ.

Participants were instructed to be in bed, and sleep, if possible, from 10:00 pm to 7:30 am.

After resting during the day, at 4:30 pm on day two, participants performed a 45-minute bout of moderate intensity continuous exercise at an intensity equivalent to ~ 50% of their heart rate (HR) reserve (17). Participants were fitted with a chest strap telemetry monitor that linked to the ActiGraph and the stationary bike. Exercise duration was announced to the DiaCon AP upon initiation of exercise. During the entirety of the study period, participants were asked to stay around the research facility, not to exercise and eat, and to only be away from the research room within the 30-minute window between plasma samples.

Venous blood samples were drawn every 30 minutes during the day, every 60 minutes during sleep (10:00 pm until 7:30 am) and every five to ten minutes during and immediately after exercise (4:30 pm until 5:30 pm). Plasma glucose (PG) was measured *via* the YSI 2300 STAT Plus Analyzer (YSI Life Sciences, Yellow Springs, OH).

If PG dropped <3.0 mmol/L at any time during the intervention, 15 grams of oral rescue carbohydrate (dextrose) tablets were provided to the participants, and plasma sampling was performed every five minutes. The treatment was repeated every 15 minutes until PG was >3.9 mmol/L. If PG was >12 mmol/L for more than two hours or >14 mmol/L (not in relation to a meal), blood ketones were measured in 15 minutely intervals and the study devices were checked for issues. After an hour, if blood ketones were ≥0.6 mmol/L and PG remained >14 mmol/L, insulin was administered with an injection pen based on the participants' insulin-sensitivity factor aiming for PG of 7.0 mmol/L.

During each visit, participants scored side effects using a visual analog scale (VAS; 0-100) at seven specified timepoints (day 1: 5:00

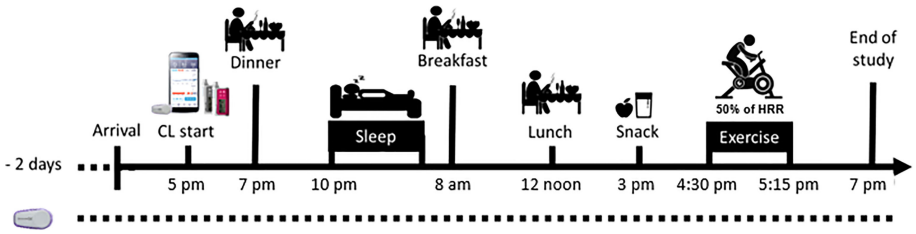

FIGURE 1
Schematic overview of the study days.

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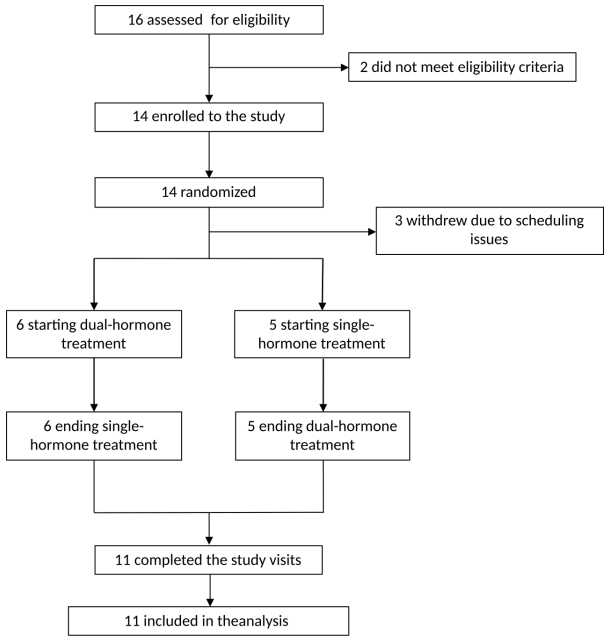

FIGURE 2
Flowchart of study.

pm and 7:00 pm and day 2: 8:00 am, 12:00 noon, 3:00 pm, 4:30 pm and 7:00 pm). Clinically significant side effects were defined as a VAS score ≥ 15 (18–20).

2.4 Outcomes

The primary outcome was percentage of TBR_{SG} during the 26-h intervention period. Secondary outcomes were: percentage of TBR_{PG}, percentage of $TIR_{SG\&PG}$ and $TAR_{SG\&PG}$, mean SG and PG, coefficient of variation (CV) and number of rescue CHO interventions. Study outcomes were also reviewed separately overnight (10:00 pm to 7:30 am) as well as during and after exercise (4:30 pm to 7:00 pm).

2.5 Statistical analysis

To be able to detect a difference in percentage of time with SG <3.9 mmol/L of 2.3%-points (approximately 30 minutes) with 90% power, a 5% significance level, and a presumed 3.0%-points standard deviation (10), it was established that 20 participants were to be included in the study (2-sided test). Categorical variables were reported as frequencies (percentage), whereas continuous variables were reported as mean (SD) or median (interquartile range [IQR]). Continuous data was assessed for normality using Shapiro-Wilk test. For normally distributed variables, paired student's t-test was used to conduct pairwise comparisons between the two groups. For skewedly distributed variables despite log-transformation, the non-parametric Wilcoxon signed-rank test was used. Missing glucose data were estimated using linear interpolation. We used McNemar's test to assess the significance of the difference in the incidence of level 2 hypoglycemia between the two study arms. Analyses were performed on an intention-to-treat basis. Statistical analyses were performed using RStudio version 1.4.

3 Results

As per protocol, we performed an interim analysis to assess efficiency of the DiaCon algorithm, where we found DH to be inferior to SH for TIR and TAR and to be non-superior for TBR, therefore the inclusion was truncated. At that time, 16 had been screened and 14 were included and enrolled in the study. Two adolescents were not eligible due to hypoglycemia unawareness. Before initiation of the first study visit, three participants withdrew due to scheduling issues [e.g., school absence and lack of time (Figure 2)]. Thus, 11 participants completed both visits (Table 1), and no differences were observed between completers and non-completers on age, sex, BMI, HbA1c, diabetes duration or daily insulin dose.

3.1 Entire study period

3.1.1 Glycemic metrics

For the 26-h study period we found no differences in TBR_{SG}, TIR_{SG}, or TAR_{SG} between the two study arms (Table 2). The mean (± SD) SG was higher during the DH compared to the SH study arm (8.7 (± 3.0) mmol/L vs. 8.1 (± 3.0) mmol/L, p<0.001), with no difference in CV. Similarly, no differences were found in PG-derived measures (Table 2), except for TAR_{PG} (33.2 [16.1, 40.7] vs. 11.5 [3.83, 23.0]%, p=0.02) and $mean_{PG}$ (8.84 (± 2.83) vs. 7.51 (± 2.98), p=0.03) both of which were higher during DH.

During DH, six events of SG-derived level 2 hypoglycemia (<3.0 mmol/L) were registered in three participants compared to two events in two participants during SH (p=0.65). In contrast, four episodes of PG-derived level 2 hypoglycemia were registered in three participants during DH compared to five episodes in four participants during SH

TABLE 1 Baseline characteristics of the 11 participants who completed both study visits.

Baseline characteristics	Mean (± SD) or median [IQR]
Sex (males [%])	9 (82%)
Age (years)	14.8 (± 1.47)
HbA1c (mmol/mol)	54.6 (± 9.20)
BMI (kg/m^2)	21.4 (± 2.42)
Diabetes duration (years)	5.73 (± 2.45)
Total daily insulin (U/kg)	0.94 (± 0.26)
Time below range (%)	3.0 [1.5, 6.5]
Time in range (%)	54.0 [46.0, 73.0]
Time above range (%)	43.0 [22.5, 52.0]

Age, HbA1c, BMI, diabetes duration and total daily insulin are expressed as mean (± SD). Time below (<3.9 mmol/L), in (3.9-10.0 mmol/L) and above range (>10.0 mmol/L) are expressed as median [interquartile range].
Sex is presented as absolute number and percentage.

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TABLE 2 Sensor and plasma glucose values during entire study, overnight and exercise and post-exercise period.

	Sensor Glucose Measures			Plasma Glucose Measures		
	Dual-Hormone (n=11)	Single-Hormone (n=11)	*P-value*	Dual-Hormone (n=11)	Single-Hormone (n=11)	*P-value*
Entire study period (5:00 pm, day 1 – 7:00 pm, day 2)						
Starting glucose for period (mmol/L)	7.33 (± 1.90)	9.35 (± 6.61)	0.64[w]	6.61 (± 1.71)	9.14 (± 4.90)	0.21
Time below range (%)	1.60 [0, 2.4]	1.28 [0.16, 3.19]	1.00[w]	0.958 [0, 3.83]	2.56 [0.479, 8.47]	0.26[w]
Time in range (%)	68.4 [48.7, 76.8]	75.7 [69.8, 87.1]	0.09	66.8 [56.9, 78.9]	79.6 [75.2, 87.4]	0.06
Time above range (%)	28.1 [18.1, 49.8]	23.3 [12.3, 27.2]	0.10	33.2 [16.1, 40.7]	11.5 [3.83, 23.0]	**0.02**
Mean glucose (mmol/L)	8.7 (± 3.02)	8.1 (± 3.0)	**<0.001**	8.84 (± 2.83)	7.51 (± 2.98)	**0.03**
Coefficient of variation (%)	34.8 (± 6.8)	37.3 (± 8.6)	0.20	33.59 (± 8.17)	39.63 (± 8.72)	0.17
Overnight period (10:00 pm – 7:30 am)						
Starting glucose for period (mmol/L)	10.3 (± 3.55)	9.34 (± 3.66)	0.54	9.66 (± 3.53)	8.22 (± 3.71)	0.24
Time below range (%)	0 [0, 0]	0 [0, 3.48]	0.10[w]	0 [0, 0]	0 [0, 5.22]	0.36[w]
Time in range (%)	73.0 [53.9, 87.4]	96.5 [84.3, 100]	***0.02***[w]	67.8 [60.4, 89.6]	89.6 [82.6, 100]	0.07[w]
Time above range (%)	27.0 [12.6, 42.6]	0 [0, 10.0]	***0.02***[w]	32.2 [5.22, 37.8]	0 [0, 5.22]	**0.02**[w]
Mean glucose (mmol/L)	8.49 (± 2.97)	7.25 (± 2.25)	**0.04**	8.34 (± 2.70)	6.88 (± 2.38)	**0.01**
Coefficient of variation (%)	35.0 (± 10.8)	31.0 (± 8.9)	0.39	32.34 (± 11.62)	34.66 (± 9.78)	0.69
Exercise and post-exercise period (4:30 pm – 7:00 pm)						
Starting glucose for period (mmol/L)	10.1 (± 3.15)	9.31 (± 2.30)	0.35	9.40 (± 2.92)	7.71 (± 2.25)	0.14
Time below range (%)	0 [0, 0]	0 [0, 0]	0.42[w]	0 [0, 4.84]	0 [0, 14.5]	0.78[w]
Time in range (%)	64.5 [50.0, 91.9]	83.9 [80.6, 100]	***0.02***[w]	67.7 [32.3, 99.4]	93.5 [82.3, 100]	0.10[w]
Time above range (%)	22.6 [0, 37.1]	12.9 [0, 17.7]	0.06[w]	1.18 [0, 58.1]	0 [0, 3.23]	0.09[w]
Mean glucose (mmol/L)	7.92 (± 2.92)	6.81 (± 1.91)	0.13	7.91 (± 2.70)	6.06 (± 1.76)	**0.05**
Coefficient of variation (%)	37.0 (± 9.6)	28.2 (± 7.7)	0.65	34.16 (± 11.51)	29.10 (± 7.49)	0.49
$Change_{during\text{-}exercise}$ (mmol/L)	-2.5 (± 1.6)	-3.2 (± 1.0)	0.14	-2.5 (± 1.8)	-2.7 (± 1.7)	0.73
Heart Rate Telemetry during exercise (4:30 pm – 5:15 pm)						
$HR\ target_{during\text{-}exercise}$ (BPM)	133 (± 4.6)	132 (± 4.8)	0.33			
$HR\ target\ accuracy_{during\text{-}exercise}$ (%)	94.6 (± 5.7)	94.2 (± 4.6)	0.52			

Time below (<3.9 mmol/L), in (3.9-10.0 mmol/L) and above range (>10.0 mmol/L) are reported as median [interquartile range], starting glucose, mean glucose and coefficient of variation are reported as mean (± SD) and level 2 hypoglycemia is presented as events/participant. Time in range 3.9-10.0 mmol/L, time below range <3.9 mmol/L and time above range >10.0 mmol/L.
[w]non-parametric test. For level 2 hypoglycemic events McNemars test was performed. HR, Heart Rate; BPM, Beats per minute. Measures notated with $_{during\text{-}exercise}$ depicts the 45-minutes bout of exercise time period.
P-values marked with w have been analysed using non-parametric test. Otherwise, paired t-tests have been applied. P-values < 0.05 were considered statistically significant (marked bold).

(p=0.71). Of those, two of the events were prolonged (>20 minutes) during DH compared to one during SH.

Figure 3 shows the SG profile during the total study period, overnight and the period during and after exercise. Individual SG profiles are provided in the Supplemental Material, Figure 1S.

3.1.2 Insulin and glucagon

There were no differences in insulin delivery, either total insulin delivery or average basal rate, between the DH and SH study arms (Table 3, Supplemental Material Figures 2-5S). For the entire study period, all received glucagon with a median [IQR] administration of 549 [229, 1034] μg (Figures 6-7S).

3.1.3 Carbohydrates (rescue interventions and meals)

The mean amount of rescue CHO provided was similar between the two arms (DH: 6.8 (± 12.3) grams/participant/visit vs. SH: 9.5 (± 15.4) grams/participant/visit, p=0.78).

The median [IQR] amount of CHO provided for each meal was 72 [70, 94] g for dinner, 60 [55, 70] g for breakfast, 60 [55, 70]g for lunch and

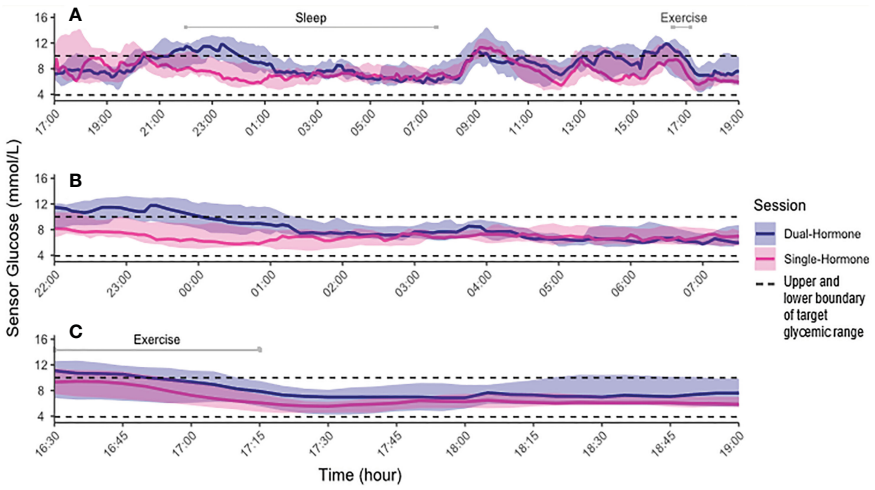

FIGURE 3
Median (interquartile range) sensor glucose values for Dual-hormone (blue) and Single-Hormone (pink) configuration. **(A)** Entire period. **(B)** Overnight period (10:00 pm-7:30 am). **(C)** Exercise and post-exercise period. Black dotted lines mark lower and upper boundary of target glycemic range of 3.9 to 10.0 mmol/L, respectively.

21 [19, 24]g for snacks, and we found no difference in the accuracy (%) of the participants' CHO estimations between the two study arms.

3.2 Overnight period

During the overnight period, the SH configuration outperformed the DH with more TIR_{SG} (96.5 [84.3, 100] vs. 73.0 [53.9, 87.4]%, p=0.02), less TAR_{SG} (0 [0.0, 10.0] vs. 27.0 [12.6, 42.6]%, p=0.02) and lower mean SG (Table 2). There were no differences in TBR_{SG} or CV. TAR_{PG} was higher during DH than during SH, with no differences in TIR_{PG} or TBR_{PG} (Table 2). The overnight amount of total insulin delivered was lower during SH than during DH (Table 3). Seven participants received glucagon overnight by the DH system (549 [409, 599] µg). Data for the entire study population is depicted in Table 3.

Overnight we found one and zero SG-derived hypoglycemic episodes, respectively, in the DH and SH arm. We did, however,

TABLE 3 Study medication administration. Insulin measures are reported as mean (SD) and glucagon as median [IQR].

Insulin and glucagon delivery	Dual-Hormone (N=11)	Single-Hormone (N=11)	*P-value*
Entire study period (5:00 pm, day 1 – 7:00 pm, day 2)			
Total insulin delivery (U/24h)	54.8 (19.0)	54.6 (15.4)	0.95
Basal insulin delivery (U/h)	1.03 (0.36)	1.01 (0.30)	0.77[w]
Bolus insulin delivery (U/24h)	29.9 (12.4)	30.3 (9.6)	0.92
Glucagon delivery (µg/26-h)	549 [229, 1034]	-	-
Overnight period (10:00 pm – 7:30 am)			
Total insulin delivery (U/night)	12.94 (4.14)	10.51 (3.08)	**0.01**
Basal insulin delivery (U/h)	1.09 (0.37)	1.05 (0.34)	0.15[w]
Bolus insulin delivery (U/night)	2.55 (2.50)	0.5 (1.13)	**0.04**[w]
Glucagon delivery (µg/night)	298 [0, 462]	-	-
Exercise and post-exercise period (4:30 pm – 7:00 pm)			
Total insulin delivery (U/exercise)	2.24 (1.07)	2.19 (0.98)	0.88
Basal insulin delivery (U/h)	0.80 (0.38)	0.87 (0.40)	0.51
Bolus insulin delivery (U/exercise)	0.25 (0.47)	0.01 (0.03)	0.14[w]
Glucagon delivery (µg/period)	1 [0, 275]	-	-

P-values marked with w have been analysed using non-parametric test. Otherwise, paired t-tests have been applied. P-values < 0.05 were considered statistically significant (marked bold).

not provide any rescue glucose interventions as PG was never <3.0 mmol/L in either arm.

3.3 Exercise period

For the exercise and post-exercise phases, the SH system performed superiorly to DH with more TIR_{SG} (83.9 [80.6, 100.0] vs. 64.5 [50.0, 91.9]%, p=0.02). There were no differences in TAR_{SG}, TBR_{SG}, mean SG, CV or in PG (Table 2). For this period, there was no difference in amount of insulin delivered. A total of five participants received glucagon during and after exercise (300 [250, 300] μg) (Table 3).

One participant received rescue CHO following exercise in the DH arm, and two participants received one and two rescue CHO interventions, respectively, in the SH arm. The participant needing two rescue interventions around exercise experienced level 2 hypoglycemia during exercise, and the exercise bout was therefore cut short. None of the DH exercise sessions were interrupted.

The exercise starting SG and drop in SG during exercise (4:30 to 5:15 pm) were comparable between the two study visits (Table 2). Furthermore, we found no difference in the estimated HR target during exercise or in the accuracy (%) of which participants reached their target between the two visits (Table 2).

3.4 Side effects

No severe adverse events were observed. Three participants (27%) reported a clinically significant measure of nausea during the DH arm compared with none in the SH arm. Headache was reported by four (36%) in both the SH and the DH arm. Stomach-ache was reported by two (18%) in the DH arm and one (9%) in the SH arm. None experienced vomiting. All reported side effects were mild in severity and, besides from hypoglycemic- and hyperglycemic episodes, required no intervention.

3.5 Technological issues

During the study, there were a few technological issues. One participant (during the DH visit) experienced prolonged pressure induced sensor attenuation causing the sensor to register level 2 hypoglycemia, while PG was well within range. This caused wrongful glucagon administration and subsequent hyperglycemia. During two DH study visits, glucagon occluded the pump which required a change of infusion set. During both study arms, the phone lost connection to the sensor. However, all connection issues were automatically reestablished without intervention from the study personnel. These phone-sensor connection issues only triggered an alarm if they lasted more than 15 minutes, thus shorter connection loses may have been present, without the study personnel being aware. Once during a DH study visit, there was a disconnection between the phone and the insulin pumps which was resolved by restarting the entire system.

4 Discussion

In this 26-h inpatient study, we compared the performance of the DiaCon DH and SH configurations in adolescents with type 1 diabetes. For the entire study period, we found no differences in $TBR_{SG/PG}$, $TIR_{SG/PG}$,$TAR_{SG/PG}$, or in the amount of rescue glucose needed. However, despite equivalency in TBR_{SG} during the overnight period and around exercise, SH achieved more TIR_{SG} and less TAR_{SG} compared to DH.

The DiaCon AP has previously been tested in adults with type 1 diabetes (15), showing that DH was superior to SH in handling hypoglycemia. These findings were in line with two systematic reviews and meta-analyses performed in 2018, finding that DH was superior to SH for TBR and TIR (4, 9). Still, only two studies have performed head-to-head comparison of DH and SH in adolescents with type 1 diabetes (21, 22). In line with our findings, the first study showed no significant differences in TIR, TAR or TBR between SH and DH for the adolescents during a 24-h observation period (21). Further, they found a tendency for SH to achieve higher TIR than DH during the overnight period (11:00 pm to 8:00 am), but it did not reach statistical significance. The other study was an overnight study showing that the addition of glucagon significantly improved TIR and reduced the time in level 1 hypoglycemia, but had similar time in level 2 hypoglycemia when compared with SH (22). Both studies included a third study arm with usual care, and generally both SH and DH achieved better glycemic control compared to this arm. Recently, a pooled analysis was performed for nocturnal control in children and adolescents using SH and DH. They found superiority in favor of the DH configuration for TIR, level 1 hypoglycemia and level 2 hyperglycemia (23).

Even though our SH configuration managed to ensure good glycemic control comparable to that of the commercially available APs with a TIR_{SG} of 75% for the whole study period, 83% during exercise and 95% overnight, hypoglycemia still posed a challenge (4, 5). Indeed, seven (64%) participants had at least one hypoglycemic event, whilst six experienced recurrent events. This issue was not resolved by addition of glucagon in our DH configuration. The reason may be attributed to the different conditions for parameter estimations in the DiaCon AP rather than glucagon *per se*. While the insulin algorithm was individualized and parameter estimates were based on individual insulin pump and CGM data, the parameter estimates for the glucagon algorithm were generic and similar for all participants. The uncertainty of the individual glucose response to glucagon (median[IQR] glucagon administration 549 [229, 1034] μg), therefore, produced equally uncertain glucose predictions for the system, resulting in less correct insulin dosing in the DH configuration. In this study, the glucagon sensitivity was not adaptive, and we did not have the data to estimate individual glucagon parameters. Whether addition of these features would have improved the DH study results remain uncertain. The DiaCon AP was further challenged by known technical issues, i.e., glucagon pump occlusions and pressure induced sensor attenuation, both of which unfortunately affected the DiaCon AP predictions and hence, it's performance during the DH study days. Glucagon occlusions happen quite commonly when using native glucagon due to rapid fibrillation after reconstitution (12, 24–26), and although we tried to avoid them by changing glucagon every 22 hours, they still occurred. Future algorithms would therefore benefit immensely from being able to detect occlusions and pressure induced sensor

attenuations to avoid miscommunication between the systems' predictions and the actual CGM data and hormonal delivery. Still, even with improved occlusion detection in the algorithms, a stable formulation of glucagon is required before it is feasible to be used in a real-world setting. New, soluble formulations have been developed (Dasiglucagon®, Baqsimi™ and Gvoke®/XeriSol®), but are still only approved for treatment of severe hypoglycemia. Recently published and ongoing clinical trials have shown promising results in using soluble glucagon for treatment of non-severe hypoglycemia, regardless of whether glucagon has been delivered through automated pumps or *via* pen-injections (13, 27, 28).

In the overnight period participants were administered higher doses of bolus insulin during DH compared to SH (2.55 [± 2.50] vs. 0.5 [± 1.13] IE). This was most likely due to a couple of different factors. During the DH arm some participants were administered glucagon right before dinner announcement, resulting in inadequate meal bolus insulin, and subsequent hyperglycemia. Due to restrictions in the DiaCon AP around meals this led to insulin corrections being administered in the night hours (14). Another possible explanation could be the occurrence of pressure induced sensor augmentations. These caused wrongful glucagon administration and hyperglycemia, which was followed by increased insulin supply when the augmentation was resolved. Furthermore, a few participants experienced oscillating sensor glucose levels, as they were overcorrected and entered a glucagon-insulin oscillating cycle. With the result from the SH arm in mind (TIR 96.5%) it is fair to speculate whether a specific night setting should be developed, making the glucagon algorithm during the night less aggressive, but as our study was rather small and the glucagon algorithm was faced with several challenges, we still think it is too unsafe to conclude (10).

Initially, we hypothesized that the addition of glucagon could have counteracted the increased risk for hypoglycemia during physical activity (8). However, we found that SH was superior to DH with more TIR_{SG}, and no difference in TAR_{SG} or $TBR_{SG/PG}$ during and after exercise. We speculate whether the higher starting glucose level before exercise for DH (10.1 [± 3.15] vs. 9.31 [± 2.30], p=0.35), though not statistically significant, may have reduced the need for glucagon around exercise – causing a systematic error when interpreting the glucose data that are in favor for SH. In fact, only five participants received glucagon in relation to exercise. Among four adult head-to-head studies, DH was found to be superior to SH in avoiding hypoglycemia during exercise, whereas only one of the studies also included adolescents (11–13, 15). In the combined adult and adolescent study, no differences were found between the two hormonal configurations (21). Unfortunately, no subgroup analysis – adult versus adolescents - for the exercise period was reported. Our study period ended 1 hour and 45 minutes after the exercise bout, and we were therefore only able to investigate a small window of the post-exercise period. Previous studies, however, have shown that SH systems could sufficiently keep glucose in range during the post-exercise period (21, 29). This could indicate that glucagon may be especially beneficial during aerobic exercise, where blood glucose changes rapidly and insulin reduction or suspension is inadequate, but requirement may waiver in the post-exercise period when the pronounced acute glycemic declines induced by exercise have passed (10).

The side effects experienced in this study were mild and self-limiting, but the adolescents reported more clinically significant events of nausea during DH than during SH (27% vs. 0%). The other side effects were equally distributed across both study visits, as reported by other study groups (26). A new outpatient study investigating dasiglucagon pen treatment for non-severe hypoglycemia found a higher occurrence of mild nausea with glucagon treatment, but the participants would still incorporate it into their regular diabetes treatment if possible (30). Taken collectively nausea remains a limitation of DH use and finding ways to resolve such should be a major consideration in future developments.

The current study had some limitations. Firstly, a small sample size makes the generalizability of the study results difficult. Furthermore, the current DiaCon AP set-up was too comprehensive for commercial pump use. Simpler set-up versions of the DiaCon AP, should be developed. The use of native glucagon limited the possibility to be used in real-world settings, but the current data show that soluble glucagon has a similar response. Furthermore, we conducted our study on adolescents of 13-17-years-old. This age group is different from all other due to the considerable hormonal and physiological changes over time. Therefore, it is important that studies with DH and SH APs also include adolescents before concluding whether it is beneficial in this age group. We chose a McDonalds meal as dinner, which is not typically classified as a balanced and healthy meal, however, we decided to do so in order to test our configurations maximally during the limited study time. Meals with a high carbohydrate and fat content are usually more difficultly handled for people with type 1 diabetes and testing our system in such a situation was therefore important. Furthermore, McDonalds is known worldwide making the approach universally applicable. Our participants were overall better regulated during the study days than in their everyday lives, though, we did not have a usual care control arm to test whether the superior glycemic control achieved by the DiaCon AP would persist an inpatient setting. This was the first 26-h inpatient study, during which the DH configuration was tested during multiple metabolic challenges.

5 Conclusion

To conclude, the two configurations performed equally well; but during night and exercise, SH achieved better glucose control than DH with equal amount of rescue glucose needed.

Whether it is justifiable to add glucagon to the AP systems remains unanswered. Our SH configuration managed to yield good glycemic control, but hypoglycemia still posed a challenge which neither of our configurations managed to overcome. However, as we become more familiar with the use of glucagon and with more stable glucagon formulations readily available, improved DH algorithms can potentially be a key player in resolving this.

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 study was approved by the Regional Committee in Health Research Ethics (H-21000207), the Danish Data Protection Agency (P-2021-326), and the Danish Medicines Agency (2020-005836-31). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

KN, JS, CL, ATR, JJ, and AGR contributed to conceive and design the study. EL, ATR, CL, AGR, and TR conducted the clinical trial. EL performed data analysis and wrote the manuscript. AGR, KN, JS, and CL supervised. All authors read and approved the final 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.2023.1073388/full#supplementary-material

FIGURE 1S:
Individual SG measures for participant during the entire study period for dual-hormone (blue) and single-hormone (pink). Black dotted lines mark the lower and upper boundary of target glycemic range of 3.9 mmol/L to 10.0 mmol/L, respectively.

FIGURE 2S:
Mean (±SD) basal insulin (IE) delivered during the entire study period for dual-hormone (blue) and single-hormone (pink). Sleep and exercise periods marked in the figure.

FIGURE 3S:
Mean (±SD) total insulin (IE) delivered during the entire study period for dual-hormone (blue) and single-hormone (pink). Sleep and exercise periods marked in the figure.

FIGURE 4S:
Mean (±SD) bolus insulin (IE) delivered during the entire study period for dual-hormone (blue) and single-hormone (pink). Sleep and exercise periods marked in the figure.

FIGURE 5S:
Individual insulin delivery for each participant during the entire study period for dual-hormone (blue) and single-hormone (pink).

FIGURE 6S:
Mean glucagon delivery during the entire dual-hormone study period. Sleep and exercise periods marked in the figure.

FIGURE 7S:
Individual glucagon delivery for each participant during the entire dual-hormone study period.

References

1. Gubitosi-Klug RA, Lachin JM, Backlund JYC, Lorenzi GM, Brillon DJ, Orchard TJ. Intensive diabetes treatment and cardiovascular outcomes in type 1 diabetes: The DCCT/EDIC study 30-year follow-up. *Diabetes Care* (2016) 39:686–93. doi: 10.2337/dc15-1990

2. Nathan DM, Cleary PA, Backlund J-YC, Genuth SM, Lachin JM, Orchard TJ, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med* (2005) 353:2643–53. doi: 10.1056/NEJMoa052187

3. Miller KM, Foster NC, Beck RW, Bergenstal RM, DuBose SN, DiMeglio LA, et al. Current state of type 1 diabetes treatment in the U.S.: Updated data from the T1D exchange clinic registry. *Diabetes Care* (2015) 38:971–8. doi: 10.2337/dc15-0078

4. Weisman A, Bai J-W, Cardinez M, Kramer CK, Perkins BA. Effect of artificial pancreas systems on glycaemic control in patients with type 1 diabetes: a systematic review and meta-analysis of outpatient randomised controlled trials. *Lancet Diabetes Endocrinol* (2017) 5:501–12. doi: 10.1016/S2213-8587(17)30167-5

5. Schiaffini R, Deodati A, Nicoletti MC, Carducci C, Ciampalini P, Lorubbio A, et al. Comparison of two advanced hybrid closed loop in a pediatric population with type 1 diabetes: a real-life observational study. *Acta Diabetol* (2022) 59:959–64. doi: 10.1007/s00592-022-01886-z

6. Phillip M, Nimri R, Barnard-Kelly K, Bergenstal R, Nørgaard K. Consensus recommendations for the use of automated insulin delivery (AID) technologies in clinical practice 2022. *Endocrine Rev* (2022) 00:1–27. doi: 10.1210/endrev/bnac022

7. Boscari F, Ferretto S, Cavallin F, Bruttomesso D. Switching from predictive low glucose suspend to advanced hybrid closed loop control: Effects on glucose control and patient reported outcomes. *Diabetes Res Clin Pract* (2022) 185:109784. doi: 10.1016/j.diabres.2022.109784

8. Sylow L, Kleinert M, Richter EA, Jensen TE. Exercise-stimulated glucose uptake — regulation and implications for glycaemic control. *Nat Rev Endocrinol* (2017) 13:133–48. doi: 10.1038/nrendo.2016.162

9. Bekiari E, Kitsios K, Thabit H, Tauschmann M, Athanasiadou E, Karagiannis T, et al. Artificial pancreas treatment for outpatients with type 1 diabetes: systematic review and meta-analysis. *BMJ* (2018) 361:k1310. doi: 10.1136/bmj.k1310

10. Peters TM, Haidar A. Dual-hormone artificial pancreas: benefits and limitations compared with single-hormone systems. *Diabetes Med* (2018) 35:450–9. doi: 10.1111/dme.13581

11. Taleb N, Emami A, Suppere C, Messier V, Legault L, Ladouceur M, et al. Efficacy of single-hormone and dual-hormone artificial pancreas during continuous and interval exercise in adult patients with type 1 diabetes: randomised controlled crossover trial. *Diabetologia* (2016) 59:2561–71. doi: 10.1007/s00125-016-4107-0

12. Castle JR, El Youssef J, Wilson LM, Reddy R, Resalat N, Branigan D, et al. Randomized outpatient trial of single- and dual-hormone closed-loop systems that adapt to exercise using wearable sensors. *Diabetes Care* (2018) 41:1471–7. doi: 10.2337/dc18-0228

13. Wilson LM, Jacobs PG, Ramsey KL, Resalat N, Reddy R, Branigan D, et al. Dual-hormone closed-loop system using a liquid stable glucagon formulation versus insulin-only closed-loop system compared with a predictive low glucose suspend system: An open-label, outpatient, single-center, crossover, randomized controlled trial. *Diabetes Care* (2020) 43:2721–9. doi: 10.2337/dc19-2267

14. Reenberg AT, Ritschel TKS, Lindkvist EB, Laugesen C, Svensson J, Ranjan AG, et al. Nonlinear model predictive control and system identification for a dual-hormone artificial pancreas. *IFAC-Pap* (2022) 55:915–21. doi: 10.1016/j.ifacol.2022.07.561

15. Ranjan AG, Boiroux D, Laugesen C, Schmidt S, Reenberg AT, Jørgensen JB, et al. Performance of a dual hormone closed-loop system vs. an insulin-onlyclosed-loop system during challenging inpatient conditions: A single-blinded randomized. *Diabetes* (2021) 70 (Supplement_1):215-OR. doi: https://doi.org/10.2337/db21-215-OR

16. *McDonald's nutrition calculator: Calories and more*. Available at: https://www.mcdonalds.com/us/en-us/about-our-food/nutrition-calculator.html.

17. Cicone ZS, Holmes CJ, Fedewa MV, MacDonald HV, Esco MR. Age-based prediction of maximal heart rate in children and adolescents: A systematic review and meta-analysis. *Res Q Exerc Sport* (2019) 90:417–28. doi: 10.1080/02701367.2019.1615605

18. Lundqvist C, Benth J, Grande R, Aaseth K, Russell M. A vertical VAS is a valid instrument for monitoring headache pain intensity. *Cephalalgia* (2009) 29:1034–41. doi: 10.1111/j.1468-2982.2008.01833.x

19. Hendey GW, Donner NF, Fuller K. Clinically significant changes in nausea as measured on a visual analog scale. *Ann Emerg Med* (2005) 45:77–81. doi: 10.1016/j.annemergmed.2004.07.446

20. Gallagher EJ, Bijur PE, Latimer C, Silver W. Reliability and validity of a visual analog scale for acute abdominal pain in the ED. *Am J Emerg Med* (2002) 20:287–90. doi: 10.1053/ajem.2002.33778

21. Haidar A, Legault L, Messier V, Mitre TM, Leroux C, Rabasa-Lhoret R. Comparison of dual-hormone artificial pancreas, single-hormone artificial pancreas, and conventional insulin pump therapy for glycaemic control in patients with type 1 diabetes: an open-label randomised controlled crossover trial. *Lancet Diabetes Endocrinol* (2015) 3:17–26. doi: 10.1016/S2213-8587(14)70226-8

22. Haidar A, Legault L, Matteau-Pelletier L, Messier V, Dallaire M, Ladouceur M, et al. Outpatient overnight glucose control with dual-hormone artificial pancreas, single-hormone artificial pancreas, or conventional insulin pump therapy in children and adolescents with type 1 diabetes: an open-label, randomised controlled trial. *Lancet Diabetes Endocrinol* (2015) 3:595–604. doi: 10.1016/S2213-8587(15)00141-2

23. Wu Z, Lebbar M, Taleb N, Legault L, Messier V, Rabasa-Lhoret R. Comparing dual-hormone and single-hormone automated insulin delivery systems on nocturnal glucose management among children and adolescents with type 1 diabetes: A pooled analysis. *Diabetes Obes Metab* (2023). 25:310–3 doi: 10.1111/dom.14850. n/a.

24. Ranjan AG, Schmidt S, Nørgaard K. Glucagon for hypoglycaemia treatment in type 1 diabetes. *Diabetes Metab Res Rev* (2021) 37:e3409. doi: 10.1002/dmrr.3409

25. Russell SJ, El-Khatib FH, Sinha M, Magyar KL, McKeon K, Goergen LG, et al. Outpatient glycemic control with a bionic pancreas in type 1 diabetes. *N Engl J Med* (2014) 371:313–25. doi: 10.1056/NEJMoa1314474

26. El-Khatib FH, Balliro C, Hillard MA, Magyar KL, Ekhlaspour L, Sinha M, et al. Home use of a bihormonal bionic pancreas versus insulin pump therapy in adults with type 1 diabetes: a multicentre randomised crossover trial. *Lancet Lond Engl* (2017) 389:369–80. doi: 10.1016/S0140-6736(16)32567-3

27. Laugesen C, Ranjan AG, Schmidt S, Nørgaard K. Low-dose dasiglucagon versus oral glucose for prevention of insulin-induced hypoglycemia in people with type 1 diabetes: A phase 2, randomized, three-arm crossover study. *Diabetes Care* (2022) 45:1391–9. doi: 10.2337/dc21-2304

28. Castellanos LE, Balliro CA, Sherwood JS, Jafri R, Hillard MA, Greaux E, et al. Performance of the insulin-only iLet bionic pancreas and the bihormonal iLet using dasiglucagon in adults with type 1 diabetes in a home-use setting. *Diabetes Care* (2021) 44: e118–20. doi: 10.2337/dc20-1086

29. Haidar A, Rabasa-Lhoret R, Legault L, Lovblom LE, Rakheja R, Messier V, et al. Single- and dual-hormone artificial pancreas for overnight glucose control in type 1 diabetes. *J Clin Endocrinol Metab* (2016) 101:214–23. doi: 10.1210/jc.2015-3003

30. Laugesen C, Ranjan A, Schmidt S, Nørgaard K. 257-OR: Pen-administered low-dose dasiglucagon vs. usual care for prevention and treatment of nonsevere hypoglycemia in people with type 1 diabetes during free-living conditions: A phase 2, randomized, two-period, crossover outpatient study. *Diabetes* (2022) 71(Supplement_1):257-OR. doi: 10.2337/db22-257-OR

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In-silico evaluation of an artificial pancreas achieving automatic glycemic control in patients with type 1 diabetes

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Artificial pancreas (AP) is a useful tool for maintaining the blood glucose (BG) of patients with type 1 diabetes (T1D) within the euglycemic range. An intelligent controller has been developed based on general predictive control (GPC) for AP. This controller exhibits good performance with the UVA/Padova T1D mellitus simulator approved by the US Food and Drug Administration. In this work, the GPC controller was further evaluated under strict conditions, including a pump with noise and error, a CGM sensor with noise and error, a high carbohydrate intake, and a large population of 100 in-silico subjects. Test results showed that the subjects are in high risk for hypoglycemia. Thus, an insulin on board (IOB) calculator, as well as an adaptive control weighting parameter (AW) strategy, was introduced. The percentage of time spent in euglycemic range of the in-silico subjects was 86.0% $\pm$ 5.8%, and the patient group had a low risk of hypoglycemia with the GPC+IOB+AW controller. Moreover, the proposed AW strategy is more effective in hypoglycemia prevention and does not require any personalized data compared with the IOB calculator. Thus, the proposed controller realized an automatic control of the BG of patients with T1D without meal announcements and complex user interaction.

KEYWORDS

automated artificial pancreas, general predictive control, adaptive control weighting parameter, hypoglycemia prevention, effective and safe glycemic control

1 Introduction

Type 1 diabetes (T1D) is an autoimmune disease characterized by chronic hyperglycemia. In patients with T1D, immune-mediated destruction of the pancreatic β cells occurs, and the pancreases produce very little or no insulin (1). In 2021, over 1.2 million children and adolescents had T1D mellitus (T1DM), and this number is increasing annually (2, 3). By 2030, 578 million people are predicted to suffer diabetes (including types 1 and 2), and this number will increase to 700 million by 2045 (4).

Although the causes of T1D are not fully understood, patients with T1D can live healthy lives with appropriate daily insulin injections. Artificial pancreas (AP) is also a useful tool for

maintaining the blood glucose (BG) of T1D patients within the euglycemic range (70–180 mg/dL) (5). It comprises a continuous glucose monitoring (CGM), an insulin pump, and an intelligent controller that connects these two devices (Figure 1A). Studies revealed that patients with T1D who used CGM and multiple insulin injections had lower hemoglobin A_{1c} levels than those receiving usual care (6). The use of AP system improves glycemic control and reduces the risk of hypoglycemia in different age groups with T1D compared with conventional or sensor-augmented pump therapy (7). The world's first AP system, the Medtronic's MiniMed 670G system, was approved by the US Food and Drug Administration (FDA) in 2016 and has been commercially available (8). This system not only reduces the patient workload and achieves good glucose control but also reduces the threat of diabetes-related complications (9). Three other AP systems, including Tandem Control-IQ in the US and Diabeloop and CamAPS FX in Europe, have received regulatory approval since then. Several AP systems are also in development or under clinical trials. The international diabetes closed-loop trial evaluated a mobile AP application that runs on Android smartphones and use Bluetooth to wirelessly communicate with the CGM and insulin pump. It achieved certain levels of reliability and wireless connection stability (10). Melissa J. Schoelwer et al. proposed a slim X2 Control-IQ hybrid closed-loop system using parameters that were based on total daily insulin, which was tested in 20 participants and proved to be effective and safe (11). Nowadays, three do-it-yourself AP systems, including OpenAPS, AndroidAPS, and Loop, are available on websites (12). Individuals can also build their own AP systems following the instructions and algorithms of those AP systems (13).

Despite the great achievement in the development of AP systems in recent years, the usability of these devices in the real-world setting is the main challenge. In other words, they are not entirely automated, thereby requiring user interaction to deliver mealtime insulin boluses (14). Compared with other control algorithms, such as proportional-integral-derivative (PID) control and model predictive control (MPC), generalized predictive control (GPC) algorithm is an adaptive control method and does not require knowledge on the initial parameters or precise glucose–insulin relationship. It could calculate the optimal insulin injection rate by minimizing the deviation of the predicted BG values from a reference glucose trajectory. The predicted BG values is calculated from an autoregressive integrated moving-average model with exogenous inputs (ARIMAX). GPC algorithm has been applied for AP system, and some improvements have been made to improve its performance. For example, Meriyan and Sato T et al. proposed a time-varying reference trajectory with fixed slopes for glucose concentration instead of a single set-point trajectory (15). Some design parameters of the GPC, such as the softening factor and forgetting factor, significantly affected the system output and should be established cautiously (15, 16). Turksoy et al. introduced some physiological signals, such as energy expenditure and galvanic skin responses, to the GPC model for post-exercise hypoglycemia prevention. Although these signals achieved a good performance, they increased the complexity of the system (17). Mirko Messori et al. introduced a novel kernel-based nonparametric approach and a constrained optimization to realize model individualization. However, model identification and validation relies on the collected patient data, and the constrained optimization further requires to postulate a model structure as prior knowledge (18). Dassau and Hyunjin Lee et al. developed several meal detection and meal size estimation algorithms for AP controller to alarm individuals or to deliver a bolus automatically (19, 20). However, it was constrained by the

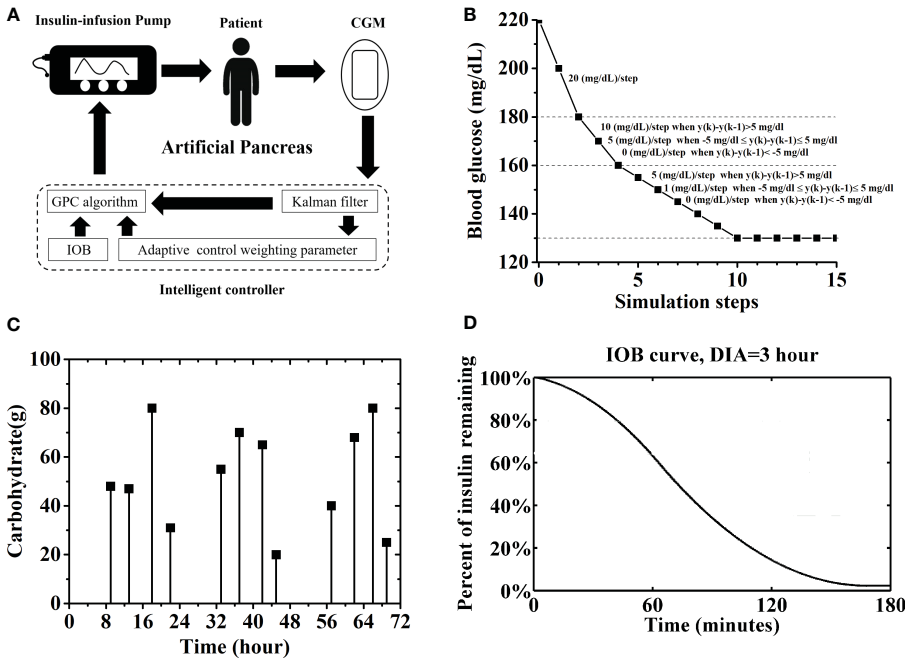

FIGURE 1
(A) Sketch map of an AP system, including a CGM, an insulin pump and an intelligent controller. Here, the intelligent controller was based on general predictive control (GPC) algorithm. A Kalman man filter, an IOB calculator and an AW strategy were introduced into the controller. **(B)** Adaptive reference glucose trajectory. **(C)** Carbohydrate and time of twelve unannounced meals. **(D)** IOB curve for prediction the active insulin amount, DIA is set as 3 hours.

threshold values set for different individuals. Thus, an intelligent controller was proposed based on GPC for AP, which calculated the insulin injection rate by only regarding the BG concentration measured by the CGM without information on the dose and timing of carbohydrates intake (21). Two adaptive strategies, including an adaptive reference glucose trajectory and an adaptive softening factor, were proposed for the GPC controller to increase system robustness when patients have normal BG values, as well as the tracking speed when patients have high hyperglycemia risk. Tests with the UVA/Padova T1DM simulator (T1DMS) approved by the FDA showed that it effectively controlled the BG of in-silico subjects under normal conditions. Here, the performance of the GPC controller was further evaluated with strict conditions, including a pump with noise and error, a CGM sensor with noise and error, a high carbohydrate (CHO) intake, and a large population of 100 in-silico subjects. Test results showed that the subjects were prone to high risk of hypoglycemia. Thus, an insulin on board (IOB) calculator, as well as an adaptive control weighting parameter (AW) strategy was introduced (Figure 1A). The performance of the GPC controller was significantly improved with them. The percentage of time spent in the euglycemic range (TIR) of the in-silico subjects was 86.0% ± 5.8%, and the patient group had a low risk of hypoglycemia with the GPC+IOB+AW controller. Moreover, the proposed GPC controller is effective in hypoglycemia prevention without the need for personalized data, complex user interaction, and meal announcements, which realizes an automatic control of the BG of patients with T1D.

Methods and materials

GPC controller

A GPC controller proposed in our previous research is applied for BG regulation (21). The GPC controller only reads the BG sent from the CGM sensor, and the optimal insulin rate was calculated. A basic Kalman filter was added to smooth the BG readings (Figure 1A). The Kalman filter was built using the Simulink tool in the MATLAB software environment without any personalized data (Details are shown in Supplemental Appendix S1).

Insulin injection rate is computed by minimizing J in the following function.

J=Σj=1n[y(k+j)−w(k+j)]2+Σj=1mλ[Δu(k+j−1)]2 w(k+j)=αjy(k)+(1−αj)yr (j=1,2,…,n)(1)

where $y(k + j)$ represents the j-step-ahead prediction of the process output, and $\Delta u\ (k + j-1)$ denotes the incremental control input at the $(k + j-1)$ sampling step. n, which denotes the output prediction horizon, is set as 8. m, which denotes the control horizon, is set as 4. Λ, which denotes the control weighting parameter, is set as 0.2. $y(k)$ represents the current BG. y_r denotes the adaptive reference glucose trajectory, which has slopes that are adjusted in accordance with variations in the BG measured in the past two steps (i.e., $y(k)$ and $y(k-1)$) (Figure 1B). α, which denotes the adaptive softening factor, is designed as $\alpha = \tau^{-|y(k)-y(k-1)|}$, $\tau = 1 + \frac{|y(k)-\bar{y}|}{\bar{y}}$, where $\bar{y}$ = 130 mg/dl (21).

The j-step-ahead prediction $y(k + j)$ is estimated using an autoregressive integrated moving-average model with exogenous inputs (Function 2) and the Diophantine equation (Function 3).$u(k)$ is the control input variable (insulin infusion rate), $\xi(k)$ denotes the zero-mean white noise, and $\Delta = (1-z^{-1})$ denotes the integration. The minimization of Function (1) provides the optimal control action (insulin infusion rate). The parameter design and the solution process are introduced in our previous research in detail (21).

$$A(z^{-1})y(k) = B(z^{-1})u(k-1) + C(z^{-1})\xi(k)/\Delta$$

$$A(z^{-1}) = 1 + a_1 z^{-1} + \ldots + a_{na} z^{-na}$$

$$B(z^{-1}) = b_0 + b_1 z^{-1} + \ldots + b_{nb} z^{-nb}$$

$$C(z^{-1}) = 1 + c_1 z^{-1} + \ldots + c_{nc} z^{-nc} \quad (2)$$

$$1 = E_j(z^{-1})A(z^{-1})\Delta + z^{-j}F_j(z^{-1})$$

$$E_j(z^{-1}) = e_{j0} + e_{j1} z^{-1} + \ldots + e_{j,j-1} z^{-j+1}$$

$$F_j(z^{-1}) = f_{j0} + f_{j1} z^{-1} + \ldots + f_{jn} z^{-n} \quad (3)$$

Software, scenario design, and data analysis

The GPC controllers were built and tested using the UVA/Padova T1DMS version 3.2.1, which embodies the biophysiological parameters of the FDA-accepted in silico populations (22). The software has two versions, namely, the academic and commercial versions. The former contains 10 adult subjects, whereas the latter contains 100 adult subjects. In the academic version, Subject 09 was excluded because the endogenous glucose production of this patient was suppressed even 6 h after meals, thereby leading to hypoglycemia (23).

In this work, the GPC controllers were first tested using 9 subjects and then sent to the Epsilon Group to test with 100 subjects. The parameter settings in the two versions of the simulator were identical, as stated below.

A CGM sensor with noise and error was used, as well as a pump with noise and error. Sensor noise and error are generated in the T1DMS with hand-written script (24). Pump noise and error are generated with two Gaussian-distributed random signal generators in the T1DMS, respectively. Details are shown in Supplemental Appendix S2. Totally, the sensor error (including noise) has a mean of 0.76 mg/dl and a standard deviation of 11 mg/dl. This sensor simulation model is believed to provide worst-case scenario sensor errors and the real sensor errors tend to be smaller during controlled inpatient clinical trials (25).

The same scenario used by Kamuran Turksoy et al. was reproduced in this study (26). All patients from the simulator were simulated over three days (72 hours), and the sampling time was set as 5 min. A total of 12 unannounced meals were provided and lasted 15 min each. Figure 1C shows the multiple meals provided during the testing period. The scenario was repeated 30 times for each in-silico subject. The BG trace of each in-silico subject was recorded. The average BG value was calculated for the patient group, as well as the percentages of time spent in the severe hypoglycemia range (BG ≤ 50

mg/dL), the hypoglycemia range (BG ≤ 70 mg/dL), the hyperglycemia range (BG > 180 mg/dL), and the severe hyperglycemia range (BG > 300 mg/dL) (27).

The percentage of time spent in euglycemic range (TIR) was used to evaluate the efficacy of the GPC controller. Two risk indexes provided by the UVA/Padova T1DMS, namely, low (LBGI) and high BG indexes (HBGI), were used to evaluate the safety of the GPC controller. LBGI refers to a measure of the frequency and extent of low BG readings, whereas HBGI refers to a measure of the frequency and extent of high BG readings. LBGI can be used to identify minimal- (LBGI< 1.1), low- (1.1 ≤ LBGI< 2.5), moderate- (2.5 ≤ LBGI< 5), and high-risk (LBGI > 5.0) of the patient subject for hypoglycemia. HBGI can be used to identify minimal- (HBGI< 5.0), low- (5.0 ≤ HBGI< 10.0), moderate- (10.0 ≤ HBGI< 15), and high-risk (HBGI > 15.0) of the patient subject for hyperglycemia.

The TIR, HBGI, and LBGI of the in-silico patient group was analyzed statistically to determine whether the GPC controller was significantly improved with the IOB or AW strategy ($p \le 0.05$ or $p \le 0.01$). The statistical analysis was performed in two steps: First, F-tests were performed to compare the variances of the BGC percentages of the two groups. Second, T-tests were conducted to compare their means. Unequal variance T-tests were applied when the variances were not equal.

IOB calculator

The insulin will take a lag to reach the bloodstream and influence cell behavior after injection. The IOB refers to the percentage of active insulin units in a patient's body. Several pump companies have considered the amount of IOB to avoid hypoglycemia and to keep the patient's BG within the euglycemic range (28). IOB is a function of the duration of insulin activity (DIA) and the number of previous insulin amount (29). DIA is an individualized data that vary because of blood flow, injection site, temperature, and exercise (17, 30). Here, the IOB calculator was built by following the instruction of the OpenAPS on the website (31). The DIA is set as 3 h, and the IOB curve is shown in Figure 1D. Thus, the estimated IOB will be subtracted from $u(k)$, and only a basal insulin infusion rate (around 60 p mol/min) will be used if $u(k)$ is smaller than IOB.

AW strategy

Λ in Function (1) determines the weight of the control input deviation. Here, its effects on the GPC-based AP system were discussed and found that the average TIR of in-silico subjects decreased gradually when Λ was above 0.5 (TIR<85%, Figure 2A). In-silico patients had a high risk of hypoglycemia (LBGI>1.1, red line in Figure 2B) when Λ is smaller than 0.125, but a hyperglycemia phenomenon would also occur (HBGI>5.0, black line in Figure 2B) when Λ is above 1.25. Therefore, more insulin will be injected, and the risk of hypoglycemia increases when Λ has a low value. In this study, an AW strategy was proposed to ensure the efficacy and safety of the GPC-controller, in which Λ varied with the BG fluctuations. The dotted lines in Figure 2A represent the efficacy range of Λ (i.e., $0.03<\Lambda<0.5$), in which the average TIR of the in-silico subjects is above 85%. The safe range of Λ (i.e., $0.125<\Lambda<1.25$), in which the average LBGI value of the in-silico subjects is below 1.1 and their average HBGI average is below 5.0, is demonstrated in Figure 2B. Thus, the selection range of the control weighting parameter should be 0.125 to 0.5. The AW strategy is designed and shown in Function 4, where $\theta = 2$ and $\overline{y} = 130$ mg/dl. $y(k)$ is the BG measured at k step. The upper and lower limits of Λ are defined as 0.125 and 0.5, respectively. In the AW strategy, the value of Λ would decrease when the BG value $y(k)$ increases above the optimal BG value $\overline{y}$. The insulin injection rate would fluctuate more violently to avoid hyperglycemia. Furthermore, Λ would increase gradually when the BG value y(k) reduces to the optimal BG value $\overline{y}$. The insulin injection rate would be more stable in that progress.

$$\lambda = \begin{cases} [\theta \times (1 + \frac{|y_{(k)} - \bar{y}|}{\bar{y}})]^{[y_{(k-1)} - y_{(k)}]} & y_{(k-1)} - y_{(k)} < 0 \\ [\theta \times (1 + \frac{|y_{(k)} - \bar{y}|}{\bar{y}})]^{-1} & y_{(k-1)} - y_{(k)} \ge 0 \end{cases} \quad (4)$$

Results

In-silico tests of the GPC controller with strict conditions

First, the proposed GPC controller in our previous research was tested with nine in-silico subjects under strict conditions, including a

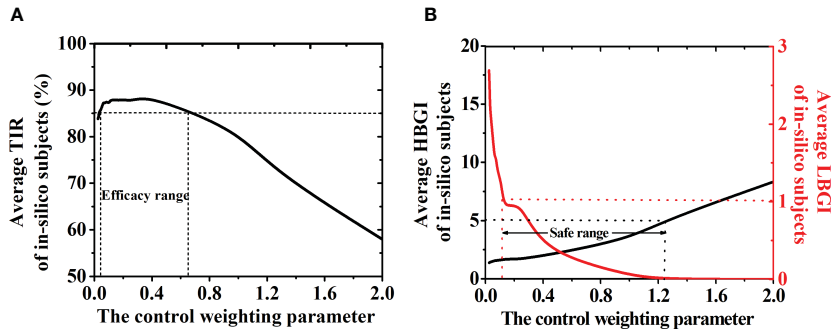

FIGURE 2
(A) TIR of the patient group with different control weighting parameter (Λ). The dotted lines represent the efficacy range of Λ (0.025 to 0.5), in which the TIR of the patient group is above 85%. **(B)** HBGI (black line) and LBGI (red line) values of the patient group with different Λ. The dotted lines represent the safe range of the control weighting parameter (0.125 to 1.25), in which the LBGI< 1.1 and HBGI<5.0. Thus, the selection range of the control weighting parameter should be 0.125 to 0.5.

pump with noise and error, a CGM sensor with noise and error, and a high CHO intake. Considering the effects of the CGM error and pump error, the scenario was repeated 30 times for each in-silico subject (Sections 2.1 and 2.2).

The BG trace of each in-silico subject was recorded, and their average BG was 103.1 ± 10.7 mg/dL. The merged BG trace and density of nine subjects were shown in Figures 3A, B (black lines). The average TIR of the patient group was 79.0% ± 8.3% (average ± standard deviation). The TIR of each subject was shown in Figure 4A (black squares). Five of them (No. 03, 05, 06, 07, and 10) had large TIR above 80% (i.e., 87.5% ± 5.2%, 86.2% ± 5.2%, 81.1% ± 1.9%, 81.3% ± 4.7% and 91.1% ± 4.8%). The TIR values of three subjects (i.e., Nos. 01, 02, and 08) were between 70% and 80% (i.e., 73.6% ± 5.3%, 70.2% ± 4.5%, and 72.3% ± 4.5%, respectively). However, the TIR of subject No. 4 was only 67.9% ± 4.4%.

Percentages of time spent in the severe hypoglycemia range (BG ≤ 50 mg/dL), the hypoglycemia range (BG ≤ 70 mg/dL), the hyperglycemia range (BG > 180 mg/dL), and the severe hyperglycemia range (BG > 300 mg/dL) of each subject are listed in Table 1. The average percentages of time spent in the severe hypoglycemia range, the hypoglycemia range, the hyperglycemia range, and the severe hyperglycemia range of the patient group were 10.1% ± 7.1%, 19.7% ± 10.2%, 3.3% ± 3.4%, and 0.0% ± 0.0%, respectively. Two indexes provided by the T1DMS software, namely, HBGI and LBGI, were calculated for each subject to identify whether they are prone to hyperglycemia or hypoglycemia (black squares in Figures 4B, C). The HBGI and LBGI values of the patient group showed normal distributions (Figures S1A, S2A, respectively). The HBGI values of 9 subjects were 0.6 ± 0.1, 0.5 ± 0.1, 0.7 ± 0.1, 0.5 ± 0.1, 0.8 ± 0.1, 1.5 ± 0.1, 1.6 ± 0.1, 0.7 ± 0.1, and 0.1 ± 0.6, indicating that all subjects had a minimal risk (HBGI<5.0) for hyperglycemia (Figures 4B, 5B). The LBGI values of these 9 subjects were 5.7 ± 1.6, 7.8 ± 1.6, 2.9 ± 1.5, 9.5 ± 1.9, 11.3 ± 2.6, 1.8 ± 0.6, 2.6 ± 1.7, 8.2 ± 1.8, and 2.1 ± 1.1, respectively. Two subjects (No. 06 and 10) had a low risk (LBGI<2.5) for hypoglycemia, whereas another two subjects (No. 03 and 07) had a moderate risk (2.5≤LBGI<5.0). The five remaining subjects (No. 01, 02, 04, 05, and 08) had a high risk for hypoglycemia (Figure 4C). Therefore, the GPC controller needs to be improved further to prevent hypoglycemia in patients with T1D.

Tests of the GPC+IOB controller

Hypoglycemia occurs because too much insulin is injected into the body. Thus, an IOB calculator was introduced to the GPC controller to calculate the insulin that remains active within the body, which will be subtracted at each step (Section 2.3).

The GPC+IOB controller was tested with the same scenario in Section 3.1. The BG trace of each in-silico subject was recorded, and their average BG was 110.5 ± 14.5 mg/dL. The merged BG trace and density of the 9 subjects were shown in Figures 3A, B (red lines), respectively. The average TIR of the patient group was 81.8% ± 7.3%. The TIR of each subject were 79.6% ± 4.8%, 72.8% ± 4.9%, 91.6% ± 4.0%, 74.6% ± 6.5%, 79.8% ± 4.4%, 83.8% ± 1.8%, 79.8% ± 5.6%, 79.1% ± 3.9%, and 95.1% ± 3.7% (red circulars in Figure 4A). Compared with the test results in the GPC controller, the TIR of seven subjects (Nos. 01, 02, 03, 04, 06, 08, and 10) increased by 6.1%, 2.7%, 4.1%, 6.7%, 2.7%, 6.7%, and 4.1%, respectively. Meanwhile, the TIR of the two other subjects (No. 05 and 07) decreased by 6.4% and 1.5%, respectively. Although the TIR of the patient group with GPC +IOB controller was higher than that with the GPC controller, the statistical analysis (i.e., F-test and T-test) showed no significant differences (Figure 5A).

The percentages of time spent in the severe hypoglycemia range, the hypoglycemia range, the hyperglycemia range, and the severe hyperglycemia range of each subject was listed in Table 2. The average percentages of time spent in the severe hypoglycemia range, the hypoglycemia range, the hyperglycemia range, and the severe hyperglycemia range of the patient group were 5.9% ± 5.4%, 13.3% ± 9.7%, 4.9% ± 6.2%, and 0.0% ± 0.0%, respectively. Thus, the hypoglycemia risk of the patient group decreased compared with the results in Section 3.1. The HBGI and LBGI values of each subject with GPC+IOB controller were demonstrated in Figures 4B, C (red circulars), respectively. The HBGI values of the 9 subjects were 0.6 ± 0.1, 0.5 ± 0.1, 1.0 ± 0.2, 0.6 ± 0.1, 1.0 ± 0.1, 1.9 ± 0.1, 3.2 ± 1.0, 0.9 ± 0.1, and 0.6 ± 0.1, respectively. All subjects had minimal risk for hyperglycemia (Figures 4B, 5B). The LBGI values of the 9 subjects were 4.1 ± 1.4, 6.6 ± 1.6, 1.8 ± 1.1, 6.3 ± 1.9, 5.3 ± 1.9, 0.7 ± 0.3, 0.5 ± 0.4, 4.9 ± 1.6, and 1.2 ± 0.8. Four of them (No. 03, 06, 07, and 10) had a low risk for hypoglycemia, two (No. 01 and 08) had a moderate risk, and the remaining three (No. 02, 04, and 05) have a high risk (Figure 4C). In relation to the test results of the GPC controller, the LBGI values of the 9 subjects decreased by 1.6%, 1.2%, 1.2%, 3.2%, 6.0%, 1.1%, 2.2%, 3.3%, and 1.0%. The HBGI and LBGI values of the patient group showed normal distributions (Figures S1B, S2B, respectively), thus F-test and T-test were performed to verify whether the GPC+IOB controller had a better performance. However, the statistical analysis showed that the improvement of the hypoglycemia was not significant (Figure 5C).

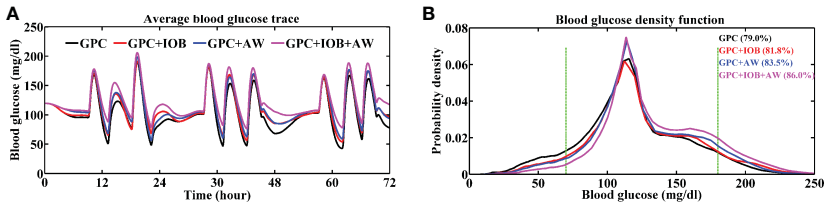

FIGURE 3
Average BG trace **(A)** and BG density **(B)** of 9 in-silico subjects. The TIR of the patient group with different controller is labeled in brackets. Green lines denote the euglycemic range. Black line represents the test with GPC controller, red line represents the test with GPC+IOB controller, blue line represents the test with GPC+AW controller and the pink line represents the test with GPC+IOB+AW controller.

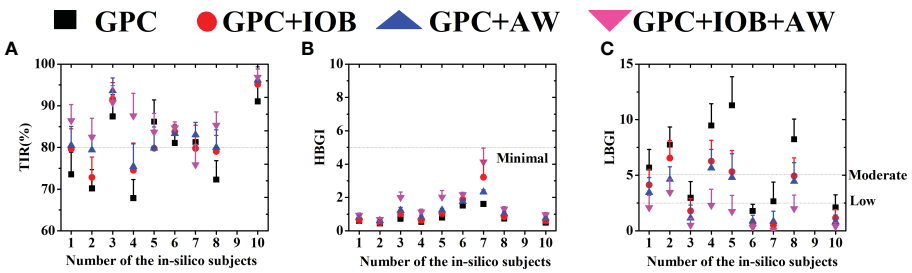

FIGURE 4
(A) The TIR of 9 in-silico subjects. **(B)** The HBGI of 9 in-silico subjects. **(C)** The LBGI of 9 in-silico subjects. Black squares represent the test with GPC controller, red circulars represent the test with GPC+IOB controller, blue triangles represent the test with GPC+AW controller and the pink triangles represent the test with GPC+IOB+AW controller.

Tests of the GPC+AW controller

Control weighting parameter, Λ, determines the weight of the control input deviation. A small Λ value means a fast change in the insulin injection rate, and a high Λ value means a stable insulin injection rate. Eren M. et al. also suggested that the Λ value played an important role in hypoglycemia prevention (15). In the present work, we discussed its effect on the efficacy and safety of the GPC-based AP. The efficacy of the GPC controller (i.e., the TIR of the patient group) greatly decreased to 60% as λ increased to 2 (Figure 2A). Although the hyperglycemia risk of the patients (i.e., HBGI value) decreased with λ (black line in Figure 2B), their hypoglycemia risk (i.e., LBGI value) rapidly increased (red line in Figure 2B). Here, an AW strategy was proposed, in which the λ value varied with the BG of the patient (Section 2.4). In short, λ would have a low value when the BG is increasing sharply but adopts a high value when the BG is decreasing gradually.

The GPC+AW controller was tested with T1DMS software following the same scenario as before. The BG trace of each in-silico subject was recorded, and their average BG was 112.9 ± 11.1 mg/dL. The merged BG trace and density of the 9 subjects were shown in Figures 3A, B (blue lines), respectively. The average TIR of the patient group was 83.5% ± 6.9%. The TIR of each subject were 80.5% ± 4.5%, 79.4% ± 3.6%, 93.6% ± 3.1%, 75.4% ± 5.4%, 79.9% ± 5.1%, 83.4% ± 1.9%, 83.0% ± 3.0%, 80.0% ± 4.2%, and 96.1% ± 3.2% (blue triangles in Figure 4A). In relation to the test results with the GPC controller, the TIR of eight subjects (No. 01, 02, 03, 04, 06, 07, 08, and 10) increased by 7.0%, 9.2%, 6.2%, 7.6%, 2.3%, 1.8%, 7.7%, and 5.1%, respectively. However, TIR of subject No. 05 decreased by 6.3%. Statistical analysis was further performed, but no significant difference was found (Figure 5A).

The percentages of time spent in the severe hypoglycemia range, the hypoglycemia range, the hyperglycemia range, and the severe hyperglycemia range of each subject were listed in Table 3. The average percentages of time spent in the severe hypoglycemia range, the hypoglycemia range, the hyperglycemia range, and the severe hyperglycemia range of the patient group were 4.9% ± 4.5%, 11.4% ± 8.2%, 5.1% ± 4.4%, and 0.0% ± 0.0%, respectively. The HBGI and LBGI values of the patient group were calculated and showed normal distributions, as shown in Figures S1C, S2C. Figures 4B, C (blue triangles) showed the HBGI and LBGI values of each subject with GPC+AW controller, respectively. The HBGI values of the 9 subjects were 0.8 ± 0.1, 0.6 ± 0.1, 1.2 ± 0.2, 0.8 ± 0.1, 1.2 ± 0.1, 1.8 ± 0.1, 2.3 ± 0.1, 1.0 ± 0.1, and 0.8 ± 0.1. All subjects had minimal risk for hyperglycemia (Figures 4B, 5B). The LBGI values of the 9 subjects were 3.5 ± 1.3, 4.6 ± 1.1, 1.2 ± 1.2, 5.7 ± 1.7, 4.8 ± 2.1, 0.9 ± 0.5, 0.9 ± 0.9, 4.5 ± 1.7, and 0.8 ± 0.6, respectively. Four of them (No. 03, 07, 08,

TABLE 1 Percentages of time spent in the severe hypoglycemia (BG ≤ 50 mg/dL) range, the hypoglycemia (BG ≤ 70 mg/dL) range, the hyperglycemia range (BG > 180 mg/dL), and the severe hyperglycemia range (BG > 300 mg/dL) of each in-silico subject with the GPC controller.

Subject No.	Percentages of time spent in the severe hypoglycemia (BG ≤ 50 mg/dL) range	Percentages of time spent in the hypoglycemia (BG ≤ 70 mg/dL) range	Percentages of time spent in the hyperglycemia (BG ≥ 180 mg/dL) range	Percentages of time spent in the severe hyperglycemia (BG ≥ 300 mg/dL) range
01	10.7% ± 3.3%	24.2% ± 5.5%	2.3% ± 1.2%	0.0% ± 0.0%
02	16.0% ± 3.5%	28.9% ± 4.5%	0.9% ± 0.6%	0.0% ± 0.0%
03	4.3% ± 2.9%	11.9% ± 5.2%	0.6% ± 0.6%	0.0% ± 0.0%
04	17.6% ± 4.7%	30.6% ± 4.5%	1.5% ± 0.9%	0.0% ± 0.0%
05	17.9% ± 3.9%	29.2% ± 5.1%	2.6% ± 0.7%	0.0% ± 0.0%
06	3.1% ± 1.5%	8.7% ± 2.2%	10.2% ± 1.3%	0.0% ± 0.0%
07	3.9% ± 3.0%	10.4% ± 4.7%	8.3% ± 1.3%	0.0% ± 0.0%
08	14.8% ± 3.6%	24.9% ± 4.7%	2.8% ± 1.3%	0.0% ± 0.0%
10	2.5% ± 2.6%	8.4% ± 4.7%	0.6% ± 0.6%	0.0% ± 0.0%

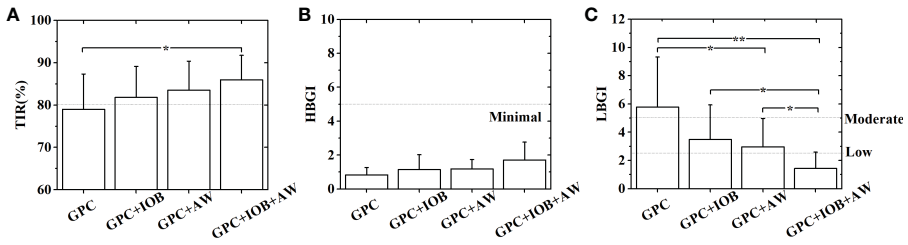

FIGURE 5
Statistical analyses of the TIR **(A)**, HBGI **(B)** and LBGI **(C)** of 9 in-silico subjects with different GPC controllers. Statistical analysis was performed in two steps: First, F-tests were performed to compare variances of the data sets. Then T-tests are conducted to further examine the differences between the data sets. Unequal variance T-tests were applied when the variances were not equal. *p< 0.05. **p< 0.01.

and 10) had a low risk for hypoglycemia, four (No. 01, 02, 05, and 08) had a moderate risk, and only one subject (No. 04) has a high risk (Figure 4C). In relation to the test results of the GPC controller, the LBGI values of the 9 subjects decreased by 2.2%, 3.1%, 1.8%, 3.8%, 6.5%, 0.9%, 1.8%, 3.8%, and 1.3%. Statistical analysis showed that the LBGI values of the patient group with GPC+AW controller was significantly lower than those with the GPC controller, indicating that the hypoglycemia was significantly improved ($p<0.05$) (Figure 5C).

Tests of the GPC+IOB+AW controller

Lastly, we tested the GPC+IOB+AW controller. The BG trace of each in-silico subject was recorded, and their average BG was 122.4 ± 14.2 mg/dL. The merged BG trace and density of the 9 subjects were shown in Figures 3A, B (pink lines), respectively. The average TIR of the patient group was 86.0% ± 5.8%. The TIR of each subject were 86.4% ± 3.9%, 82.5% ± 4.6%, 90.8% ± 4.0%, 87.6% ± 5.4%, 83.7% ± 4.5%, 84.7% ± 1.4%, 75.9% ± 4.9%, 85.3% ± 3.2%, and 96.9% ± 2.0%, respectively (pink triangles in Figure 4A). In relation to the test results with the GPC controller, the TIR of seven subjects (No. 01, 02, 03, 04, 06, 08, and 10) increased by 12.9%, 12.3%, 3.3%, 19.7%, 3.6%, 13.0%, and 5.8%, respectively, whereas TIR of two subjects (No. 05 and 07) decreased by 2.5% and 5.4%, respectively. Statistical analysis showed that the TIR of the patient group with the GPC+AW+IOB controller was significantly higher than that with the GPC controller ($p<0.05$). Thus, the efficacy of the GPC controller was significantly improved with the IOB and AW strategies (Figure 5A).

The percentages of time spent in the severe hypoglycemia range, the hypoglycemia range, the hyperglycemia range, and the severe hyperglycemia range of each subject were listed in Table 4. The average percentages of time spent in the severe hypoglycemia range, the hypoglycemia range, the hyperglycemia range, and the severe hyperglycemia range of the patient group were 2.1% ± 2.9%, 5.8% ± 6.0%, 8.2% ± 7.1%, and 0.0% ± 0.0%, respectively. Seemingly, the hypoglycemia risk declined further. The HBGI and LBGI values of the patient group were calculated. As shown in Figures S1D, S2D, the data showed normal distributions. The HBGI and LBGI values of each subject with GPC+IOB+AW controller were shown in Figures 4B, C (pink triangles), respectively. The HBGI values of the 9 subjects were 0.9 ± 0.1, 0.7 ± 0.1, 2.0 ± 0.3, 1.1 ± 0.2, 2.0 ± 0.4, 2.2 ± 0.1, 4.1 ± 0.8, 1.3 ± 0.2, and 1.0 ± 0.1, respectively. All subjects had minimal risk for hyperglycemia (Figures 4B, 5B). The LBGI values of the 9 subjects were 2.1 ± 1.1, 3.5 ± 1.2, 0.5 ± 0.6, 2.3 ± 1.4, 1.8 ± 1.4, 0.3 ± 0.3, 0.1 ± 0.2, 2.0 ± 1.2, and 0.4 ± 0.3, respectively. Eight of them (No. 01, 03, 04, 05, 06, 07, 08, and 10) had a low risk for hypoglycemia, and only one subject (No. 02) had a moderate risk (Figure 4C). In relation to the test results of the GPC controller, the LBGI values of the 9 subjects decreased by 3.6%, 4.3%, 2.5%, 7.2%, 9.5%, 1.5%, 2.6%, 6.2%, and 1.7%, respectively. Statistical analysis (i.e., F-test and T-test) showed

TABLE 2 Percentages of time spent in the severe hypoglycemia (BG ≤ 50 mg/dL) range, the hypoglycemia (BG ≤ 70 mg/dL) range, the hyperglycemia range (BG > 180 mg/dL), and the severe hyperglycemia range (BG > 300 mg/dL) of each in-silico subject with the GPC+IOB controller.

Subject No.	Percentages of time spent in the severe hypoglycemia (BG ≤ 50 mg/dL) range	Percentages of time spent in the hypoglycemia (BG ≤ 70 mg/dL) range	Percentages of time spent in the hyperglycemia (BG ≥ 180 mg/dL) range	Percentages of time spent in the severe hyperglycemia (BG ≥ 300 mg/dL) range
01	7.4% ± 2.7%	18.4% ± 5.0%	1.9% ± 1.0%	0.0% ± 0.0%
02	13.6% ± 3.6%	26.0% ± 4.8%	1.1% ± 0.7%	0.0% ± 0.0%
03	2.6% ± 2.5%	7.1% ± 4.0%	1.3% ± 0.8%	0.0% ± 0.0%
04	10.7% ± 4.1%	23.8% ± 6.6%	1.7% ± 0.9%	0.0% ± 0.0%
05	8.3% ± 3.2%	16.7% ± 4.9%	3.4% ± 1.0%	0.0% ± 0.0%
06	0.3% ± 0.7%	3.7% ± 1.9%	12.5% ± 0.9%	0.0% ± 0.0%
07	0.3% ± 0.8%	1.9% ± 2.1%	18.3% ± 5.7%	0.0% ± 0.0%
08	9.0% ± 3.4%	17.7% ± 4.2%	3.2% ± 1.3%	0.0% ± 0.0%
10	1.0% ± 1.3%	4.4% ± 3.7%	0.5% ± 0.6%	0.0% ± 0.0%

TABLE 3 Percentages of time spent in the severe hypoglycemia (BG ≤ 50 mg/dL) range, the hypoglycemia (BG ≤ 70 mg/dL) range, the hyperglycemia range (BG > 180 mg/dL), and the severe hyperglycemia range (BG > 300 mg/dL) of each in-silico subject with the GPC+AW controller.

Subject No.	Percentages of time spent in the severe hypoglycemia (BG ≤ 50 mg/dL) range	Percentages of time spent in the hypoglycemia (BG ≤ 70 mg/dL) range	Percentages of time spent in the hyperglycemia (BG ≥ 180 mg/dL) range	Percentages of time spent in the severe hyperglycemia (BG ≥ 300 mg/dL) range
01	5.4% ± 3.0%	16.2% ± 4.5%	3.2% ± 1.3%	0.0% ± 0.0%
02	9.5% ± 2.7%	18.9% ± 3.7%	1.7% ± 0.8%	0.0% ± 0.0%
03	1.6% ± 2.2%	4.1% ± 3.2%	2.3% ± 1.2%	0.0% ± 0.0%
04	9.8% ± 3.7%	22.3% ± 5.4%	2.2% ± 1.1%	0.0% ± 0.0%
05	7.2% ± 3.5%	14.9% ± 5.1%	5.2% ± 0.9%	0.0% ± 0.0%
06	0.9% ± 1.4%	4.8% ± 2.1%	11.8% ± 1.0%	0.0% ± 0.0%
07	1.1% ± 1.7%	3.2% ± 3.2%	13.7% ± 1.3%	0.0% ± 0.0%
08	7.9% ± 3.1%	15.2% ± 4.2%	4.7% ± 1.2%	0.0% ± 0.0%
10	0.6% ± 1.1%	2.7% ± 3.3%	1.1% ± 0.6%	0.0% ± 0.0%

that the LBGI values of the patient group with GPC+IOB+AW controller were significantly lower than those with the GPC controller ($p<0.01$), GPC+IOB controller ($p<0.05$), and GPC+AW controller ($p<0.05$) (Figure 5C). Thus, the GPC+IOB+AW controller achieves optimal glycemic control and minimizes the risk of hypoglycemia in patients with T1D.

Evaluation of the GPC+IOB+AW controller with 100 in-silico subjects and normal CHO intakes

The GPC+IOB+AW controller was further sent to the Epsilon Group and evaluated with 100 in-silico subjects. The DIA of the IOB calculator was set as 3 h without any personalized data. The same scenario and parameter settings in Section 3.1 were repeated 10 times for each in-silico subject. The merged BG trace and density of 100 subjects were shown in Figures 6A, B, respectively. The average TIR value of the patient group is 81.3% ± 8.6%, and TIR values of 85 subjects were above 70% (Figure 6C). The percentages of time spent in the severe hypoglycemia range, the hypoglycemia range, the hyperglycemia range, and the severe hyperglycemia range of each subject was listed in Table S1. The average percentages of the time spent in the severe hypoglycemia range, the hypoglycemia range, the hyperglycemia range, and the severe hyperglycemia range of the patient group were 1.7% ± 3.6%, 4.4% ± 6.2%, 14.7% ± 9.0%, and 0.1% ± 0.6%, respectively. All 100 in-silico subjects had a low risk for hyperglycemia, with the average HBGI value of 2.7 ± 1.4 (Figure 6D). A total of 87 subjects had a low risk, 9 subjects had a moderate risk, and 4 subjects had a high risk for hypoglycemia (Figure 6E). The average LGBI value of the patient group is 1.2 ± 1.6. Therefore, the GPC+IOB+AW controller realized effective and safe BG control for the majority of the population.

People might be concerned that the GPC+IOB+AW is only applicable to those patients with high CHO intake. Hence, we also tested its performance with normal CHO intakes, as stated as follows: 30 g of CHO at 7 AM, 30 g at 12 PM, and 30 g at 6 PM daily, 3 days. Figures 7A, B show the merged BGC trace and density of the nine subjects, respectively. The average TIR of the patient group was 95.6% ± 3.9% (Figure 7C). The percentages of the time spent in the severe

TABLE 4 Percentages of time spent in the severe hypoglycemia (BG ≤ 50 mg/dL) range, the hypoglycemia (BG ≤ 70 mg/dL) range, the hyperglycemia range (BG > 180 mg/dL), and the severe hyperglycemia range (BG > 300 mg/dL) of each in-silico subject with the GPC+IOB+AW controller.

Subject No.	Percentages of time spent in the severe hypoglycemia (BG ≤ 50 mg/dL) range	Percentages of time spent in the hypoglycemia (BG ≤ 70 mg/dL) range	Percentages of time spent in the hyperglycemia (BG ≥ 180 mg/dL) range	Percentages of time spent in the severe hyperglycemia (BG ≥ 300 mg/dL) range
01	2.7% ± 2.6%	9.4% ± 4.4%	4.1% ± 1.2%	0.0% ± 0.0%
02	6.6% ± 2.4%	15.3% ± 4.6%	2.2% ± 0.8%	0.0% ± 0.0%
03	0.7% ± 1.3%	2.1% ± 2.6%	7.1% ± 3.3%	0.0% ± 0.0%
04	2.8% ± 2.8%	9.2% ± 5.6%	3.2% ± 1.1%	0.0% ± 0.0%
05	2.6% ± 2.7%	5.9% ± 4.6%	10.4% ± 2.8%	0.0% ± 0.0%
06	0.1% ± 0.5%	1.3% ± 1.7%	14.0% ± 0.9%	0.0% ± 0.0%
07	0.0% ± 0.2%	0.2% ± 0.9%	23.8% ± 4.9%	0.0% ± 0.0%
08	3.7% ± 2.6%	8.1% ± 3.6%	6.6% ± 1.3%	0.0% ± 0.0%
10	0.1% ± 0.4%	1.1% ± 1.8%	2.0% ± 0.9%	0.0% ± 0.0%

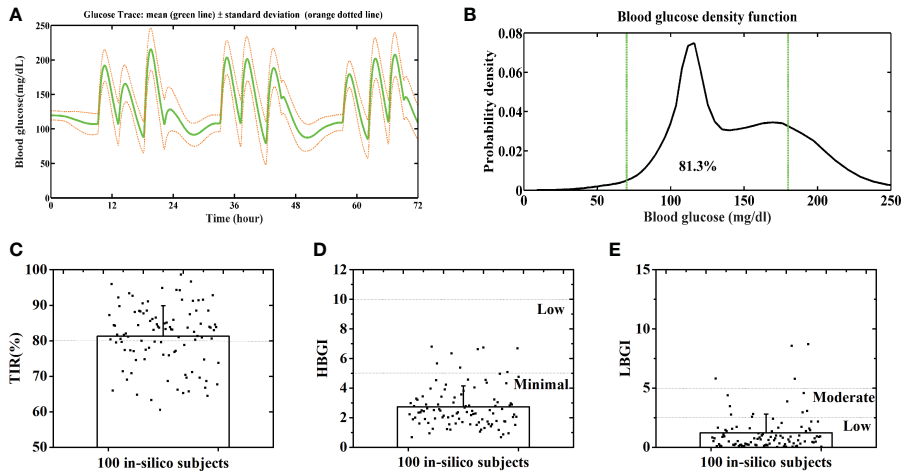

FIGURE 6
Average BG trace **(A)** and BG density **(B)** of 100 in-silico subjects. Distributions of the TIR **(C)**, HBGI **(D)** and LBGI **(E)** of 100 in-silico subjects. Green lines denote the euglycemic range.

hypoglycemia range, the hypoglycemia range, the hyperglycemia range, and the severe hyperglycemia range of each subject were listed in Table S2. The average percentages of time spent in the severe hypoglycemia range, the hypoglycemia range, the hyperglycemia range, and the severe hyperglycemia range of the patient group were 1.4% ± 2.4%, 4.4% ± 5.2%, 0.0% ± 0.0%, and 0.0% ± 0.0%, respectively. All subjects had low risks for hyperglycemia and hypoglycemia, with the average HBGI and LBGI of 0.66 ± 0.23 and 1.05 ± 0.75, respectively (Figures 7D, E). Thus, the GPC+IOB+AW controller only focuses on the BGC variances and can handle different CHO intakes.

Discussions

T1D occurs most frequently in children and young adults. In 2021, over 1.2 million children and adolescents had T1DM, and that number is increasing annually. Multiple daily injections, glucose monitoring, structured diabetes education, and expert medical care were great challenges for these young people and had affected their normal lives seriously. An automatic AP is a promising tool to solve that problem (2, 3).

The aim of our research is to develop an automated AP without any user interaction or personalized data. Thus, we proposed an intelligent controller based on GPC for AP, which only regards the BG levels provided by the CGM without meal announcements. It realized effective BG control in our previous research and was further tested here with more strict conditions. Although it is effective in hyperglycemia prevention, hypoglycemia risk for patients increased to a high level. Thus, the GPC controller needs further improvement.

The IOB calculator, which could estimate the insulin amount that remains active within the patient's body, was introduced into the GPC controller. For instance, the estimated active insulin amount for subject No. 01 is shown in Figure 8A (black dotted line), which was subtracted from the ideal insulin injection rate calculated by the GPC

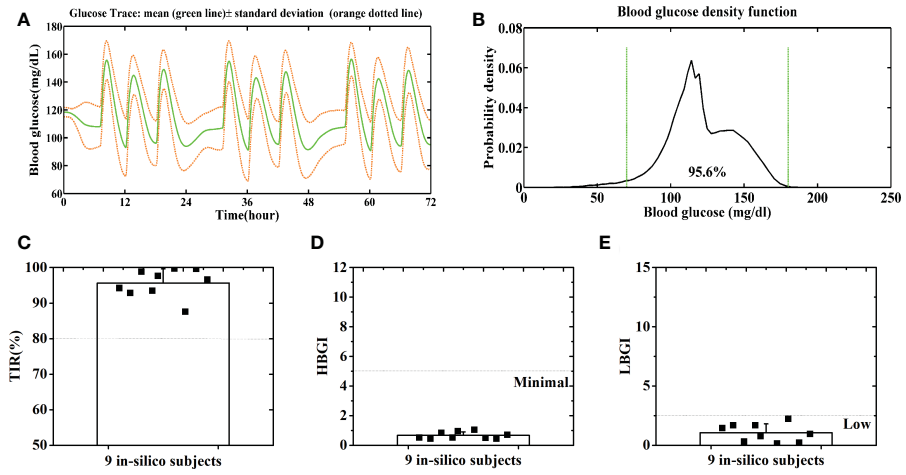

FIGURE 7
Average BG trace **(A)** and BG density **(B)** of 9 in-silico subjects with a normal CHO intake. Distributions of the TIR **(C)**, HBGI **(D)** and LBGI **(E)** of 9 in-silico subjects with a normal CHO intake. Green lines denote the euglycemic range.

algorithm (blue dotted line in Figure 8A) at each step (Section 2.3). Thus, less insulin would be injected into the patient's body (red line in Figure 8A). The test results with T1DMS showed that the hypoglycemia risk for each in-silico subject reduced with the GPC +IOB controller. However, we found that the IOB calculator needs a personalized data, DIA. The incorrect estimation of the DIA induces mismatch in the IOB and insulin injection, thereby resulting in hypoglycemia or hyperglycemia. Hence, determining the individualized DIA still remains a critical point nowadays (29).

To overcome the aforementioned problem, we proposed an AW strategy without requiring any individualized data. As shown in Figures 8B, C, the value of Λ sharply decreases when the BG is greatly increasing but adopts a high value when the BG is gradually decreasing. Thus, a large dose of insulin will be injected when the BG is increasing, but a smaller dose of insulin will be injected when the BG is decreasing. The test results with T1DMS showed that the hypoglycemia of the patient group was significantly improved with the GPC+AW controller, which is more effective than the GPC+IOB controller (Figure 5C). Therefore, the AW strategy has high usability and performance.

The efficacy of our GPC+IOB+AW controller is comparable with other controllers, such as the proportional integral derivative with double phase lead (PIDD) controller, the proportional-integral-derivative (PID) controller, the model predictive controller (MPC), the extended model predictive controller (EMPC), and the conventional proportional-derivative (PD) controller with the fuzzy P part (Fuzzy P+D). The TIR of the patient group with the aforementioned controllers were 77%, 72.6%, 79.6%, 84.3%, and 83%, respectively (23, 32, 33). However, its performance still needs improvement compared with current standard-of-care, such as the basal-bolus therapy. The TIR, HBGI, and LBGI of the in-silico subjects was 92%, 0.49, and 0.78 using the basal/bolus controller proposed by Fraser Cameron with the similar scenario, which is better than the results here, as well as the results obtained by other controllers (23). However, the basal-bolus therapy design requires user interaction and personalized data, including meal information, the carbohydrate-to-insulin ratio, and the correction factor. Moreover, the values of these factor may vary during the day (34). The most outstanding feature of our GPC+IOB+AW is that it realizes

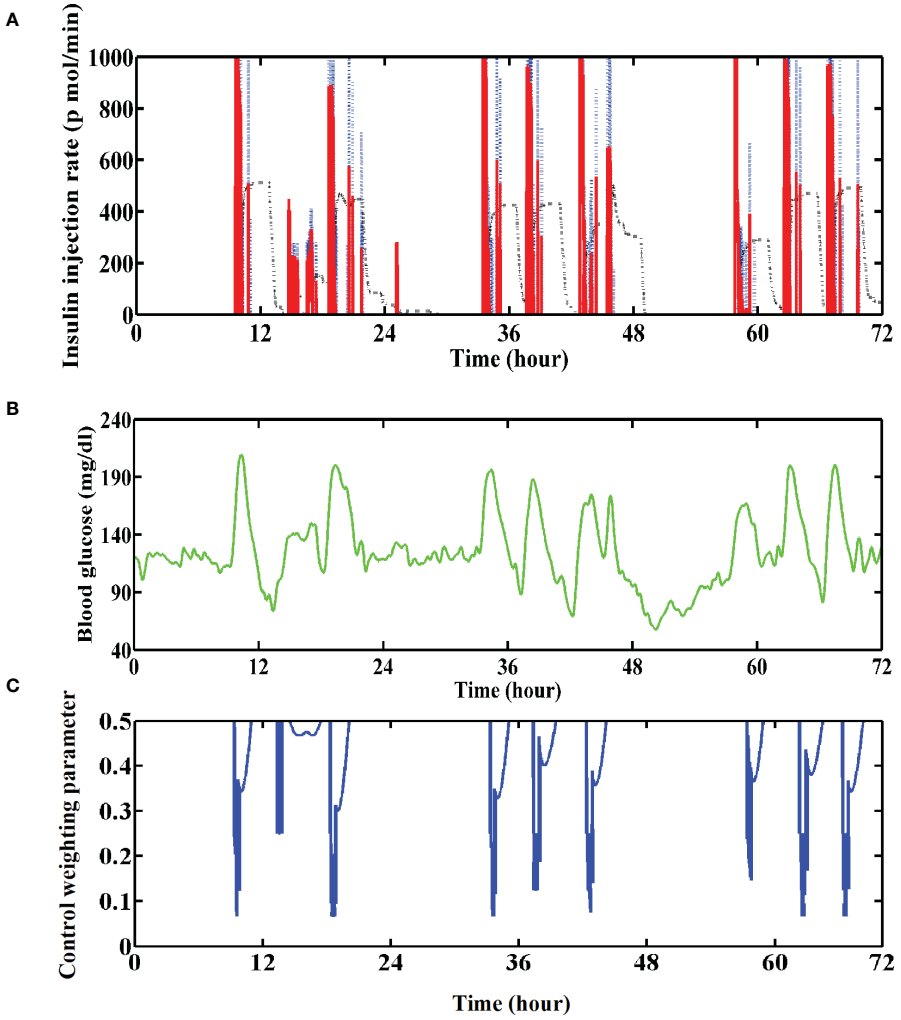

FIGURE 8
(A) Insulin injection rate for the No. 01 subject with the GPC+IOB+AW controller. The blue dotted line denotes the ideal insulin injection rate calculated by the GPC controller, the black dotted line denotes the estimated active insulin amount in the body and the red line denotes their subtraction. **(B)** Variations of the BG trace of the No. 01 subject with GPC+IOB+AW controller. **(C)** Variations of the control weighting parameter of the No. 01 subject.

an automatic control and does not require user interaction, personalized data, and meal announcements.

Moreover, subsequent tests with real patients are still substantially needed because an in-silico population with an average TIR of 86% is only representative to a very limited proportion of real patients. Other modules, such as the carbohydrate on board, should also be considered in future research.

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.

Author contributionss

HJ and WL conceived and designed the experiments. Modeling and simulation were conducted by WL, BL and TC. WL and YW analyzed the results and wrote 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.2023.1115436/full#supplementary-material

References

1. DiMeglio LA, Evans-Molina C, Oram RA. Type 1 diabetes. *Lancet* (2018) 391 (10138):2449–62. doi: 10.1016/S0140-6736(18)31320-5

2. Wang H, Li N, Chivese T, Werfalli M, Sun H, Yuen L, et al. IDF diabetes atlas: Estimation of global and regional gestational diabetes mellitus prevalence for 2021 by international association of diabetes in pregnancy study group's criteria. *Diabetes Res Clin Pract* (2022) 183:109050. doi: 10.1016/j.diabres.2021.109050

3. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF diabetes atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract* (2022) 183:109119. doi: 10.1016/j.diabres.2021.109119

4. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF diabetes atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract* (2018) 138:271–81. doi: 10.1016/j.diabres.2018.02.023

5. El Fathi A, Raef Smaoui M, Gingras V, Boulet B, Haidar A. The artificial pancreas and meal control: An overview of postprandial glucose regulation in type 1 diabetes. *IEEE Control Systems* (2018) 38(1):67–85. doi: 10.1109/MCS.2017.2766323

6. Beck RW, Riddlesworth T, Ruedy K, Ahmann A, Bergenstal R, Haller S, et al. Effect of continuous glucose monitoring on glycemic control in adults with type 1 diabetes using insulin injections: The DIAMOND randomized clinical trial. *JAMA* (2017) 317(4):371–8. doi: 10.1001/jama.2016.19975

7. Tauschmann M, Thabit H, Bally L, Allen JM, Hartnell S, Wilinska ME, et al. Closed-loop insulin delivery in suboptimally controlled type 1 diabetes: A multicentre, 12-week randomised trial. *Lancet* (2018) 392(10155):1321–9. doi: 10.1016/S0140-6736(18)31947-0

8. Smalley E. Medtronic automated insulin delivery device gets FDA nod. *Nat Biotechnol* (2016) 34(12):1220. doi: 10.1038/nbt1216-1220

9. Saunders A, Messer LH, Forlenza GP. MiniMed 670G hybrid closed loop artificial pancreas system for the treatment of type 1 diabetes mellitus: Overview of its safety and efficacy. *Expert Rev Med Devices* (2019) 16(10):845–53. doi: 10.1080/17434440.2019.1670639

10. Anderson SM, Dassau E, Raghinaru D, Lum J, Brown SA, Pinsker JE, et al. The international diabetes closed-loop study: Testing artificial pancreas component interoperability. *Diabetes Technol Ther* (2019) 21(2):73–80. doi: 10.1089/dia.2018.0308

11. Schoelwer MJ, Robic JL, Gautier T, Fabris C, Carr K, Clancy-Oliveri M, et al. Safety and efficacy of initializing the control-IQ artificial pancreas system based on total daily insulin in adolescents with type 1 diabetes. *Diabetes Technol Ther* (2020) 22(8):594–601. doi: 10.1089/dia.2019.0471

12. Toffanin C, Kozak M, Sumnik Z, Cobelli C, Petruzelkova L. In silico trials of an open-source android-based artificial pancreas: A new paradigm to test safety and efficacy of do-It-Yourself systems. *Diabetes Technol Ther* (2020) 22(2):112–20. doi: 10.1089/dia.2019.0375

13. Braune K, May A, Thurm U. Safe and successful completion of a half marathon by an adult with type 1 diabetes using a personalized open source artificial pancreas system. *J Diabetes Sci Technol* (2020) 14(6):1137–8. doi: 10.1177/1932296819884922

14. Boughton CK, Hovorka R. The artificial pancreas. *Curr Opin Organ Transplant* (2020) 25(4):336–42. doi: 10.1097/MOT.0000000000000786

15. Eren M. Adaptive control strategy for regulation of blood glucose levels in patients with type 1 diabetes. *J Process Control* (2009) 19(8):1333–46. doi: 10.1016/j.jprocont.2009.04.004

16. Sato T, Inoue A. Future reference trajectory improvement in self-tuning I-PD controller based on generalized predictive control law. *IFAC Proc Volumes* (2005) 38 (1):24–9. doi: 10.3182/20050703-6-CZ-1902.00404

17. Turksoy K, Bayrak ES, Quinn L, Littlejohn E, Cinar A. Multivariable adaptive closed-loop control of an artificial pancreas without meal and activity announcement. *Diabetes Technol Ther* (2013) 15(5):386–400. doi: 10.1089/dia.2012.0283

18. Messori M, Toffanin C, Del Favero S, De Nicolao G, Cobelli C, Magni L. Model individualization for artificial pancreas. *Comput Methods Programs BioMed* (2019) 171:133–40. doi: 10.1016/j.cmpb.2016.06.006

19. Dassau E, Bequette BW, Buckingham BA, Doyle FJ3rd. Detection of a meal using continuous glucose monitoring: implications for an artificial beta-cell. *Diabetes Care* (2008) 31(2):295–300. doi: 10.2337/dc07-1293

20. Lee H, Buckingham BA, Wilson DM, Bequette BW. A closed-loop artificial pancreas using model predictive control and a sliding meal size estimator. *J Diabetes Sci Technol* (2009) 3(5):1082–90. doi: 10.1177/193229680900300511

21. Liu W, Zhang G, Yu L, Xu B, Jin H. Improved generalized predictive control algorithm for blood glucose control of type 1 diabetes. *Artif Organs* (2019) 43(4):386–98. doi: 10.1111/aor.13350

22. Man CD, Micheletto F, Lv D, Breton M, Kovatchev B, Cobelli C. The UVA/PADOVA type 1 diabetes simulator: New features. *J Diabetes Sci Technol* (2014) 8(1):26–34. doi: 10.1177/1932296813514502

23. Cameron F, Bequette BW, Wilson DM, Buckingham BA, Lee H, Niemeyer G. A closed-loop artificial pancreas based on risk management. *J Diabetes Sci Technol* (2011) 5 (2):368–79. doi: 10.1177/193229681100500226

24. Breton M, Kovatchev B. Analysis, modeling, and simulation of the accuracy of continuous glucose sensors. *J Diabetes Sci Technol* (2008) 2(5):853–62. doi: 10.1177/193229680800200517

25. Kovatchev BP, Breton M, Man CD, Cobelli C. In silico preclinical trials: A proof of concept in closed-loop control of type 1 diabetes. *J Diabetes Sci Technol* (2009) 3(1):44–55. doi: 10.1177/193229680900300106

26. Turksoy K, Quinn L, Littlejohn E, Cinar A. Multivariable adaptive identification and control for artificial pancreas systems. *IEEE Trans BioMed Eng* (2014) 61(3):883–91. doi: 10.1109/TBME.2013.2291777

27. Battelino T, Alexander CM, Amiel SA, Arreaza-Rubin G, Beck RW, Bergenstal RM, et al. Continuous glucose monitoring and metrics for clinical trials: An international consensus statement. *Lancet Diabetes Endocrinol* (2023) 11(1):42–57. doi: 10.1016/S2213-8587(22)00319-9

28. Zisser H, Robinson L, Bevier W, Dassau E, Ellingsen C, Doyle FJ, et al. Bolus calculator: A review of four "smart" insulin pumps. *Diabetes Technol Ther* (2008) 10 (6):441–4. doi: 10.1089/dia.2007.0284

29. Magdelaine N, Rivadeneira PS, Chaillous L, Fournier-Guilloux AL, Krempf M, MohammadRidha T, et al. Hypoglycaemia-free artificial pancreas project. *IET Syst Biol* (2020) 14(1):16–23. doi: 10.1049/iet-syb.2018.5069

30. Turksoy K, Bayrak ES, Quinn L, Littlejohn E, Cinar A. (2013). Adaptive multivariable closed-loop control of blood glucose concentration in patients with type 1 diabetes, in: *American Control Conference*, Washington, DC, USA: IEE

31. Lewis DM. Do-It-Yourself artificial pancreas system and the OpenAPS movement. *Endocrinol Metab Clin North Am* (2020) 49(1):203–13. doi: 10.1016/j.ecl.2019.10.005

32. Ilham Ben A, Pierre-Yves R, Marie-Anne L, Isabelle G, Jean-Yves P. A closed-loop artificial pancreas using a proportional integral derivative with double phase lead controller based on a new nonlinear model of glucose metabolism. *J Diabetes Sci Technol* (2013) 7(3):699–707. doi: 10.1177/193229681300700315

33. Soylu S, Danisman K. In silico testing of optimized fuzzy p + d controller for artificial pancreas. *Biocybernetics Biomed Eng* (2018) 38(2). doi: 10.1016/j.bbe.2018.02.009

34. Alcantara-Aragon V, Gonzalez C, Corcoy R, Ubeda J, Chico A. Carbohydrate-to-Insulin ratio in a Mediterranean population of type 1 diabetic patients on continuous subcutaneous insulin infusion therapy. *J Diabetes Sci Technol* (2015) 9(3):588–92. doi: 10.1177/1932296814563571

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The prevalence of depression among parents of children/adolescents with type 1 diabetes: A systematic review and meta-analysis

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Background: Emerging research indicates that depression among parents of children/adolescents with type 1 diabetes mellitus (T1DM) has increased significantly. However, the prevalence rates reported by different studies vary substantially.

Methods: Seven databases were systematically searched (Pubmed, Embase, MEDLINE, Scopus, Web of Science, Cochrane Library, PsycInfo) from the inception to 15th October 2022. We pooled prevalence rates from each study with a random-effect model. We conducted a stratified meta-analysis to identify the potential sources of heterogeneity among studies. The GRADE (Grading of Recommendations, Assessment, Development and Evaluations) approach was utilized to evaluate the quality of evidence.

Results: Twenty-two studies were included, with a total of 4639 parents living with type 1 diabetic children. Overall, the pooled prevalence rate of depression or depressive symptoms was 22.4% (95%CI 17.2% to 28.7%; I^2 = 96.8%). The prevalence was higher among mothers (31.5%) than fathers (16.3%) as well as parents of children (aged < 12 years) with T1DM (32.3%) than those with adolescents (aged ≥ 12 years) (16.0%).

Conclusion: Our research suggests that more than 1 in 5 parents of type 1 diabetic children/adolescents worldwide suffer from depression or depressive symptom. Depression screening and interventions are required for parents of children with T1DM.

Systematic review registration: https://www.crd.york.ac.uk/prospero/, identifier (CRD42022368702).

KEYWORDS

parental depression, parental mental illness, type 1 diabetes mellitus (T1DM), children, adolescents, systematic review and meta-analysis

Introduction

The prevalence of T1DM is increasing globally, making it the third most prevalent chronic childhood condition (1). A recent study suggests that approximately 1.2 million children and adolescents under the age of 20 have T1DM worldwide (2). T1DM is a complex disease requiring daily insulin injections, glucose monitoring and a strict diet (3). During childhood, parents play a crucial role in managing and monitoring T1DM in their children or adolescents. Type 1 diabetes usually occurs without warning, compelling parents to make multiple life changes in a short period of time. For the parents of those children, stress and numerous life changes can be overwhelming or even worse (4, 5). Indeed, caring for a child with T1DM is a massive challenge for parents due to their children's cognitive and verbal immaturity (6). Meanwhile, Parents frequently express grave concerns and feelings of guilt regarding hypoglycaemia and other complications. All of these factors contribute to an increased risk of depression and parental stress among parents of type 1 diabetic children/adolescents (7). In a European study of parents coping with children with T1DM, it showed that a significant proportion of parents suffered from post-traumatic stress disorder (22% of fathers and 24% of mothers) (8). However, relatively few studies exist to explore the feelings and mental problems that parents ascribe to those experiences.

Evidence indicates that depression is prevalent among the parents of children with T1DM compared to the parents of healthy children (9–11). The prevalence rates reported by various countries and regions ranged from 5% to 73.5% (12–16). Several factors contribute to the wide variation in depression prevalence, including (a) different assessment tools for evaluating parental depression and depressive symptoms across studies (b) variation in social health care systems in supporting type 1 diabetes and (c) the differences in the characteristics of children, adolescents and their parents included in studies. Consequently, it was improbable that a single study would yield a relatively precise prevalence of depression.

Parental psychological problems are frequently connected with an increased risk of childhood depression, anxiety and internalised problems in the general population (17, 18). In the population of T1DM, parents with psychological problems have negatively impacted their children's mental health and worsened T1DM outcomes and diabetes management (19, 20). In the meantime, parental psychological problems may also lead to insufficient or excessive involvement in the diabetes care routine, resulting in distress and poor diabetes management in their children (21, 22). Indeed, focusing on the severity of depression in parents is essential for implementing possible early screening and appropriate intervention strategies to improve the management of T1DM in children and adolescents.

Until now, there are no reviews and meta-analysis to analyze systematically and summarise the prevalence of depression among parents of children/adolescents with T1DM. Thus, we conducted this meta-analysis to assess the prevalence of depression among parents of children/adolescents with T1DM. We also aimed to survey whether gender, type of tools for assessment, location, age of children or income level influenced the prevalence rates. Hopefully, it can raise awareness of the problem among society and clinicians, promoting a screening of patients of children with T1DM for the symptoms of depression and other mental problems.

Materials and methods

This systematic review and meta-analysis followed the protocols from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (23) and was registered in the PROSPERO database (number: CRD42022368702). The PRISMA checklist is available in the Supplementary Material (see Supplementary File 1).

Literature search

Systematically searches were conducted in the following electronic databases: Pubmed, Embase, MEDLINE, Scopus, Web of Science, Cochrane Library and PsycInfo from the inception to 15th October 2022. The keywords we used for searching were "depression," "depressive disorder," "depression symptom(s)," "parent(s)," "parental," "caregiver(s)," "mother," "father," "diabetes mellitus, type 1," "insulin-dependent diabetes mellitus," and "type 1 diabetes". We also manually searched the reference lists for the additional records (see Supplementary File 2).

Inclusion and exclusion criteria

Included studies satisfied the following criteria:

1) Participants included parents of children/adolescents with type 1 diabetes;
2) The prevalence rates of depression among parents or the data for calculating were provided;
3) The tools for assessing depression and diagnostic criteria were clarified in the articles;
4) Study population ≥ 50 participants to reduce bias in results due to small sample sizes;
5) Participants' children were younger than 18 years old;
6) Studies were published in English.

Studies were excluded if they 1) were reviews, commentaries, letters to the editor, conference papers and books; 2) included participants with cognitive impairment and inability to complete a depression assessment.

Study selection

Two investigators (ZC and JW) independently searched the literature and reviewed study titles and abstracts. Studies that met the inclusion criteria were assessed for full-text review. Two

investigators were responsible for the detailed analysis, quality assessment and data extraction of the included studies. A third independent investigator (CC) was consulted in the event of any disagreement between the investigator. Figure 1 illustrates a summary of the study selection procedure.

Data extraction

Two independent investigators (ZC, JW) extracted the following data from each study included: author, year of publication, country, depression assessment tools for parents, age of participants, age of children/adolescents, race, sample population, positive cases of depression and the corresponding prevalence rate. In addition, we analyzed corresponding data from mothers and fathers as well as from children and adolescents.

Risk of bias

We evaluated the quality of each study by using the Joanna Briggs Institute (JBI) critical appraisal tool and a nine-items questionnaire for prevalence studies (24). The score of each study was calculated according to JBI checklist by selecting "yes", "no", "not clear" and "not applicable" for each item. The total quality score ranges between 0 and 9.

Statistical analysis

We used the Comprehensive Meta-analysis Software (version 3.0) to calculate pooled prevalence rates and 95% confidence intervals (95% CI) of participants living with children/adolescents with T1DM. P values < 0.05 was considered statistically significant in all analyses. We used a random-effects model to pool the prevalence rates from each study due to variability across studies. Pooled prevalence is presented as an event rate (i.e., 0.20) in figures, whereas it is reported as prevalence (i.e., 20.0%) in tables and text. The heterogeneity of prevalence rates across studies was evaluated using the Q test, and the values of I^2 statistics, such as 25%, 50% and 75%, were regarded as the cutoffs for low, medium and high heterogeneity, respectively (25). Stratified meta-analysis (gender, tools for depression assessment, quality score, regions, age of parents, age of T1DM children) were performed to investigate the potential source of heterogeneity across studies. One-way ANOVA was used to detect variation between different subgroups. Sensitivity analysis was conducted to determine the influence of individual studies on the pooled prevalence by sequentially removing one

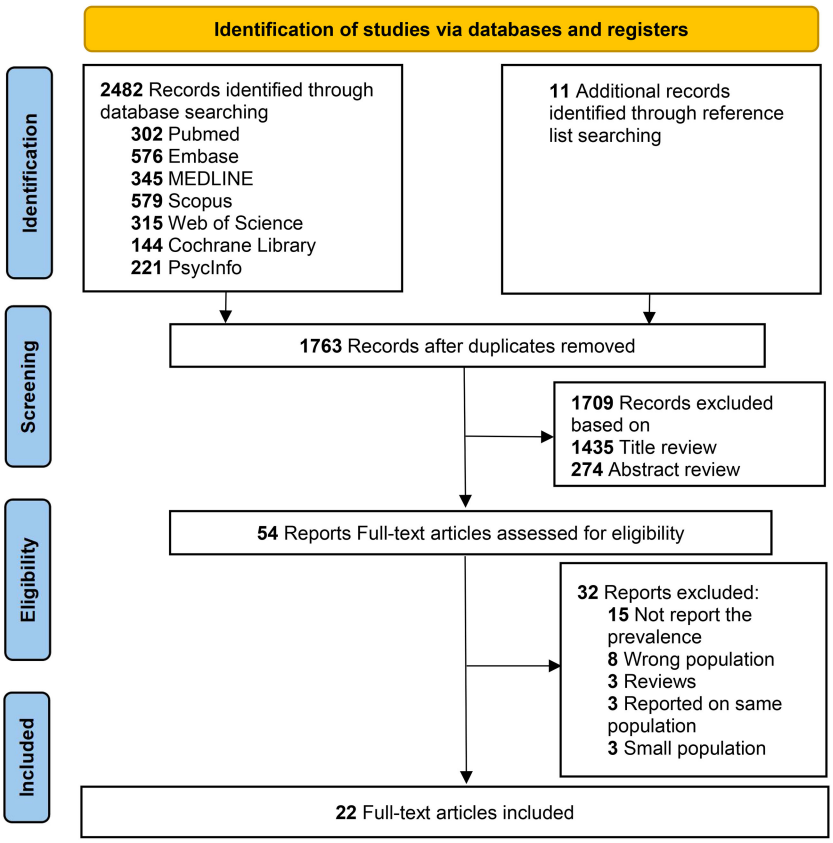

FIGURE 1
PRISMA flow diagram.

study at each time (26). Potential publication bias was examined by funnel plots and Egger's test (27).

Level of evidence

The quality of evidence for prevalence rate was categorized into the following level: high quality, moderate quality, low quality and very low quality by using GRADE approaches, which includes five factors that can degrade the quality of evidence: Risk of bias, indirectness, inconsistency, imprecision, publication bias (28). We used the GRADEpro software to perform the quality assessment.

Results

Study selection

As shown in the flow diagram from PRISMA (Figure 1), 2482 articles were identified according to our search strategy from electronic databases. 11 more records were manually acquired from the reference lists. 54 studies were relevant for a full-text review and 32 studies were excluded due to wrong population, small population, type of research, duplicate population data and lack of data on prevalence. Finally, a total of 22 non-duplicated studies met the inclusion criteria (12–15, 19, 29–44).

Characteristics of included studies

Table 1 demonstrates the characteristics of the 22 included studies. All studies included were published from 1985 to 2022, with a total of 1389 cases of depression and 4639 participants. The sample sizes below 50 were excluded and ranged from 60 to 1079 participants. 13 studies (59.1%) were from North America, 5 studies (22.7%) were from Europe, 2 studies were from Asia (9.1%) and 2 studies were from South America (9.1%). 7 studies (31.8%) reported the prevalence rates of both genders of parents. Seven instruments were used across studies for depression assessment, including the Center for Epidemiologic Studies Depression Scale (CES-D), Beck depression inventory (BDI-II), Patient health questionnaire (PHQ-9), Hospital Anxiety and Depression Scale (HADS), Brief symptom inventory 18 (BSI 18), EuroQol-5D (EQ-5D), World Health Organization-five well-being index (WHO-5).

Risk of bias

Table 2 shows the risk of bias and the quality assessment of the included studies in our meta-analysis using the JBI checklist. 17 studies (77.3%) were missing sample size estimates and adequate sample sizes and 2 studies (9.1%) did not report the source of participants. The vast majority of the included studies (18/22) described the sources and characteristics of participants in detail. All studies (100%) used standard and valid instruments for assessing depression. Overall speaking, the quality of the studies was fair (6–7) to high (8–9).

Pooled prevalence of depression among parents of children/adolescent with T1DM

The pooled prevalence of depression among parents of children/adolescents with T1DM was 0.224 (95%CI 0.172 to 0.287; I^2 = 96.8%) or 22.4% using a random-effect model (Figure 2). The quality assessment of GRADE approaches reported that the level of evidence was moderate (Figure 3).

Subgroup analysis

Stratified meta-analysis was conducted by the following categories: gender of parents (father or mother), tools for assessment (CES-D, BDI-II, PHQ-9, HADS, others), quality score (fair or high), location, age groups of parents (≥40 years or <40 years), age groups of children (≥12 years or <12 years).

As shown in Table 3, Mothers were associated with significantly higher prevalence rates of depression than fathers (31.5% *vs* 16.3%, F=7.3, *P*=0.019). We found the prevalence rates increased in studies that used other instruments (BSI 18, EQ-5Q, WHO-5) (29.6%), followed by CES-D (28.9%) and BDI-II (24.1%), although heterogeneity between groups was not statistically significant (F= 1.43, *P*=0.268).

The prevalence estimated from studies limited to South America (38.5%) and North America (27.5%) was higher than those limited to Europe (14.8%) and Asia (11.4%). However, the difference between groups of location was not statistically significant (F= 2.39, *P*=0.102).

Concerning the quality of studies, an increased prevalence was seen among studies with better quality scores (high quality 24.1% *vs* fair quality 16.7%, F= 6.02, *P*=0.023). Moreover, we found that the prevalence was higher among parents of children (aged < 12 years) than those living with adolescents (aged ≥ 12 years) (32.3% *vs* 16.0%, F= 8.92, *P*=0.007).

The prevalence were higher as the annual household income increased (29.6% *vs* 21.9%, F= 0.40, *P*=0.543) and as those parents with better education background (24.2% *vs* 13.5%, F= 4.07, *P*=0.090), which were not statistically significant.

Sensitivity analysis and publication bias

Sensitivity analysis was conducted using an omitting-one-out analysis to estimate the possible source of heterogeneity across studies included in our study. This approach suggested that the pooled prevalence rates of depression among parents remained stable and consistent. The pooled prevalence rates of depression among parents vary from 20.6% (95%CI 15.9% to 26.3) to 23.6% (95%CI 18.2% to 30.1%) (Figure 4).

There was a significant asymmetry in the funnel plot, representing the presence of publication bias by visual examination (Figure S1). However, there was no potential publication bias detected with the result of Egger's test (B = -1.97, SE = 3.10, *P* = 0.534).

TABLE 1 Selected Characteristics of 22 studies included in meta-analysis

Source	Country	Publication Year	Size, (n)	Prevalence (%), cases (n)	Fathers, No. (n) Prevalence (%)	Mothers, No. (n) Prevalence (%)	Age, Parents	Age, Children	Tool, Cutoff
Kovacs et al	US	1985	107	29.0%, 31	38, 13.2%	69, 37.7%	39.6 (6.8)	11.0 (1.5)	BDI-II 10
Horsch et al	UK	2007	60	16.7%, 10	NA	60, 16.7%	40.2 (5.9)	10.3 (4.1)	HADS 8
de Wit et al	Netherlands	2007	91	14.3%, 13	NA	NA	NA	14.9 (1.1)	CES-D 16
Jaser et al	US	2008	108	22.2%, 24	NA	108, 22.2%	39.8 (5.5)	9.9 (1.5)	CES-D 16
Streisand et al	US	2008	102	73.5%, 75	NA	NA	40.2 (7.2)	9.7 (4.0)	CES-D 16
Williams et al	US	2009	180	23.3%, 42	NA	NA	NA	14.4 (2.4)	CES-D 16
Eckshtain et al	US	2009	100	10%, 10	NA	NA	NA	14.4 (2.2)	BSI-18 65
Grey et al	US	2009	67	23.9%, 16	NA	67, 23.9%	37.2 (5.6)	4.77 (1.5)	CES-D 16
Driscoll et al	US	2010	108	33.3%, 36	18, 16.7%	90, 36.7%	37.6 (8.4)	8.1 (2.5)	CES-D 16
Hansen et al	US	2012	125	23.2%, 29	43, 18.6%	82, 25.6%	42.3 (5.7)	10.8 (1.6)	HADS 8
Malerbi et al	Brazil	2012	1079	51.2%, 547	115, 32.2%	964, 52.9%	38.6 (7.6)	11.4 (3.9)	EQ-5D NA
Moreira et al	Portugal	2013	104	10.6%, 11	NA	NA	42.0 (6.0)	12.4 (3.7)	HADS 11
Mackey et al	US	2014	225	20.9%, 47	NA	225, 20.9%	NA	12.7 (1.2)	BDI-II 14
Jaser et al	US	2014	118	18.0%, 21	NA	118, 18.0%	44.2 (5.8)	12.8 (2.0)	CES-D 16
Capistrant et al	India	2018	165	9.7%, 16	82, 6.1%	83, 13.3%	41.3 (6.5)	13.1 (3.2)	PHQ-9 10
McConville et al	US	2020	128	29.7%, 38	16, 12.5%	112, 26.8%	36.6 (6.4)	7.5 (1.30	CES-D 16
von Borries et al	Chile	2020	86	25.6%, 22	NA	86, 25.6%	43.0 (8.1)	14.0 (2.3)	BDI-II 14
Noser et al	US	2020	126	25.4%, 32	14, 14.3%	112, 26.8%	36.6 (6.4)	7.5 (1.3)	CES-D 16
Nguyen et al	Netherlands	2021	121	5%, 6	NA	NA	46.0 (4.6)	15.0 (1.9)	PHQ-9 10
Inverso et al	US	2022	157	26.8%, 42	NA	NA	34.1 (7.0)	4.5 (1.7)	CES-D 16
Stahl–Pehe et al	German	2022	1058	27.6%, 292	NA	NA	NA	14.3 (1.5)	WHO-5 50
Luo et al	China	2022	224	12.9%, 29	NA	NA	39.9 (5.0)	13.5 (2.5)	PHQ-9 10

BDI-II Beck depression inventory; HADS Hospital Anxiety and Depression Scale; CES-D Center for Epidemiologic Studies Depression Scale; BSI-18 Brief symptom inventory 18; EQ-5D EuroQol-5D; PHQ-9 Patient health questionnaire; WHO-5 World Health Organization-five well-being index; NA Not applicable.

Discussion

Main findings

This study, to our knowledge, is the first systematic review and meta-analysis on the prevalence of depression among parents of type 1 diabetic children/adolescents. Our review has assembled data from 22 studies involving 4639 subjects in ten countries from four continents. Meanwhile, we reported the prevalence of depression among parent males and females and parents caring for children and adolescents. At the same time, we found that the variability between the finding across different studies was strongly associated with three factors: the gender of parents, the quality of the studies judged by the JBI checklist and the age of the participants' children.

Prevalence of depression

Overall, the present analysis demonstrated that a surprisingly high proportion of parents caring for their children with T1DM had depression disorder or depressive symptoms (22.4%). The summary prevalence rates (31.5% in mothers *vs* 16.3% in fathers) suggested that depression affected every family member with significant variability. In addition, a comparison of the prevalence of

TABLE 2 Qualities of studies included in meta-analysis.

Study name	Response									
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Score
Kovacs et al	N	Y	N	Y	Y	Y	Y	Y	Y	7
Horsch et al	Y	U	N	Y	Y	Y	Y	Y	U	6
de Wit et al	Y	Y	N	N	Y	Y	Y	Y	Y	7
Jaser et al	Y	Y	N	Y	Y	Y	Y	N	Y	7
Streisand et al	Y	Y	U	Y	Y	Y	Y	Y	Y	8
Williams et al	Y	Y	U	N	Y	Y	Y	U	Y	6
Eckshtain et al	Y	Y	N	N	Y	Y	Y	U	Y	6
Grey et al	Y	Y	N	Y	Y	Y	Y	Y	Y	8
Driscoll et al	Y	Y	N	Y	Y	Y	Y	Y	Y	8
Hansen et al	Y	Y	N	Y	Y	Y	Y	Y	U	7
Malerbi et al	Y	Y	Y	Y	Y	Y	Y	Y	Y	9
Moreira et al	Y	Y	N	Y	Y	Y	Y	Y	U	7
Mackey et al	Y	Y	Y	Y	Y	Y	Y	Y	Y	9
Jaser et al	Y	Y	U	Y	Y	Y	Y	Y	Y	8
Capistrant et al	N	Y	U	Y	Y	Y	Y	Y	Y	7
McConville et al	Y	Y	N	Y	Y	Y	Y	Y	Y	9
von Borries et al	Y	Y	N	Y	Y	Y	Y	Y	U	7
Noser et al	Y	Y	N	Y	Y	Y	Y	Y	Y	8
Nguyen et al	Y	Y	N	Y	Y	Y	Y	Y	U	6
Inverso et al	Y	Y	Y	Y	Y	Y	Y	Y	U	8
Stahl–Pehe et al	Y	Y	Y	N	Y	Y	Y	Y	Y	8
Luo et al	Y	Y	Y	Y	Y	Y	Y	Y	Y	9

Q1-Q9: Question 1 to question 9 used to assessment the quality of studies reporting prevalence data in the JBI critical appraisal checklist.
Q1. Was the sample frame appropriate to address the target population? Q2. Were study participants sampled in an appropriate way?
Q3. Was the sample size adequate? Q4. Were the study subjects and the setting described in detail?
Q5. Was the data analysis conducted with sufficient coverage of the identified sample?
Q6. Were valid methods used for the identification of the condition?
Q7. Was the condition measured in a standard, reliable way for all participants? Q8. Was there appropriate statistical analysis?
Q9. Was the response rate adequate, and if not, was the low response rate managed appropriately?
Y, yes; N, no; U, unclear; NA, not applicable.

depression among parents with T1DM children (aged < 12 years) and parents with T1DM adolescents (aged ≥ 12 years) suggested that the incidence of depression in parents is likely to double when raising younger children (32.3% *vs* 16.0%).

Comparison, explanation and connection

Depression is a significant cause of disability worldwide and it is estimated that roughly 5% of adults are suffering from depression according to an extensive global survey in 2022 (45). The prevalence of depression among parents of children/adolescents with T1DM in our study (22.4%) is 4.48 times more prevalent than the general population among adults. There are a couple of reasonable explanations for the higher prevalence of depression among parents of children with paediatric diabetes compared to the general population. One of the potential reasons for the question would be the substantial emotional devastation in parents according to the announcement of the diagnosis of their children with diabetes. Over 1 in 3 parents reported distress at the diagnosis and the effect would last for years (20, 46). The other possible reason is the overwhelming sense of responsibility in managing children's blood glucose levels even though they had mastered executing a complex and demanding daily diabetes treatment for their children. High-stress levels associated with childhood diabetes have been described as risk factors for depression (47). Finally, children/adolescents with type 1 diabetes had great opportunities to experience social discrimination, marginalization and stigma as well as their parents when compared to the healthy population (48, 49).

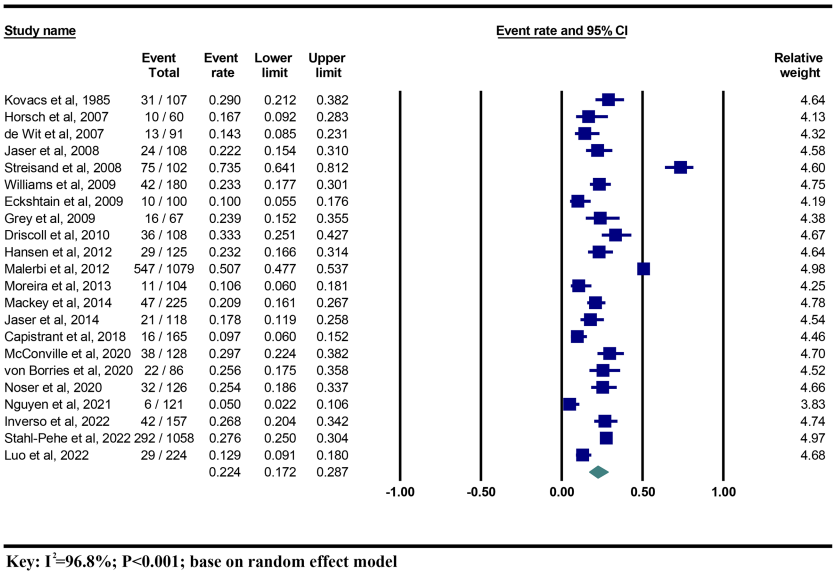

FIGURE 2
The prevalence of parental depression among children/adolescents with T1DM.

As we expected, our review showed that a higher proportion of mothers experienced depressive symptoms or depressive feelings than fathers. Indeed, this was because mothers were the prime caregivers in most families, took on a higher task of diabetes management and paid more attention to the inner pain of children with T1DM (50, 51). Furthermore, our meta-analysis also showed that the prevalence of depression increased in parents caring for younger children than those living with older adolescents of T1DM. The possible reason for the differences was higher levels of paediatric parenting stress which was related to more significant parental depressive symptoms when raising a younger child with type 1 diabetes (41). In addition, it is essential to notice that other moderators (i.e., socio-economic status, regions and age of the parents) might potentially impact the prevalence of depression among parents of children with T1DM.

In terms of practice, parental depressive symptoms and diabetes-related stress would contribute to the weakening of parental functioning, impacting the management effectiveness of diabetes. For instance, several studies reported that parental emotional problems were directly and indirectly associated with a level of HbA1c, one of the leading indicators for diabetes control (34, 52). Evidence indicated that children of parents with depression

The prevalence of depression among parents of children/adolescents with T1DM

Patient or population: parents of children/adolescents with T1DM

Settings:

Intervention: None

Outcomes	Illustrative comparative	Effect (95% CI)	Prevalence Rate (%) (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
		Prevalence Rate*				
Prevalence of depression	The prevalence rate of depression among parents was 22.4% (17.2% to 28.7%)		22.4 (17.2 to 28.7)	4639 (22 studies)	⊕⊕⊕⊖ **moderate**[1,2,3]	

***Prevalence Rate** (and its 95% confidence interval) was pooled from studies included in the meta-analysis, performed by the Comprehensive Meta-analysis Software (version 3.0) and based on a random effect model.

CI: Confidence interval;

GRADE Working Group grades of evidence

High quality: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: We are very uncertain about the estimate.

[1] High heterogeneity among studies

[2] based on consistent evidence from 22 studies

[3] Sensitivity analysis showed no confounding factors and the results were continuous and reliable

FIGURE 3
Level of evidence (GRADE).

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TABLE 3 Stratified prevalence of depression among parents of chlidren/adolescent with type 1 diabetes.

Subgroups	Study, n	Prevalence	Heterogeneity among the studies		Heterogeneity between	
		(95%CI)	I^2%	*P* value	groups (*F* and *P* value)	
Gender					7.3	0.019
Mother	7	31.5 (17.9-45.0)	95.9	<0.001		
Father	7	16.3 (7.5-25.1)	77.9	<0.001		
Tools for assessment					1.43	0.268
CES-D	10	28.9 (19.5-38.4)	93.5	<0.001		
BDI-II	3	24.1 (19.2-29.0)	25.3	0.262		
PHQ-9	3	9.1 (4.4-13.8)	72.8	0.025		
HADS	3	16.6 (8.6-24.6)	70.9	0.032		
Others	3	29.6 (9.1-50.1)	99.0	<0.001		
Quality Score					6.02	0.023
6-7	11	16.7 (11.8-21.6)	84.5	<0.001		
8-9	11	24.1 (17.4-30.8)	97.3	<0.001		
Location					2.39	0.102
North America	13	27.5 (20.0-34.9)	92.6	<0.001		
South America	2	38.5 (13.9-63.1)	96.1	<0.001		
Asia	2	11.4 (8.2-14.5)	2.1	0.312		
Europe	5	14.8 (3.9-25.8)	96.0	<0.001		
Age group of parents					0.46	0.508
≥40 years old	8	22.5 (10.0-35.0)	96.9	<0.001		
<40 years old	9	28.3 (17.0-39.5)	96.6	<0.001		
Age group of children					8.92	0.007
≥12 years old	11	16.0 (10.6-21.5)	92.3	<0.001		
<12 years old	11	32.3 (22.3-42.3)	95.5	<0.001		
Annual Family Income					0.40	0.543
> $30,000	6	29.6 (12.4-46.7)	96.7	<0.001		
< $30,000	5	21.9 (1.8-42.0)	98.9	<0.001		
Average Education Level					4.07	0.090
≤ High school	4	13.5 (8.5-18.6)	59.1	0.062		
≥ College	4	24.2 (18.0-30.3)	68.9	0.022		

were at higher risk of developing mental disorders, including depression, in childhood and adolescence (53). Thus, parental depression issues should not be treated as an isolated problem but rather an essential part of managing type 1 diabetes.

Strength and limitations

Our systematic review and meta-analysis has a couple of strength: 1. It was the first study to quantify and summarise the prevalence of depression among parents of children with type 1diabetes using scientific and statistical methods. 2. We conducted subgroup analyses of multiple factors to show differences between the prevalence of parental depression by gender, by the age of children, by region, and by assessment tool. 3. We applied the JBI critical appraisal tool to assess the risk bias of each included study and the GRADE approaches to evaluate the quality of evidence.

We have to admit there are several limitations in our meta-analysis. First, although we analyzed data from 10 countries worldwide, it will become more comprehensive to extract additional data from more countries and regions. Second, all studies included in this meta-analysis used self-report questionnaires to assess the prevalence of depression

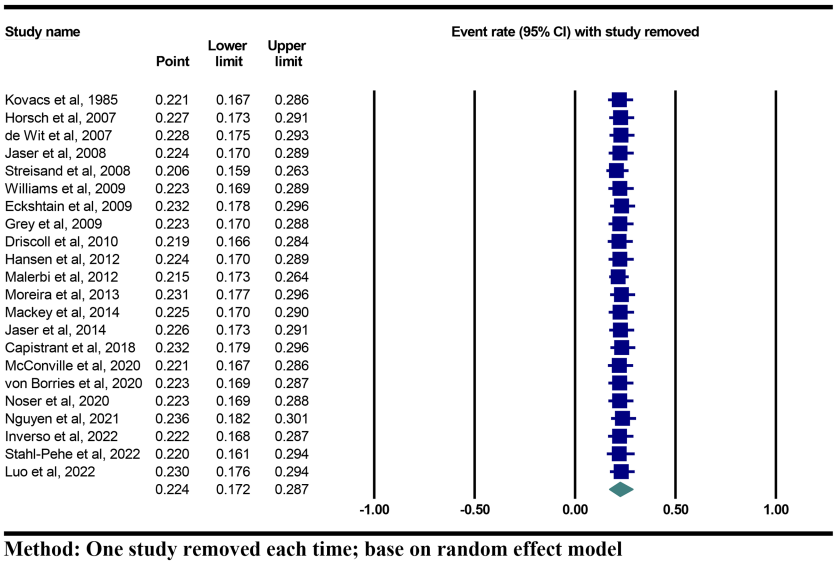

FIGURE 4
Forest plots of leave-one-out sensitivity analysis: based on random effect model.

instead of diagnostic interviews. Third, since we included studies conducted in English, potential data from studies published in other languages could not be extracted.

The impacts of the findings

Our systematic review and meta-analysis has the following clinical impacts: 1) Further studies are required to investigate the possible sources for the high prevalence of depression among parents of type 1 diabetic children compared to the general population. 2) Public health and medical resources should be coordinated for early diagnosis and intervention of depression among that kind of parents. 3) Since numerous studies have confirmed the association of parental depression with poor diabetes management in children, parental mental health issues should be considered an essential component of paediatric diabetes.

Conclusions

Our study indicated that the prevalence of depression is distinctly higher among parents of children with T1DM compared to the general population. Encouraging clinicians, schools and work units to detect, interfere and focus on the mental disorders of those parents of children with T1DM might improve the results, leading to a healthier family environment and better management of type 1 diabetes. Moreover, future research is needed to investigate the causes and mechanisms of depression in parents of children with paediatric diabetes.

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

ZC and JW contributed equally to the study. ZC and JW had the original idea for the study. ZC and JW searched databases and performed the selection of studies. ZC and JW did the statistical analyses and wrote the article's first draft. CC, DC and ZL contributed to writing and critically editing the manuscript and approved the last version. ZL was the supervisor of this review. 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.2023.1095729/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Funnel plot of publication bias for the prevalence of parental depression among children/adolescents with T1DM.

References

1. Miller GF, Coffield E, Leroy Z, Wallin R. Prevalence and costs of five chronic conditions in children. *J School Nurs* (2016) 32:357–64. doi: 10.1177/1059840516641190

2. Ogle GD, James S, Dabelea D, Pihoker C, Svennson J, Maniam J, et al. Global estimates of incidence of type 1 diabetes in children and adolescents: Results from the international diabetes federation atlas, 10th edition. *Diabetes Res Clin Pract* (2022) 183:109083. doi: 10.1016/j.diabres.2021.109083

3. Besser RE, Bell KJ, Couper JJ, Ziegler AG, Wherrett DK, Knip M, et al. ISPAD clinical practice consensus guidelines 2022: Stages of type 1 diabetes in children and adolescents. *Pediatr Diabetes* (2022) 23:1175-1187. doi: 10.1111/pedi.13410

4. Iversen AS, Graue M, Haugstvedt A, Råheim M. Being mothers and fathers of a child with type 1 diabetes aged 1 to 7 years: A phenomenological study of parents' experiences. *Int J Qual Stud Health Well-being* (2018) 13:1487758. doi: 10.1080/17482631.2018.1487758

5. Hessler D, Fisher L, Polonsky W, Johnson N. Understanding the areas and correlates of diabetes-related distress in parents of teens with type 1 diabetes. *J Pediatr Psychol* (2016) 41:750–8. doi: 10.1093/jpepsy/jsw002

6. Lowes L, Eddy D, Channon S, McNamara R, Robling M, Gregory JW. The experience of living with type 1 diabetes and attending clinic from the perception of children, adolescents and carers: Analysis of qualitative data from the depicted study. *J Pediatr Nurs* (2015) 30:54–62. doi: 10.1016/j.pedn.2014.09.006

7. Patton SR, Dolan LM, Smith LB, Thomas IH, Powers SW. Pediatric parenting stress and its relation to depressive symptoms and fear of hypoglycemia in parents of young children with type 1 diabetes mellitus. *J Clin Psychol Med Settings* (2011) 18:345–52. doi: 10.1007/s10880-011-9256-1

8. Landolt MA. Posttraumatic stress disorder in parents of children with newly diagnosed type 1 diabetes. *J Pediatr Psychol* (2002) 27:647–52. doi: 10.1093/jpepsy/27.7.647

9. Silina E. Prevalence of anxiety and depression symptoms in adolescents with type 1 diabetes (T1D) and their parents. *Nordic J Psychiatry* (2021) 75(sup 1):S26-S26. doi: 10.1080/08039488.2021.2019940

10. Moreira H, Frontini R, Bullinger M, Canavarro MC. Family cohesion and health-related quality of life of children with type 1 diabetes: The mediating role of parental adjustment. *J Child Family Stud* (2013) 23:347–59. doi: 10.1007/s10826-013-9758-6

11. Bassi G, Mancinelli E, Di Riso D, Salcuni S. Parental stress, anxiety and depression symptoms associated with self-efficacy in paediatric type 1 diabetes: A literature review. *Int J Environ Res Public Health* (2020) 18:152. doi: 10.3390/ijerph18010152

12. Nguyen LA, Pouwer F, Lodder P, Hartman E, Winterdijk P, Aanstoot H-J, et al. Depression and anxiety in adolescents with type 1 diabetes and their parents. *Pediatr Res* (2021) 91:188–96. doi: 10.1038/s41390-021-01392-y

13. Jaser SS, Linsky R, Grey M. Coping and psychological distress in mothers of adolescents with type 1 diabetes. *Maternal Child Health J* (2014) 18:101–8. doi: 10.1007/s10995-013-1239-4

14. Von Borries D, Astudillo P, Perez V, García FH, Rumié K, García BH. Association between depressive symptoms in mothers and metabolic control in adolescents with type 1 diabetes. *Rev Chil Pediatría* (2020) 91:190. doi: 10.32641/rchped.v91i2.1151

15. Malerbi FE, Negrato CA, Gomes MB. Assessment of psychosocial variables by parents of youth with type 1 diabetes mellitus. *Diabetol Metab Syndrome* (2012) 4(1):1–10. doi: 10.1186/1758-5996-4-48

16. Streisand R, Mackey ER, Elliot BM, Mednick L, Slaughter IM, Turek J, et al. Parental anxiety and depression associated with caring for a child newly diagnosed with type 1 diabetes: Opportunities for education and counseling. *Patient Educ Couns* (2008) 73:333–8. doi: 10.1016/j.pec.2008.06.014

17. Micco JA, Henin A, Mick E, Kim S, Hopkins CA, Biederman J, et al. Anxiety and depressive disorders in offspring at high risk for anxiety: A meta-analysis. *J Anxiety Disord* (2009) 23:1158–64. doi: 10.1016/j.janxdis.2009.07.021

18. Rasic D, Hajek T, Alda M, Uher R. Risk of mental illness in offspring of parents with schizophrenia, bipolar disorder, and major depressive disorder: A meta-analysis of family high-risk studies. *Schizophr Bull* (2013) 40:28–38. doi: 10.1093/schbul/sbt114

19. Capistrant B, Friedemann-Sánchez G, Pendsey S. Diabetes stigma, parent depressive symptoms and type-1 diabetes glycemic control in India. *Soc Work Health Care* (2019) 58:919–35. doi: 10.1080/00981389.2019.1679321

20. Whittemore R, Jaser S, Chao A, Jang M, Grey M. Psychological experience of parents of children with type 1 diabetes. *Diabetes Educator* (2012) 38:562–79. doi: 10.1177/0145721712445216

21. Wiebe DJ, Gelfand D, Butler JM, Korbel C, Fortenberry KT, McCabe JE, et al. Longitudinal associations of maternal depressive symptoms, maternal involvement, and diabetes management across adolescence. *J Pediatr Psychol* (2011) 36:837–46. doi: 10.1093/jpepsy/jsr002

22. Tong H, Qiu F, Fan L. Characterising common challenges faced by parental caregivers of children with type 1 diabetes mellitus in mainland China: A qualitative study. *BMJ Open* (2022) 12(1):e048763. doi: 10.1136/bmjopen-2021-048763

23. Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-p) 2015: Elaboration and explanation. *BMJ* (2015) 349:g7647. doi: 10.1136/bmj.g7647

24. Munn Z, Moola S, Lisy K, Riitano D, Tufanaru C. Chapter 5: Systematic reviews of prevalence and incidence. In: Aromataris E, Munn Z, editors. *JBI manual for evidence synthesis*. JBI (2020). Available at: https://synthesismanual.jbi.global.doi:10.46658/JBIMES-20-06. (Accessed Nov 1st 2022)

25. Higgins JP. Measuring inconsistency in meta-analyses. *BMJ* (2003) 327:557–60. doi: 10.1136/bmj.327.7414.557

26. Wallace BC, Schmid CH, Lau J, Trikalinos TA. Meta-analyst: Software for meta-analysis of binary, continuous and diagnostic data. *BMC Med Res Method* (2009) 9:1-12. doi: 10.1186/1471-2288-9-80

27. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* (1997) 315:629–34. doi: 10.1136/bmj.315.7109.629

28. Schünemann HJ, Vist GE, Jaeschke R, Glasziou P, Brozek J, Oxman AD. Grade: Grading quality of evidence and strength of recommendations for diagnostic tests and strategies. *BMJ* (2008) 336(7653):1106-1110. doi: 10.1136/bmj.a139

29. Kovacs M, Finkelstein R, Feinberg TL, Crouse-Novak M, Paulauskas S, Pollock M. Initial psychologic responses of parents to the diagnosis of insulin-dependent diabetes mellitus in their children. *Diabetes Care* (1985) 8:568–75. doi: 10.2337/diacare.8.6.568

30. Horsch A, McManus F, Kennedy P, Edge J. Anxiety, depressive, and posttraumatic stress symptoms in mothers of children with type 1 diabetes. *J Traumatic Stress* (2007) 20:881–91. doi: 10.1002/jts.20247

31. de Wit M, Delemarre-van de Waal HA, Bokma JA, Haasnoot K, Houdijk MC, Gemke RJ, et al. Self-report and parent-report of physical and psychosocial well-being in Dutch adolescents with type 1 diabetes in relation to glycemic control. *Health Qual Life Outcomes* (2007) 5:1-8. doi: 10.1186/1477-7525-5-10

32. Jaser SS, Whittemore R, Ambrosino JM, Lindemann E, Grey M. Mediators of depressive symptoms in children with type 1 diabetes and their mothers. *J Pediatr Psychol* (2007) 33:509–19. doi: 10.1093/jpepsy/jsm104

33. Williams LB, Laffel LM, Hood KK. Diabetes-specific family conflict and psychological distress in paediatric type 1 diabetes. *Diabetic Med* (2009) 26:908–14. doi: 10.1111/j.1464-5491.2009.02794.x

34. Eckshtain D, Ellis DA, Kolmodin K, Naar-King S. The effects of parental depression and parenting practices on depressive symptoms and metabolic control in urban youth with insulin dependent diabetes. *J Pediatr Psychol* (2009) 35:426–35. doi: 10.1093/jpepsy/jsp068

35. Grey M. Coping and psychosocial adjustment in mothers of young children with type 1 diabetes. *Children's Health Care* (2009) 38:91–106. doi: 10.1080/02739610902813229

36. Driscoll KA, Johnson SB, Barker D, Quittner AL, Deeb LC, Geller DE, et al. Risk factors associated with depressive symptoms in caregivers of children with type 1 diabetes or cystic fibrosis. *J Pediatr Psychol* (2010) 35:814–22. doi: 10.1093/jpepsy/jsp138

37. Hansen JA, Weissbrod C, Schwartz DD, Taylor WP. Paternal involvement in pediatric type 1 diabetes: Fathers' and mothers' psychological functioning and disease management. *Families Systems Health* (2012) 30:47–59. doi: 10.1037/a0027519

38. Moreira H, Frontini R, Bullinger M, Canavarro MC. Caring for a child with type 1 diabetes: Links between family cohesion, perceived impact, and parental adjustment. *J Family Psychol* (2013) 27:731–42. doi: 10.1037/a0034198

39. Mackey ER, Struemph K, Powell PW, Chen R, Streisand R, Holmes CS. Maternal depressive symptoms and disease care status in youth with type 1 diabetes. *Health Psychol* (2014) 33:783–91. doi: 10.1037/hea0000066

40. McConville A, Noser AE, Nelson EL, Clements MA, Majidi S, Patton SR. Depression as a predictor of hypoglycemia worry in parents of youth with recent-onset type 1 diabetes. *Pediatr Diabetes* (2020) 21:909–16. doi: 10.1111/pedi.13039

41. Noser AE, Dai H, Marker AM, Raymond JK, Majidi S, Clements MA, et al. Parental depression and diabetes-specific distress after the onset of type 1 diabetes in children. *Health Psychol* (2019) 38:103–12. doi: 10.1037/hea0000699

42. Inverso H, Moore HR, Rooney KA, Tully CB, Monaghan M, Hilliard ME, et al. Clinically elevated parent depressive symptoms and stress at child type 1 diabetes diagnosis: Associations with parent diabetes self-efficacy at 18-months post-diagnosis. *Pediatr Diabetes* (2022) 23:809–14. doi: 10.1111/pedi.13365

43. Stahl-Pehe A, Selinski S, Bächle C, Castillo K, Lange K, Holl RW, et al. Overestimation and underestimation of youths' health-related quality of life are associated with youth and caregiver positive screens for depression: Results of a population-based study among youths with longstanding type 1 diabetes. *Diabetol Metab Syndrome* (2022) 14(1):1-14. doi: 10.1186/s13098-022-00809-w

44. Luo D, Wang Y, Cai X, Li R, Li M, Liu H, et al. Resilience among parents of adolescents with type 1 diabetes: Associated with fewer parental depressive symptoms and better pediatric glycemic control. *Front Psychiatry* (2022) 13:834398. doi: 10.3389/fpsyt.2022.834398

45. Herrman H, Patel V, Kieling C, Berk M, Buchweitz C, Cuijpers P, et al. Time for united action on depression: A lancet–world psychiatric association commission. *Lancet* (2022) 399:957–1022. doi: 10.1016/s0140-6736(21)02141-3

46. Lowes L, Gregory JW, Lyne P. Newly diagnosed childhood diabetes: A psychosocial transition for parents? *J Advanced Nurs* (2005) 50:253–61. doi: 10.1111/j.1365-2648.2005.03388.x

47. Streisand R, Mackey ER, Herge W. Associations of parent coping, stress, and well-being in mothers of children with diabetes: Examination of data from a national sample. *Maternal Child Health J* (2009) 14:612–7. doi: 10.1007/s10995-009-0497-7

48. Delamater AM, de Wit M, McDarby V, Malik JA, Hilliard ME, Northam E, et al. Ispad clinical practice consensus guidelines 2018: Psychological care of children and adolescents with type 1 diabetes. *Pediatr Diabetes* (2018) 19:237–49. doi: 10.1111/pedi.12736

49. Liu NF, Brown AS, Folias AE, Younge MF, Guzman SJ, Close KL, et al. Stigma in people with type 1 or type 2 diabetes. *Clin Diabetes* (2017) 35:27–34. doi: 10.2337/cd16-0020

50. Di Riso D, Bassi G, Mancinelli E, Zaffani S, Salcuni S, Maffeis C. Mothers and fathers parenting stress and their perception of children's psychosocial functioning in paediatric diabetes: A pilot study. *Int J Environ Res Public Health* (2020) 17:4734. doi: 10.3390/ijerph17134734

51. Van Gampelaere C, Luyckx K, Straaten S, Laridaen J, Goethals ER, Casteels K, et al. Families with pediatric type 1 diabetes: A comparison with the general population on child well-being, parental distress, and parenting behavior. *Pediatr Diabetes* (2019) 21:395–408. doi: 10.1111/pedi.12942

52. Rumburg TM, Lord JH, Savin KL, Jaser SS. Maternal diabetes distress is linked to maternal depressive symptoms and adolescents' glycemic control. *Pediatr Diabetes* (2015) 18:67–70. doi: 10.1111/pedi.12350

53. Goodman SH, Rouse MH, Connell AM, Broth MR, Hall CM, Heyward D. Maternal depression and child psychopathology: A meta-analytic review. *Clin Child Family Psychol Rev* (2011) 14:1–27. doi: 10.1007/s10567-010-0080-1

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Fecal metabolomics combined with 16S rRNA gene sequencing to analyze the effect of Jiaotai pill intervention in type 2 diabetes mellitus rats

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The occurrence and development of type 2 diabetes mellitus (T2DM) are closely related to gut microbiota. Jiaotai pill (JTP) is used to treat type 2 diabetes mellitus, with definite efficacy in clinical practice. However, it is not clear whether the therapeutic effect is produced by regulating the changes in gut microbiota and its metabolism. In this study, T2DM rat models were established by a high-fat diet and low-dose streptozotocin (STZ). Based on the pharmacodynamic evaluation, the mechanism of JTP in the treatment of type 2 diabetes mellitus was investigated by fecal metabolism and 16S rRNA gene sequencing. The results showed that JTP decreased blood glucose (FBG, HbA1c) and blood lipid (TC, TG, and LDL) levels and alleviated insulin resistance (FINS, IL-10) in T2DM rats. 16S rRNA gene sequencing results revealed that JTP increased microbiota diversity and reversed the disorder of gut microbiota in T2DM rats, and therefore achieved the therapeutic effect in T2DM. JTP regulated 13 differential flora, which were Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Eubacteriaceae, Prevotellaceae, Ruminococcaceae, Clostridium_IV, Clostridium_XlVa, *Eubacterium*, *Fusicatenibacter*, *Romboutsia*, and *Roseburia*. Metabolomics analysis showed that JTP interfered with 13 biomarkers to play a therapeutic role in type 2 diabetes mellitus. They were L-Valine, Choline, L-Aspartic acid, Serotonin, L-Lysine, L-Histidine, 3-Hydroxybutyric acid, Pyruvic acid, N-Acetylornithine, Arachidonic acid, L-Tryptophan, L-Alanine, and L-Methionine. KEGG metabolic pathway analysis of the above differential metabolites and gut microbiota by using the MetaboAnalyst database and Picrust software. It was found that JTP treated type 2 diabetes mellitus by affecting metabolic pathways such as amino acid metabolism, carbohydrate metabolism, and lipid metabolism. Spearman correlation analysis revealed high correlations for 7 pharmacological indicators, 12 biomarkers, and 11 gut microbiota. In this study, the therapeutic effect and potential mechanism

of JTP on type 2 diabetes mellitus were preliminarily demonstrated by gut microbiota and metabolomics, which could provide a theoretical basis for the treatment of T2DM with JTP.

KEYWORDS

Jiaotai pill, type 2 diabetes mellitus, 16S rRNA gene sequencing, UPLC-Q-exactive focus MS, metabolomics

1. Introduction

Type 2 diabetes mellitus is the most common metabolic disease (1). With the development of the social economy, the change in people's lifestyles (increased energy intake and reduced exercise, etc.), and the aging of the population, the incidence morbidity of type 2 diabetes mellitus is increasing year by year worldwide and has become one of the biggest killers of human health (2). Glucose and lipid metabolism disorders are the main features of type 2 diabetes mellitus, and glucose and lipid metabolism disorders are closely related to the composition of the gut microbiota (3). Studies have confirmed that glucose and lipid metabolism disorders can lead to gut microbiota imbalance, while gut microbiota imbalance aggravates glucose and lipid metabolism disorders (4). The structure of gut microbiota in type 2 diabetes mellitus is significantly different compared to healthy subjects. Gut microbiota imbalance and activation of toll-like receptor 4/stress-activated protein kinase leads to insulin resistance (5). These observations highlight that gut microbiota may be a novel diagnostic and therapeutic target for the treatment of type 2 diabetes mellitus.

Traditional Chinese medicine (TCM) has accumulated a wealth of experience in the treatment of diabetes in thousands of years of clinical practice, and TCM can reduce blood glucose through multiple pathways and multiple targets, with obvious advantages in the treatment of diabetes and its complications. Increasing evidence suggests a close association between TCM and gut microbes, which interact with each other. TCM intervenes in intestinal microecology by regulating the number and proportion of gut microbiota, inflammatory factors, signaling pathways, genes, etc., which has the effects of regulating gut microbiota, protecting the intestinal mucosal barrier, restoring intestinal microbial diversity, and enhancing immune function. Intestinal microecology also affects the metabolism and absorption of TCM in the body, which can enhance or reduce the efficacy and change the toxicity of herbal medicines. Jiaotai Pill was first recorded in "Han's Medical Circular," and consists of Coptis chinensis, and Cinnamon. It is clinically effective in the treatment of type 2 diabetes mellitus. Modern research has found that Coptis chinensis significantly inhibits the growth of pathogenic bacteria and promotes the growth of beneficial bacteria (6, 7). Meanwhile, gut microbiota converts berberine and safranine in Coptis chinensis into hydrogenated products and the palmatine in Coptis chinensis into demethoxylated products, making it easier to be absorbed and to exert therapeutic effects (8, 9). The cinnamic acid and cinnamic aldehyde in Cinnamon can regulate the imbalance of gut microbiota (10, 11). Therefore, it is speculated that JTP can play a role in the treatment of diabetes by regulating changes in gut microbiota.

The gut microbiota is both a player and a regulator of metabolic processes (12). The metabolism of the host is not only regulated by its genome, but also by symbiotic bacteria. Metabolomics can efficiently screen biomarkers and deeply analyze the molecular mechanisms of host health or disease. Combined metabolomics with microbiomics, a scientific language explaining the effectiveness of TCM has been established, and it has a good guiding role in the diagnosis and treatment of clinical diseases (13, 14).

In this study, based on the pharmacodynamic evaluation of the efficacy of JTP in the treatment of type 2 diabetes mellitus, we performed untargeted metabolomics and 16S rRNA gene sequencing studies on stool samples to investigate the changes in endogenous metabolites and intestinal bacteria during the treatment of type 2 diabetes mellitus with JTP. Then, the relationship between host phenotype, intestinal microbiota, and metabolites was analyzed by calculating Spearman correlation coefficients. To investigate the mechanism of JTP for the treatment of type 2 diabetes mellitus from the perspective of multi-level integration of biomarkers and gut microbiota.

2. Materials and methods

2.1. Preparation of drugs

Coptis chinensis and Cinnamon were purchased from the Harbin Branch of Beijing Tong Ren Tang Pharmacy and identified as the dried rhizome of *Coptis chinensis* Franch of Buttercup family and the dried bark of *Cinnamomi cortex* Presl of Camphoraceous family by the Teaching and Research Department of Chinese Medicine Identification of Heilongjiang University of Chinese Medicine, respectively.

According to the ratio of Coptis chinensis: Cinnamon (10:1), weigh 500 g of Coptis chinensis and 50 g of Cinnamon, decoct and extract twice, add 10 times the amount of water each time, decoct for 40 min. The two decoctions were combined, concentrated and freeze-dried. A total of 88 g of lyophilized powder was obtained, the powder yield was 16%. Metformin hydrochloride was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (batch number: 201812161).

2.2. Animals and experimental design

In this study, 32 men SD rats, weighing 150 ± 20 g, 7–8 weeks old, provided by Liaoning Changsheng Biotechnology Co., Ltd.

were used, license number: SCXK (Liao) 2020–0001. The rats were housed in an SPF class animal room with a room temperature of 25 ± 1°C, relative humidity of 50 ± 5%, and a light cycle of 12 h light/night. Rats were acclimatized for a week with a standard rodent diet, and water was available *ad libitum*. The level of fasting blood glucose (FBG) and body mass were measured. Rats with normal blood glucose values were screened and randomly divided into four groups, which were the control group (Con), model group (Mod), metformin group (MET), and Jiaotai Pill group (JTP). The model and treatment groups were fed a high-fat diet for 4 weeks with two consecutive injections of 25 mg/kg of STZ (Sigma Corporation, lot number NO. 18888664). The control group was fed a conventional maintenance diet for 4 weeks and injected with an equal volume of citrate buffer intraperitoneally. After 1 week, blood glucose was stabilized, Jiaotai Pill (6.8 g of raw drug/kg) was gavaged in the JTP group, metformin (0.2 g/kg) was gavaged once daily for 10 weeks in the metformin group, and an equal volume of distilled water was gavaged in the remaining groups. All experiments were performed following the Declaration of Helsinki and were approved by the Animal Health and Ethics Committee of Heilongjiang University of Chinese Medicine (2021012709).

2.3. Determination of FBG, TC, TG, LDL, HbA1c, FINS, and IL-10 in serum

After the rats fasted for 12 h but water *ad libitum*, blood was taken by the tail break method, and the level of fasting blood glucose (FBG) was measured.

Blood was taken from the abdominal aorta of the rats and centrifuged at 3,000 rpm for 10 min at 4°C to collect serum. Total cholesterol (TC), Triglyceride (TG), and Low-density lipoprotein (LDL) in rat serum were measured by an automated biochemical instrument. The enzyme-linked immunoassay was used to detect glycosylated hemoglobin (HbA1c), fasting serum insulin level (FINS), and interleukin 10 (IL-10) in rats.

2.4. Histopathology analysis of rat pancreas

After blood collection, the rat pancreas was carefully separated and the pancreatic tissues were immersed in 10% neutral-buffered formalin to prepare pathological sections.

2.5. Fecal DNA extraction and high-throughput 16S rRNA sequencing

The feces of rats were collected by stimulated defecation and placed into sterilized 2 ml EP tubes. The collected feces samples were uniformly sent to Wuhan UW Medical Laboratory Co., Ltd. for testing, and the Illumina Miseq platform (Illumina Inc., San Diego, CA, USA) was used for standard bioinformatic analysis of the bacterial 16S rRNA V3-V4 region. The main procedure is as follows: take 30 ng of qualified genomic DNA samples and corresponding fusion primers to configure the PCR reaction system, set the PCR reaction parameters for PCR amplification, use Agencourt AMPure XP magnetic beads to purify the PCR amplification products, dissolve them in Elution Buffer, label them, and complete the library construction. The libraries were tested for fragment range and concentration using an Agilent 2100 Bioanalyzer. The libraries that passed the assay were selected for sequencing on the HiSeq platform (Illumina) according to the insert size.

2.6. Fecal metabolomics

The collected fecal samples were weighed about 100 mg, placed in 2 ml centrifuge tubes, and thawed on ice. Added 500 μL of ultrapure water, extracted by ultrasonication at 4°C for 5 min, vortex shaking for 30 s, centrifuged at 10,000 rpm for 15 min at 4°C. A total of 300 μL of supernatant was placed in 1.5 ml centrifuge tubes as the first step of extraction. Discard the remaining supernatant in the fecal sediment, add 500 μL of methanol, ultrasonic extract at 4°C for 5 min, vortex shake for 30 s, and centrifuge at 10,000 rpm for 15 min at 4°C. A total of 300 μL of supernatant was taken as the second extraction solution, and the two extracts were combined. After vortex shaking for 30 s and filtration using a 0.22 μm filter membrane, the samples were transferred to the injection vial and all samples were analyzed in positive and negative ion modes. A total of 10 mg of each sample to be tested was mixed and then processed as QC samples according to the pre-treatment method described above.

Chromatographic separation was performed on a Thermo Scientific^{TM} Ultra Performance Liquid Chromatograph (Thermo Fisher Scientific, Waltham, MA, USA), We used an Acquity HSS T3 (1.8 μm, 100 mm × 2.1 mm; Waters, USA) at 40°C, The mobile phase was optimized, and 0.1% formic acid in water and 0.1% formic acid in acetonitrile was selected as mobile phases A and B, respectively. The injection volume was 1 μL in both positive and negative ion modes. The mobile phases were used at a flow rate of 0.4 ml/min with a gradient of 0–3 min at 1.0–10.0% B, 3–5 min at 10.0–20.0% B, 5–8.5 min at 20.0–40.0% B, 8.5–13.5 min at 40.0–99.0% B, 13.5–14 min at 99.0% B, 14–15 min at 99.0–1.0% B.

Mass spectrometry (MS) data were acquired by using Q-Exactive focus mass spectrometer (Thermo Fisher Inc., Waltham, MA, USA) in both positive and negative ion modes. The optimized conditions were as follows: spray voltage was set to 3,500/3,200 V (±), capillary ion transport temperature was set to 350°C, sheath gas flow rate was 45 arb, the auxiliary gas flow rate was 15 arb, capillary (ion transport) temperature was set to 320°C, S-lens voltage was set to 75. The full scan range was set to 60–900 m/z, the resolution was set to 70,000 FWHM on MS^1 and 17,500 FWHM on MS^2, the AGC target was set to $1E^6$ on MS^1 and $2E^5$ on MS^2, maximum allowed ion injection time was set to 100 ms on MS^1 and 50 ms on MS^2, Top three ddms2 for identification, isolation window was set to 1.5 m/z, secondary mass spectrometry collision energy was set to 20, 40, and 60; vertex excitation was 4–8 s, dynamic exclusion was 8 s, acquisition data type was profile mode. The multivariate statistical analysis was used to qualify the potential biomarkers in both positive and negative ion modes.

2.7. Statistical analysis

2.7.1. Gut microbiota analysis

The spliced Tags were clustered into Operational taxonomic unit (OTUs) using the software USEARCH (v7.0.1090_i86linux32). Sequence Variants (ASVs), ASVs are sequences that are 100% similar. In turn, the feature table (Feature, a collective term for ASVs/ASVs, etc.) is obtained. The RDP classifier Bayesian algorithm was used to analyze the OTU representative sequences taxonomically, and the cluster composition of each sample was counted. The function of the microbiota was predicted using Picrust software.

2.7.2. Metabolomics analysis

Raw data were analyzed using Compound Discoverer 3.2 (CD, Waltham, MA, USA) for matching and identification of differential biomarkers. Between-group difference analysis was performed using SIMCA-P (version 14.1, Umetrics, Umea, Sweden). Metabolic Pathway Analysis was performed by using MetaboAnalyst 5.0.[1]

Spearman correlation was used to analyze the association of differential biomarkers with differential gut microbiota.

Data in a normal distribution were expressed as (mean ± SD). Student's *t*-test or Mann-Whitney *U*-test was performed to test the differences between the two groups. Statistical analyses were performed using SPSS Statistics (version.20.0; SPSS Inc., Chicago, IL, USA), and $p < 0.05$ were considered significant.

3. Results

3.1. Rat FBG results

During the model establishment period, the fasting blood glucose (FBG) of rats showed an increasing trend in the high-fat diet stage. Compared with the control group, the FBG of the T2DM model group was significantly increased after the first injection of STZ ($p < 0.01$), and the FBG was significantly higher than 11.1 mmol/L after the second injection of STZ. After 7 days, the FBG of T2DM rats tended to be stable and reached the evaluation criteria of the type 2 diabetes mellitus model (**Figure 1A**).

After treatment, compared with the control group, the FBG level in the model group was significantly higher ($p < 0.01$). FBG in the metformin group (MET) and Jiaotai pill (JTP) groups was significantly lower than that in the model group ($p < 0.01$) (**Figure 1B**).

3.2. Serum levels of HbA1c, FINS, TC, TG, LDL, and IL-10

Compared with the control group, the serum levels of HbA1c, FINS, TG, and LDL were highly significantly increased ($p < 0.01$), TC levels were significantly increased ($p < 0.05$), and IL-10 levels were highly significantly decreased ($p < 0.01$) in the T2DM model rats.

Compared with the model group, the serum levels of FINS, TG, and LDL in the JTP group rats were highly significantly lower ($p < 0.01$), the level of TC had a decreasing trend, the level of HbA1c was significantly lower ($p < 0.05$), and the level of IL-10 was highly significant higher ($p < 0.01$), the levels of HbA1c, FINS, TC, TG, and LDL in the MET group were highly significantly lower ($p < 0.01$), and IL-10 levels were highly significantly increased ($p < 0.01$) (**Figures 1C–H**).

3.3. Histopathology analysis of rat pancreas

In the control group, the islets had clear boundaries, regular morphology, neatly arranged cells, and uniform size, while in the type 2 diabetes mellitus (T2DM) model group, the islets had fewer cells, disorganized cell bodies, irregular morphology, most nuclei of unequal size, and vacuolar degeneration. Compared with the model group, the islets in metformin group (MET) and Jiaotai pill (JTP) groups were observed to have a regular shape, more β-cells, neat edges, and clear boundaries (**Figure 1I**).

3.4. High-throughput sequencing of 16S rRNA

In this experiment, based on the 97% similarity OTU obtained, the trends of the species accumulation curve (**Figure 2A**) and species dilution curve (**Figure 2B**) showed that the sequencing results had high abundance and uniform species distribution, which were sufficient to reflect the microbial information in all samples, and the sequencing data were reasonable for data analysis. As shown in the figure, sobs (**Figure 2E**), Chao (**Figure 2F**), Shannon (**Figure 2G**), and ace (**Figure 2H**) index analysis showed that the microbial community richness and microbial community diversity decreased in the model group compared with the control group, and the microbial community richness showed an increasing trend after JTP treatment. In addition, we investigated the similarity of the overall microbial community structure by β-diversity. The two axes of variation (PCo1 and PCo2) accounted for 25.66 and 19.21% of the total variance of PCoA, respectively. And the PCoA plots exhibited clear separation and obvious spatial clustering (**Figure 2C**). In addition, similar observations were obtained in the Non-metric multidimensional scaling (NMDS) analysis. The JTP intervention shifted the microbial community structure from the model group to the control group (**Figure 2D**).

At the phylum level (**Figure 2I**), the relative abundance of Actinobacteria, Proteobacteria, and Bacteroidetes was increased and the relative abundance of Firmicutes was decreased in the T2DM model group compared to the control group. The relative abundance of T2DM-induced disruptions in bacterial phylum levels was reversed after JTP treatment. At the genus level (**Figure 2J**), the relative abundances of Desulfovibrio, Romboutsia, Fusicatenibacter, and Lactobacillus were higher, and the relative abundances of Roseburia, Clostridium_IV, Eubacterium, Clostridium_XlVa were lower in the T2DM model

1 https://www.metaboanalyst.ca/

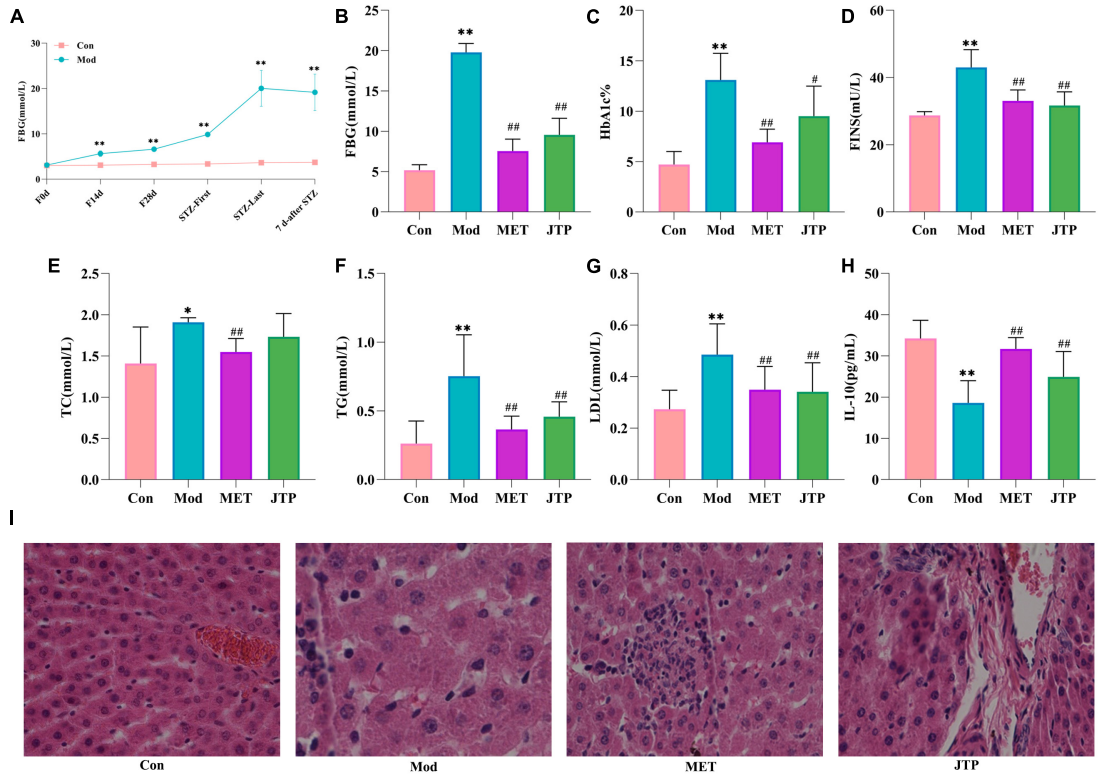

FIGURE 1
(A) Trends in fasting blood glucose (FBG) values in the control and model groups during the establishment of the type 2 diabetes mellitus (T2DM) model. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the control group ($n = 8$). **(B–H)** Changes in FBG, HbA1c, FINS, TC, TG, LDL, and IL-10 in the control, model, MET, and JTP groups ($n = 8$). $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the model group. **(I)** HE staining results of pancreatic tissue in the control, model, MET, and JTP groups (200×).

group compared to the control group. JTP significantly reduced the relative abundance of Romboutsia and Fusicatenibacter and significantly enriched Roseburia, Clostridium_IV, Eubacterium, and Clostridium_XlVa. At the family level (Figure 2K), the relative abundance of Desulfovibrionaceae, Prevotellaceae, and Lachnospiraceae was elevated in the T2DM model group and the relative abundance of Eubacteriaceae and Ruminococcaceae was reduced compared to the control group. JTP significantly reduced the relative abundance of Prevotellaceae and had a significant enrichment effect on Ruminococcaceae and Eubacteriaceae.

We performed PICRUST analysis to predict the function of gut microbiota based on the KEGG database. The relative abundance of 30 pathways was predicted. The top ten pathways were Carbohydrate metabolism, Amino acid metabolism, Metabolism of cofactors and vitamins, Metabolism of terpenoids and polyketides, Replication and repair, Metabolism of other amino acids, Lipid metabolism, Energy metabolism, Cell motility, and Glycan biosynthesis and metabolism. And among them, the expression of Cell motility in the model group was lower than that of the control group. A callback trend was observed after the intervention with JTP. The expression of Carbohydrate metabolism, Amino acid metabolism, Metabolism of cofactors and vitamins, Metabolism of terpenoids and polyketides, Replication and repair, Metabolism of other amino acids, Lipid metabolism, Energy metabolism, Cell motility, and Glycan biosynthesis and metabolism were enriched in the model group. After JTP intervention reversed the abundance of Metabolism of cofactors and vitamins, Replication and repair, Metabolism of other amino acids, Energy metabolism, Glycan biosynthesis and metabolism (Figure 2L).

3.5. Fecal metabolomics

Total Ion Chromatography (TIC) of quality control (QC) samples were obtained in both positive and negative ion modes, consistently showing good peak shape and relatively uniform distribution, thus verifying that the UPLC-Q-Exactive focus was stable throughout the detection process (Supplementary Figure 1).

The data of the fecal metabolic profile of rats at key time points (0, 14, 28, and 35 days) in the T2DM model replication cycle were analyzed, and a certain grouping of model and control rats appeared from day 14 of modeling, but there was an intersection and a significant difference between day 35 of modeling and day 0. This indicates that endogenous substances were changed during the transformation of healthy rats into T2DM rats (Figures 3A, D). Analyzing the metabolic profiles of each group after treatment, the JTP and MET groups were closer to the control group. QC proved that the method has good stability and repeatability, and the obtained data are reliable (Figures 3B, E).

To better reveal the differences between the control and T2DM model group, Orthogonal partial least squares discrimination analysis (OPLS-DA) analysis [ESI^{+}: R^2Y-0.881, Q^2-0.779; ESI^{-}:

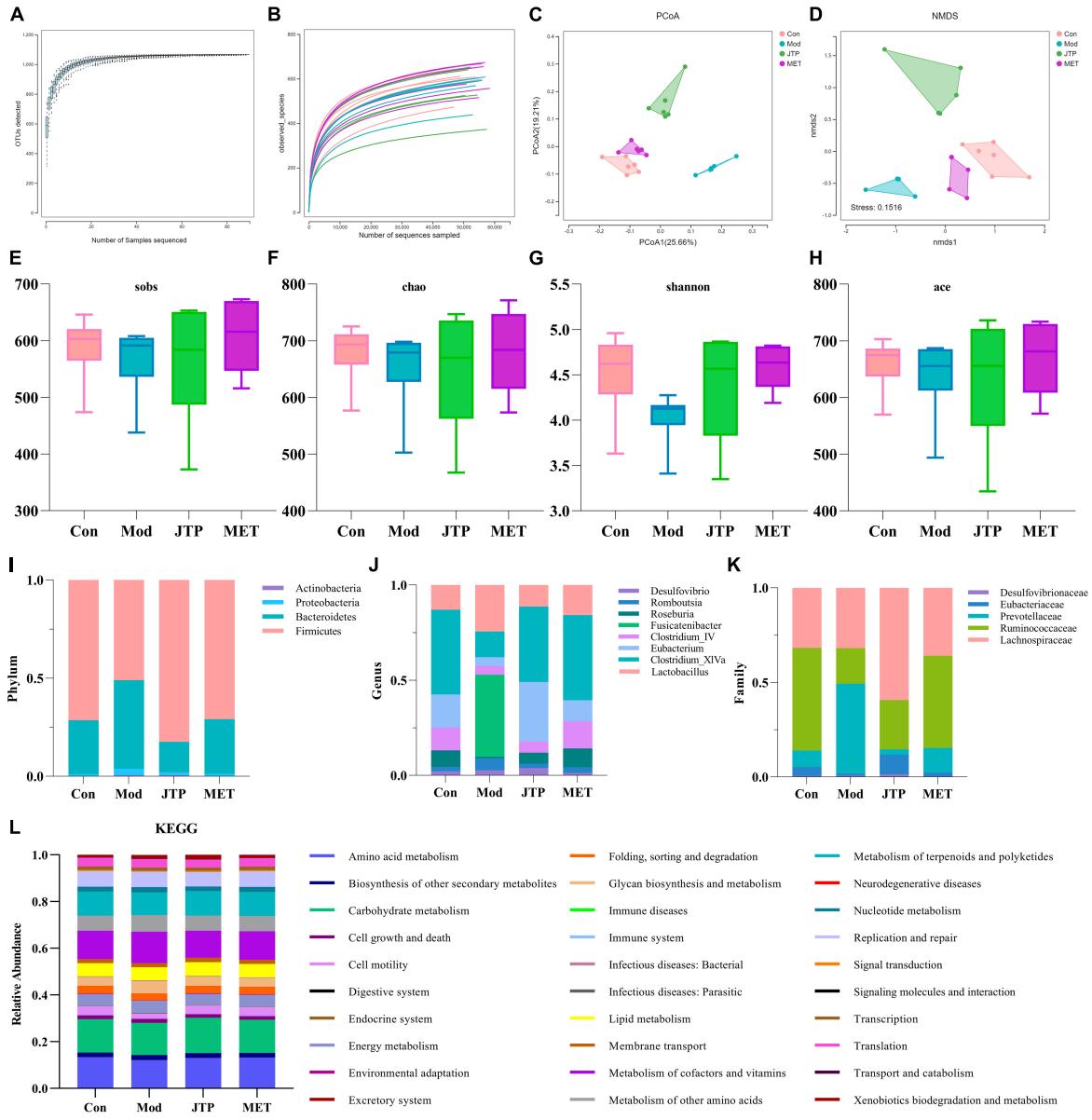

FIGURE 2

(A) The species accumulation curves. **(B)** Species dilution curve. **(C)** PCoA analysis of each group $n = 6$. **(D)** NMDS analysis of each group $n = 6$. **(E)** Sobs indexes $n = 6$. **(F)** Chao indexes $n = 6$. **(G)** Shannon indexes $n = 6$. **(H)** Ace indexes $n = 6$. **(I)** Differences in the abundance of bacteria in each group at the phylum level. Abscissa is sample grouping and ordinate is the relative abundance of annotated species. **(J)** Differences in the abundance of bacteria in each group at the family level. Abscissa is sample grouping and ordinate is the relative abundance of annotated species. **(K)** Differences in the abundance of bacteria in each group at the genus level. Abscissa is sample grouping and ordinate is the relative abundance of annotated species. **(L)** PICRUST analysis to predict the function of gut microbiota based on the KEGG database. PCoA, principal coordinates analysis; NMDS, non-metric multidimensional scaling.

R^2Y-1.000, Q^2-0.981 (Supplementary Figure 2)] was used to show that both the T2DM model group and the control group clustered significantly and there was a clear separation between the groups (Figures 3C, F). The data of control and T2DM model groups were further analyzed to obtain S-splot loading plots (Figures 3G, J), Variable important in projection plots (Figures 3H, K), and volcano plots (Figures 3I, L), and finally, the ions with VIP > 1, $p < 0.05$, and FC > 1.2 were selected as Candidate biomarkers. A total of 23 potential biomarkers in T2DM rats were finally identified (Figure 4A and Supplementary Tables 1, 2), 11 in positive ion mode, namely: L-Phenylalanine, L-Valine, Choline, L-Aspartic acid, Serotonin, L-Histidinol, Ureidopropionic acid, D-Serine, D-Tryptophan, L-Glutamine, and L-Lysine. A total of 12 in the negative ion mode, namely: L-Histidine, L-Threonine, L-Glutamic acid, 3-Hydroxybutyric acid, Pyruvic acid, Citric acid, N-Acetylornithine, Fumaric acid, Arachidonic acid, L-Tryptophan, L-Alanine, and L-Methionine. It involved 34 metabolic pathways associated with type 2 diabetes mellitus (Figure 4B and Supplementary Table 3), including 13 with impact >0.1000, which were: Alanine, aspartate and glutamate metabolism, D-Glutamine and D-glutamate metabolism, Phenylalanine, tyrosine and tryptophan biosynthesis, Phenylalanine metabolism,

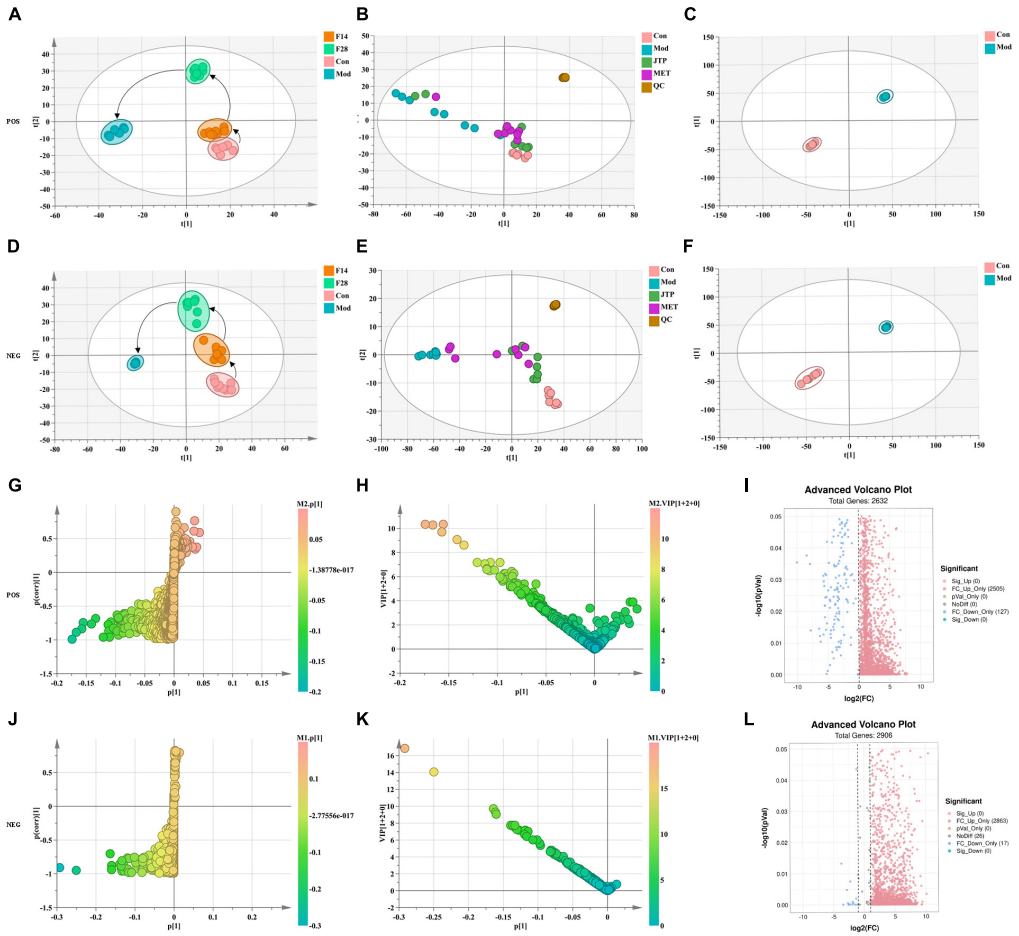

FIGURE 3
(A) Metabolic profile during type 2 diabetes mellitus (T2DM) modeling in positive ion mode. **(B)** Metabolic profile after treatment positive ion mode. **(C)** OPLS-DA score plots for the control group and the model group in positive ion mode. **(D)** Metabolic profile during T2DM modeling in negative ion mode. **(E)** Metabolic profile after treatment negative ion mode. **(F)** OPLS-DA score plots for the control group and the model group in negative ion mode. **(G)** Fecal biomarkers in the S-plot between the control group and the model group in positive ion mode. **(H)** Fecal biomarkers in the VIP between the control group and the model group in positive ion mode. **(I)** Fecal biomarkers in the FC between the control group and the model group in positive ion mode. **(J)** Fecal biomarkers in the S-plot between the control group and the model group in negative ion mode. **(K)** Fecal biomarkers in the VIP between the control group and the model group in negative ion mode. **(L)** Fecal biomarkers in the FC between the control group and the model group in negative ion mode.

Arachidonic acid metabolism, Tryptophan metabolism, Histidine metabolism, Pyruvate metabolism, Citrate cycle (TCA cycle), Arginine biosynthesis, beta-Alanine metabolism, Cysteine, and methionine metabolism, Glycolysis/Gluconeogenesis.

Partial least squares discrimination analysis (PLS-DA) analysis [ESI^+: R^2Y-0.995, Q^2-0.973; ESI^-: R^2Y-0.952, Q^2-0.900 (Supplementary Figure 3)] of the control, model, and JTP groups on the last day of drug administration showed that the fecal metabolic profiles of the JTP group were significantly farther from the model group and closer to the control group (Figures 5A, B). The results indicated that JTP could significantly regulate the metabolic profile of T2DM model rats to a healthy state, which further suggested that JTP could interfere with the occurrence and development of T2DM. Based on 23 potential biomarkers, the statistical analysis identified 21 of these biomarkers in the JTP callback and 13 were statistically significant (Figure 5D). JTP significantly modulates 8 of these metabolic pathways, namely: Arachidonic acid metabolism, Tryptophan metabolism, Alanine, aspartate and glutamate metabolism, Histidine metabolism, Pyruvate metabolism, Cysteine and methionine metabolism, Glycolysis/Gluconeogenesis, and Citrate cycle (TCA cycle) (Figure 5C).

3.6. Correlation analysis for gut microbiota, biomarkers, and pharmacological indices

Correlations were calculated using spearman for thirteen Jiaotai pill (JTP)-regulated potential biomarkers and the seven pharmacological indicators (Figure 6A). Positive correlations are shown in red, negative correlations in blue, and those with $p < 0.05$ and $|r| > 0.5$ were selected as correlating.

3-Hydroxybutyric acid was positively correlated with FBG, HbA1c, FINS, TG, LDL and negatively correlated with IL-10. Arachidonic acid was positively correlated with IL-10 and

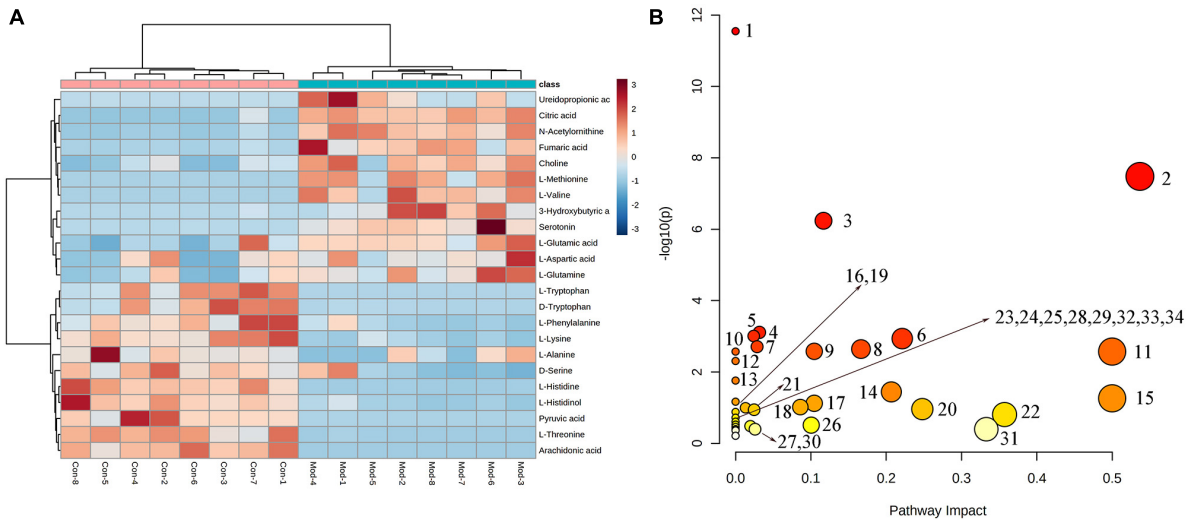

FIGURE 4
(A) Heatmap of changes in levels of 23 potential biomarkers in control and model groups. **(B)** Metabolism pathway analysis of 23 potential biomarkers with MetPA. MetPA, metabolic pathway analysis.

negatively correlated with FBG, FINS, HbA1c, and TG. Choline was positively correlated with LDL, FBG, and FINS. L-Aspartic acid was positively correlated with LDL. L-Histidine was positively correlated with IL-10 and negatively correlated with FINS, FBG, and LDL. L-Methionine was positively correlated with LDL, FINS, FBG, HbA1c, and TG, and negatively correlated with IL-10. L-Lysine was negatively correlated with FBG, FINS, and HbA1c. L- Tryptophan was positively correlated with IL-10, and negatively correlated with FBG, HbA1c, FINS, LDL, and TG. L-Valine was positively correlated with FBG, FINS, HbA1c, and LDL, and negatively correlated with IL-10. N-Acetylornithine was positively correlated with FBG, FINS, HbA1c, LDL, TG, and TC, and negatively correlated with IL-10. Pyruvic acid was positively correlated with IL-10, and negatively correlated with FINS, FBG, HbA1c, and LDL. Serotonin was positively correlated with FBG, FINS, and TG, and negatively correlated with IL-10.

Thirteen JTP-regulated potential biomarkers were correlated with the 13 differential gut microbiota using spearman calculations (Figure 6B). Positive correlations are shown in red, negative correlations in blue, and those with $p < 0.05$ and $|r| > 0.5$ were selected as correlating.

At the phylum level, the Actinobacteria were positively correlated with N-Acetylornithine, L-Methionine, L-Valine, 3-Hydroxybutyric acid, Choline, L-Aspartic acid, Serotonin, and negatively correlated with Arachidonic acid, L-Tryptophan, L-Lysine, Pyruvic acid, L-Histidine. Bacteroidetes were negatively correlated with L-Histidine, Pyruvic acid. Firmicutes were positively correlated with L-Histidine, Pyruvic acid, and Arachidonic acid and negatively correlated with L-Methionine. Proteobacteria were positively correlated with Serotonin, L-Valine, N-Acetylornithine, 3-Hydroxybutyric acid, L-Methionine, and Choline, and negatively correlated with L-Tryptophan, L-Histidine, Arachidonic acid, L-Lysine, Pyruvic acid was negatively correlated.

At the genus level, the Clostridium_IV was positively correlated with Arachidonic acid, L-Tryptophan, Pyruvic acid, and L-Histidine, and negatively correlated with L-Valine, N-Acetylornithine, 3-Hydroxybutyric acid, Serotonin, and L-Methionine were negatively correlated. Clostridium_XlVa was positively correlated with Pyruvic acid, L-Histidine, Arachidonic acid, and L-Tryptophan and negatively correlated with 3-Hydroxybutyric acid. Eubacterium was positively correlated with L-Lysine. Fusicatenibacter was positively correlated with L-Methionine, Serotonin, 3-Hydroxybutyric acid, and N-Acetylornithine, and negatively correlated with L-Histidine, L-Lysine, Pyruvic acid, L-Alanine, L-Tryptophan, and Arachidonic acid. Romboutsia was positively correlated with L-Valine and N-Acetylornithine and negatively correlated with L-Tryptophan, Arachidonic acid, Pyruvic acid, and L-Lysine. Roseburia was positively correlated with L-Histidine, Arachidonic acid, Pyruvic acid, and L-Tryptophan, and negatively correlated with 3-Hydroxybutyric acid, Serotonin, L-Methionine, N-Acetylornithine, L-Valine.

At the family level, the Eubacteriaceae were positively correlated with L-Lysine. Prevotellaceae was positively correlated with L-Methionine, and negatively correlated with L-Histidine, L-Lysine, Pyruvic acid, and Arachidonic acid. Ruminococcaceae was positively correlated with Arachidonic acid, L-Tryptophan, and negatively correlated with N-Acetylornithine, 3-Hydroxybutyric acid, Serotonin, L-Methionine, L-Valine.

The biomarkers and gut microbiota with correlation were correlated with pharmacological indicators, and the Sankey diagram (Figure 6C) shows that 7 pharmacological indicators, 12 biomarkers, and 11 gut microbiota were highly correlated.

4. Discussion

Type 2 diabetes mellitus model rats established by high-fat diet and low dose STZ had dry, gray, and loose hair, depression, gradual loss of body shape, irritability, high urine output, and sour and pungent taste. The study showed that the rats fed with high-fat diets exhibited insulin resistance, increased pancreatic islet β cells, and

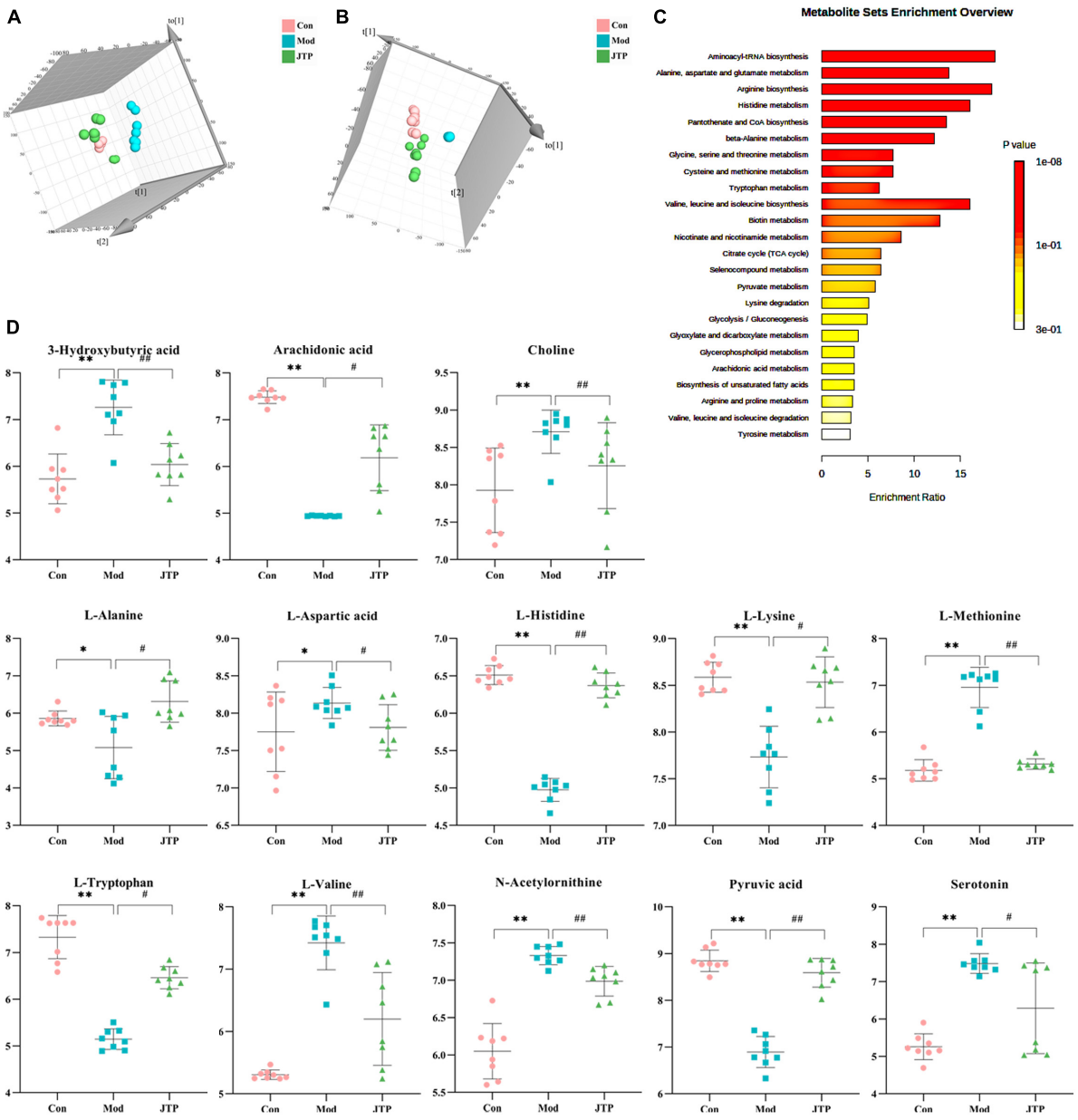

FIGURE 5
(A) PLS-DA analysis of the control, model, and JTP groups in positive ion mode. **(B)** PCA analysis of the control, model, and JTP groups in negative ion mode. **(C)** Metabolism pathway analysis of 13 biomarkers of JTP back regulation with MetPA. **(D)** Scatter plots of the changes in the 13 biomarkers of JTP back regulation in the control, model, and JTP groups, $^{*}p < 0.05$, $^{*}p < 0.01$ compared to the control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the model group ($n = 8$).

impaired islet β-cell function (15). It was consistent with the results of HE staining of pancreatic tissues. In the study, it was found that the levels of FBG, HbA1c, and FINS were increased in the model rats, which were consistent with the clinical manifestations of hyperglycemia and insulin resistance in type 2 diabetes mellitus. The levels of TC, TG, and LDL were increased in the model rats, which were consistent with the manifestation of dyslipidemia in type 2 diabetes mellitus. IL-10, which is involved in the regulation of insulin secretion, β-cell apoptosis, and peripheral insulin resistance, was reduced in the T2DM model. The successful establishment of the T2DM model was confirmed by a combination of multiple aspects. JTP reversed the symptoms of hyperglycemia, insulin resistance, dyslipidemia, and tissue damage. The efficacy of JTP in the treatment of type 2 diabetes mellitus was demonstrated.

Based on 16S rRNA sequencing analysis, we observed a decrease in alpha diversity of gut microbiota in T2DM group rats through sobs, Chao, Shannon, and ace indexes. Microbial species diversity is the basis of intestinal nutrient absorption, metabolism, and immune barrier regulation (16), and a decrease in flora species diversity affects intestinal function and induces various diseases. In the JTP group, the above indices increased, the diversity of flora increased, and the gut microbiota disorder was restored. Alterations in the gut microbiota were closely associated with T2DM, consistent with previous findings (17, 18).

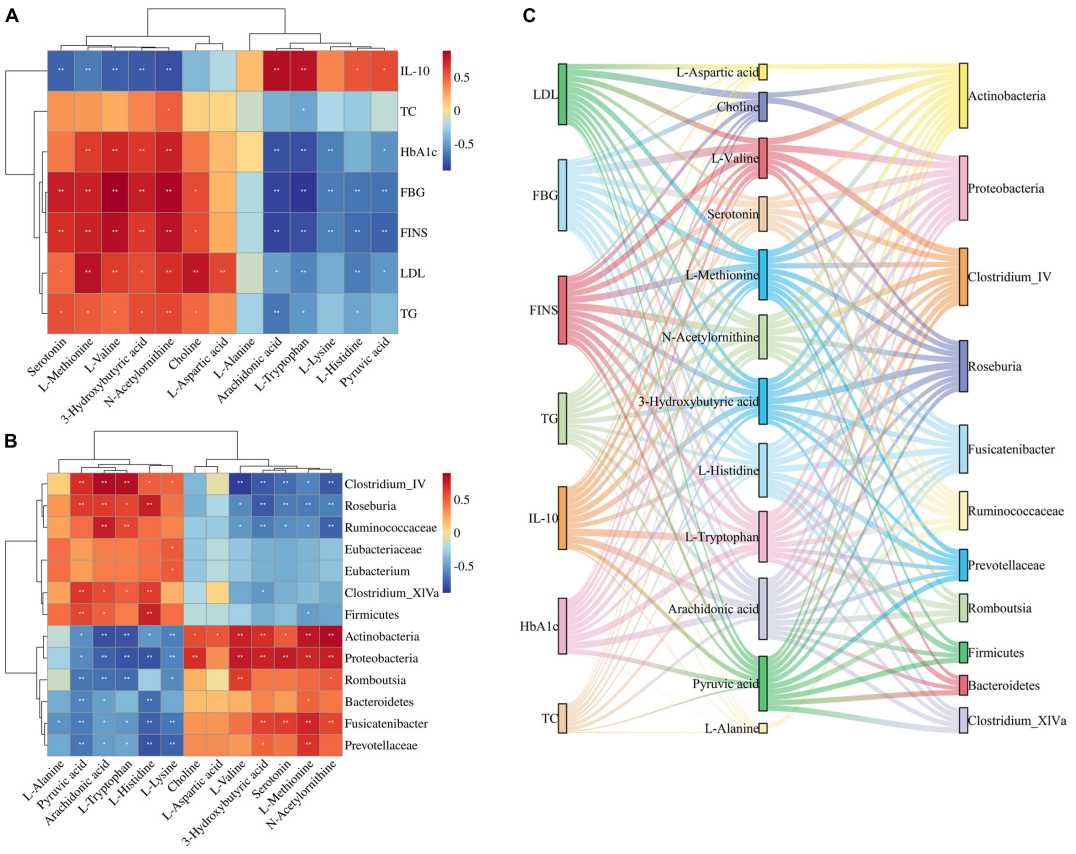

FIGURE 6
(A) Spearman correlation analysis of biomarkers and pharmacological indices. **(B)** Spearman correlation analysis of biomarkers and gut microbiota. **(C)** Sankey diagrams for the interconnection of pharmacological indicators, biomarkers, and gut microbiota.

In this study, PCoA and NMDS analysis were used to analyze the similarity (β-diversity) of the gut microbiota of the samples. Two-dimensional plots of both PCoA and NMDS showed that samples from the T2DM group were far from the other three groups, and samples from the control and T2DM groups clustered on the left and right sides of the horizontal axis, respectively. The samples of the JTP group and MET group were distributed on the side of the control group, indicating that the structure of intestinal microflora after JTP and MET treatment was more similar to that of the control group.

In our study, the dominant microorganisms in the rat gut mainly included Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria. The stability of these flora plays an important role in immune regulation, energy metabolism, and substance metabolism, which are essential factors for the maintenance of human health and a mirror reflecting the internal environment of the organism (19). Compared to the control group, the relative abundance of Actinobacteria, Proteobacteria, and Bacteroidetes in the gut microbiota was increased and the relative abundance of Firmicutes was decreased in the T2DM group. Carbohydrates and proteins in the gut are metabolized and hydrolyzed primarily by Firmicutes, whereas steroids, polysaccharides, and bile acids are metabolized by Bacteroidetes (20, 21). Studies have shown that short-chain fatty acids not only provide energy for intestinal epithelial cells but also enhance the intestinal defense barrier. Firmicutes are the main species that ferment carbohydrates into various short-chain fatty acids (21). As the abundance of Firmicutes decreases, these protective effects are weakened, which is likely to induce the occurrence of type 2 diabetes mellitus. The increase in abundance of Bacteroidetes and the disturbance of lipid and energy metabolism further accelerate gut microbiota disorders and contribute to the development of type 2 diabetes mellitus (22). Bacteroidetes accelerate the secretion of lipopolysaccharides and thus cause insulin resistance (23). The increased abundance of Actinobacteria and Proteobacteria in the T2DM group, with gut microbiota imbalance, triggers increased intestinal wall permeability which allows a large number of intestinal bacteria to translocate and distribute in blood and tissues, causing insulin resistance.

The study also confirmed that the abundance of Gram-negative pathogenic bacteria (family level: Prevotellaceae; genus level: Romboutsia, Desulfovibrio)was increased when type 2 diabetes mellitus occurred. Gram-positive bacteria (family level: Eubacteriaceae, Ruminococcaceae; genus level: Clostridium_IV, Clostridium_XlVa, Eubacterium, Roseburia) decreased in abundance. The increase of Gram-negative bacteria will cause an increase in cytosolic toxicogenic LPS (24). In particular, there is an increase in the abundance of endotoxin-producing Desulfovibrio (Desulfovibrio), which impairs intestinal barrier function and leads to high levels of circulating LPS (25). The

reduction of beneficial bacteria such as Clostridium increases intestinal permeability. At this point, gut microbiota toxins enter the circulatory system, causing inflammation and then leading to insulin resistance.

PICRUST analysis revealed that gut microbiota is involved in metabolic pathways such as amino acid metabolism, carbohydrate metabolism, and lipid metabolism affecting type 2 diabetes mellitus. Metabolomics was used to further analyze the metabolism of rats. The JTP callback of metabolic pathways associated with type 2 diabetes mellitus is described below.

Metabolomics studies have shown that T2DM rats are always accompanied by abnormal amino acid metabolism. L-Phenylalanine and L-Tryptophan belong to the aromatic amino acids (AAA), which affect insulin resistance and dyslipidemia, and play an important role in type 2 diabetes mellitus (26). L-Phenylalanine is involved in glucose and fat metabolism in the body through oxidative conversion to tyrosine catalyzed by phenylalanine hydroxylase. It has been reported that L-Phenylalanine levels are elevated in type 2 diabetic patients and T2DM rat models (27). This is consistent with the results of our results that the level of L-Phenylalanine in feces of T2DM group increased. L-Tryptophan is an important metabolite involved in the regulation of inflammation and may reduce blood glucose levels in T2DM rats by relieving inflammation and promoting insulin sensitivity (28, 29). Gut microbiota can decompose tryptophan to produce indole, and long-term increased indole levels can inhibit mitochondrial metabolism, reduce intracellular ATP concentration, and reduce ATP-sensitive potassium channel opening. This inhibits the secretion of gastrointestinal hormones such as pancreatic hyperglycemic polypeptide (30). In the T2DM model group, Actinobacteria, Proteobacteria, Fusicatenibacter, and Romboutsia were elevated in abundance, and L-Tryptophan and Pyruvic acid levels decreased, which were correlated and negatively correlated. The levels of L-Phenylalanine and L-Tryptophan were increased after JTP treatment. Valine belongs to the branched chain amino acids (BCAA). In our study, it was higher in the T2DM model group than in the control group. It was shown that increased BCAA levels can lead to increased insulin secretion and islet β-cell depletion in T2DM patients (31). Abnormal BCAA metabolism can lead to the accumulation of valine, resulting in β-cell mitochondrial dysfunction and high sensitivity to insulin resistance. It can be used as a characteristic biomarker of type 2 diabetes mellitus. Aspartate and glutamate metabolism can inhibit Akt phosphorylation (32), and activation of Akt phosphorylated insulin promotes glycogen synthesis and inhibits gluconeogenesis in the liver, thereby lowering blood glucose (33). L-Aspartic acid and L-Glutamic acid, as core aspartate and glutamate metabolism metabolites, were increased in the T2DM model group. Histidine metabolism is a key metabolic pathway affecting type 2 diabetes mellitus, and L-Histidine is a core metabolite of histidine metabolism. Histidine supplementation has been found to suppress the inflammatory response and improve insulin resistance. L-Lysine significantly improves the structure and function of glycosylated lysozyme *in vitro* and is an effective therapeutic supplement for T2DM (34). L-Histidine and L-Lysine levels are decreased in the T2DM model. l-Methionine undergoes demethylation and further hydrolysis to form cysteine. Agullo-Ortuno et al found that homocysteine levels are an indicator of diabetes risk (35). In this study, L-Methionine levels were increased in the T2DM model group. In summary, the regulation of amino acid metabolism may have significant implications for the treatment of diabetes, and JTP plays a therapeutic role in type 2 diabetes mellitus by regulating Tryptophan metabolism, Alanine, aspartate and glutamate metabolism, Histidine metabolism, Cysteine and methionine metabolism.

Carbohydrate metabolism was significantly altered in T2DM rats. Pyruvic acid is the end product of glycolysis and the starting point for gluconeogenesis, and can be generated by transamination and participate in energy metabolism to provide energy to living cells and organisms (36, 37). The pyruvate dehydrogenase complex converts Pyruvic acid to acetyl coenzyme A and enters the tricarboxylic acid cycle (38).

At the same time, Pyruvic acid can be fermented into succinate, lactate, and acetyl-CoA by gut microbiota and further metabolized into SCFAs. This affects changes in levels of glycolysis/gluconeogenesis and pyruvate metabolism (39). In this study, Firmicutes was found to be positively correlated with Pyruvic acid, and Actinobacteria, Bacteroidetes, and Proteobacteria were negatively correlated with Pyruvic acid. Pyruvic acid levels are decreased and energy metabolism is disturbed in diabetic patients. Pyruvic acid levels reverted to normal after JTP treatment.

Lipid metabolism is highly associated with the development of type 2 diabetes mellitus (T2DM). Arachidonic acid is a polyunsaturated fatty acid that inhibits lipogenesis and promotes lipolysis (40)and plays an important role in regulating lipid metabolism in the body (41). A decrease in Arachidonic acid has been reported to affect lipid metabolism disorders in type 2 diabetes mellitus. At the same time, our experimental results showed that TC, TG, and LDL levels were increased in T2DM model rats, which is consistent with previous reports. Arachidonic acid is present in the structural phospholipids of cell membranes (42)and delays the progression of type 2 diabetes mellitus by increasing the fluidity of cell membranes and increasing the number of insulin receptors and their affinity for insulin. Our study found a positive correlation between Ruminococcaceae and Arachidonic acid, consistent with previous studies (43). Ruminococcaceae produces SCFAs that stimulate glucagon secretion and increase satiety, thereby regulating fat and cholesterol synthesis and regulating lipid metabolism in humans (44). After 4 weeks of JTP treatment, the level of Arachidonic acid in feces increased and the abundance of Ruminococcaceae increased, suggesting that JTP improves type 2 diabetes mellitus by regulating Arachidonic acid and Ruminococcaceae to improve lipid metabolism disorders, inflammation, and insulin secretion.

This study mainly focuses on the changes in gut microbiota and metabolite detection in JTP intervention in T2DM rats. The animal indicators are currently limited to the phenotypic part. In the future, experiments are needed to further improve the exploration of pathways and mechanisms.

5. Conclusion

In summary, Jiaotai pill (JTP) decreased blood glucose and lipid levels and reduced insulin resistance in type 2 diabetes mellitus (T2DM) rats. Potential biomarkers and key functional bacteria were identified for JTP treatment in T2DM rats. These results suggest that JTP can increase microbiota diversity and restore gut

microbiota balance. It improves metabolic pathways such as amino acid metabolism, carbohydrate metabolism, and lipid metabolism associated with type 2 diabetes mellitus, and plays a therapeutic role in T2DM rats.

Data availability statement

The datasets presented in this study can be found in online repositories. The name of the repository and accession number can be found below: https://www.ncbi.nlm.nih.gov/bioproject/922696.

Ethics statement

This animal study was reviewed and approved. All experiments were performed following the Declaration of Helsinki and were approved by the Animal Health and Ethics Committee of Heilongjiang University of Chinese Medicine (2021012709).

Author contributions

XhW and JL designed the study. XW conducted the animal trial, sample collection, and physical analysis. QL and SY performed the bioinformatics analysis of 16S rRNA sequencing. JL and YZ performed the untargeted metabolomics data. JL and CP wrote the manuscript. XhW, JL, and ZF contributed to the discussion of the work and assisted in drafting the manuscript. All authors read and approved the final manuscript.

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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/fnut.2023.1135343/full#supplementary-material

References

1. Rohm TV, Meier DT, Olefsky JM, Donath MY. Inflammation in obesity, diabetes, and related disorders. *Immunity.* (2022) 55:31–55. doi: 10.1016/j.immuni.2021.12.013

2. GBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet.* (2020) 396:1204–22.

3. Qi Q, Li J, Yu B, Moon JY, Chai JC, Merino J, et al. Host and gut microbial tryptophan metabolism and type 2 diabetes: an integrative analysis of host genetics, diet, gut microbiome and circulating metabolites in cohort studies. *Gut.* (2022) 71:1095–105. doi: 10.1136/gutjnl-2021-324053

4. Wang Y, Ai Z, Xing X, Fan Y, Zhang Y, Nan B, et al. The ameliorative effect of probiotics on diet-induced lipid metabolism disorders: A review. *Crit Rev Food Sci Nutr.* (2022) doi: 10.1080/10408398.2022.2132377 [Epub ahead of print].

5. Wang M, Li S, Wang F, Zou J, Zhang Y. Aerobic exercise regulates blood lipid and insulin resistance via the toll-like receptor 4-mediated extracellular signal-regulated kinases/AMP-activated protein kinases signaling pathway. *Mol Med Rep.* (2018) 17:8339–48. doi: 10.3892/mmr.2018.8863

6. Lyu Y, Lin L, Xie Y, Li D, Xiao M, Zhang Y, et al. Blood-Glucose-Lowering effect of coptidis rhizoma extracts from different origins via gut microbiota modulation in db/db Mice. *Front Pharmacol.* (2021) 12:684358. doi: 10.3389/fphar.2021.684358

7. Yang Y, Lu W, Zhang X, Wu C. Gut fungi differentially response to the antipyretic (heat-clearing) and diaphoretic (exterior-releasing) traditional Chinese medicines in Coptis chinensis-conditioned gut microbiota. *Front Pharmacol.* (2022) 13:1032919. doi: 10.3389/fphar.2022.1032919

8. Feng R, Shou JW, Zhao ZX, He CY, Ma C, Huang M, et al. Transforming berberine into its intestine-absorbable form by the gut microbiota. *Sci Rep.* (2015) 5:12155. doi: 10.1038/srep12155

9. Zhang ZW, Cong L, Peng R, Han P, Ma SR, Pan LB, et al. Transformation of berberine to its demethylated metabolites by the CYP51 enzyme in the gut microbiota. *J Pharm Anal.* (2021) 11:628–37. doi: 10.1016/j.jpha.2020.10.001

10. Qi L, Mao H, Lu X, Shi T, Wang J. Cinnamaldehyde promotes the intestinal barrier functions and reshapes gut microbiome in early weaned rats. *Front Nutr.* (2021) 8:748503. doi: 10.3389/fnut.2021.748503

11. Hong S, Cha KH, Park JH, Jung DS, Choi JH, Yoo G, et al. Cinnamic acid suppresses bone loss via induction of osteoblast differentiation with alteration of gut microbiota. *J Nutr Biochem.* (2022) 101:108900. doi: 10.1016/j.jnutbio.2021.108900

12. Smits LP, Bouter KE, de Vos WM, Borody TJ, Nieuwdorp M. Therapeutic potential of fecal microbiota transplantation. *Gastroenterology.* (2013) 145:946–53. doi: 10.1053/j.gastro.2013.08.058

13. Feng WW, Liu J, Cheng H, Peng C. Integration of gut microbiota and metabolomics for Chinese medicines research: opportunities and challenges. *Chin J Integr Med.* (2021) 28:1032–9. doi: 10.1007/s11655-021-3305-x

14. Hou Z, Song F, Xing J, Zheng Z, Liu S, Liu Z. Comprehensive fecal metabolomics and gut microbiota for the evaluation of the mechanism of Panax Ginseng in the treatment of Qi-deficiency liver cancer. *J Ethnopharmacol.* (2022) 292:115222. doi: 10.1016/j.jep.2022.115222

15. Le L, Jiang B, Wan W, Zhai W, Xu L, Hu K, et al. Metabolomics reveals the protective of Dihydromyricetin on glucose homeostasis by enhancing insulin sensitivity. *Sci Rep.* (2016) 6:36184. doi: 10.1038/srep36184

16. Capasso R, Matias I, Lutz B, Borrelli F, Capasso F, Marsicano G, et al. Fatty acid amide hydrolase controls mouse intestinal motility in vivo. *Gastroenterology.* (2005) 129:941–51. doi: 10.1053/j.gastro.2005.06.018

17. Wang Z, Du H, Peng W, Yang S, Feng Y, Ouyang H, et al. Efficacy and Mechanism of Pueraria lobata and Pueraria thomsonii Polysaccharides in the Treatment of Type 2 Diabetes. *Nutrients.* (2022) 14:3926. doi: 10.3390/nu14193926

18. Bai Z, Huang X, Wu G, Ye H, Huang W, Nie Q, et al. Polysaccharides from red kidney bean alleviating hyperglycemia and hyperlipidemia in type 2 diabetic rats via gut microbiota and lipid metabolic modulation. *Food Chem.* (2023) 404:134598. doi: 10.1016/j.foodchem.2022.134598

19. Gao T, Tian C, Tian G, Ma L, Xu L, Liu W, et al. Excessive fructose intake inhibits skeletal development in adolescent rats via gut microbiota and energy metabolism. *Front Microbiol.* (2022) 13:952892. doi: 10.3389/fmicb.2022.952892

20. Xu J, Bjursell MK, Himrod J, Deng S, Carmichael LK, Chiang HC, et al. A genomic view of the human-*Bacteroides* thetaiotaomicron symbiosis. *Science.* (2003) 299:2074–6. doi: 10.1126/science.1080029

21. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *Science.* (2005) 307:1915–20. doi: 10.1126/science.1104816

22. Chen K, Nakasone Y, Yi S, Ibrahim HR, Sakao K, Hossain MA, et al. Natural Garlic organosulfur compounds prevent metabolic disorder of lipid and glucose by increasing gut commensal *Bacteroides* acidifaciens. *J Agric Food Chem.* (2022) 70:5829–37. doi: 10.1021/acs.jafc.2c00555

23. Zhao X, Fu Z, Yao M, Cao Y, Zhu T, Mao R, et al. Mulberry (*Morus alba* L.) leaf polysaccharide ameliorates insulin resistance- and adipose deposition-associated gut microbiota and lipid metabolites in high-fat diet-induced obese mice. *Food Sci Nutr.* (2022) 10:617–30. doi: 10.1002/fsn3.2689

24. Salguero MV, Al-Obaide MAI, Singh R, Siepmann T, Vasylyeva TL. Dysbiosis of Gram-negative gut microbiota and the associated serum lipopolysaccharide exacerbates inflammation in type 2 diabetic patients with chronic kidney disease. *Exp Ther Med.* (2019) 18:3461–9. doi: 10.3892/etm.2019.7943

25. Zhang C, Zhang M, Wang S, Han R, Cao Y, Hua W, et al. Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. *ISME J.* (2010) 4:232–41. doi: 10.1038/ismej.2009.112

26. Ikeda H. The Effect of Mild Renal Dysfunction on the Assessment of Plasma Amino Acid Concentration and Insulin Resistance in Patients with Type 2 Diabetes Mellitus. *J Diabetes Res.* (2022) 2022:2048300. doi: 10.1155/2022/2048300

27. Macias-Acosta MP, Valerdi-Contreras L, Bustos-Angel ED, Garcia-Reyes RA, Alvarez-Zavala M, Gonzalez-Avila M. Involvement of the fecal amino acid profile in a clinical and anthropometric study of Mexican patients with insulin resistance and type 2 diabetes mellitus. *Amino Acids.* (2022) 54:47–55. doi: 10.1007/s00726-021-03107-3

28. Madella AM, Van Bergenhenegouwen J, Garssen J, Masereeuw R, Overbeek SA. Microbial-Derived tryptophan catabolites, kidney disease and gut inflammation. *Toxins.* (2022) 14:645. doi: 10.3390/toxins14090645

29. Su X, Gao Y, Yang R. Gut Microbiota-Derived tryptophan metabolites maintain gut and systemic homeostasis. *Cells.* (2022) 11:2296. doi: 10.3390/cells11152296

30. Cabreiro F. Metformin joins forces with microbes. *Cell Host Microbe.* (2016) 19:1–3. doi: 10.1016/j.chom.2015.12.012

31. Lee CC, Watkins SM, Lorenzo C, Wagenknecht LE, Il'yasova D, Chen YD, et al. Branched-Chain Amino Acids and Insulin Metabolism: The Insulin Resistance Atherosclerosis Study (IRAS). *Diabetes Care.* (2016) 39:582–8. doi: 10.2337/dc15-2284

32. Yamaguchi Y, Yamamoto K, Sato Y, Inoue S, Morinaga T, Hirano E. Combination of aspartic acid and glutamic acid inhibits tumor cell proliferation. *Biomed Res.* (2016) 37:153–9. doi: 10.2220/biomedres.37.153

33. Zhang K, Guo X, Yan H, Wu Y, Pan Q, Shen JZ, et al. Phosphorylation of forkhead protein FoxO1 at S253 regulates glucose homeostasis in mice. *Endocrinology.* (2019) 160:1333–47. doi: 10.1210/en.2018-00853

34. Mirmiranpour H, Khaghani S, Bathaie SZ, Nakhjavani M, Kebriaeezadeh A, Ebadi M, et al. The Preventive Effect of L-Lysine on Lysozyme Glycation in Type 2 Diabetes. *Acta Med Iran.* (2016) 54:24–31.

35. Agullo-Ortuno MT, Albaladejo MD, Parra S, Rodriguez-Manotas M, Fenollar M, Ruiz-Espejo F, et al. Plasmatic homocysteine concentration and its relationship with complications associated to diabetes mellitus. *Clin Chim Acta.* (2002) 326:105–12. doi: 10.1016/S0009-8981(02)00287-5

36. Schell JC, Wisidagama DR, Bensard C, Zhao H, Wei P, Tanner J, et al. Control of intestinal stem cell function and proliferation by mitochondrial pyruvate metabolism. *Nat Cell Biol.* (2017) 19:1027–36. doi: 10.1038/ncb3593

37. Guasch-Ferre M, Santos JL, Martinez-Gonzalez MA, Clish CB, Razquin C, Wang D, et al. Glycolysis/gluconeogenesis- and tricarboxylic acid cycle-related metabolites, Mediterranean diet, and type 2 diabetes. *Am J Clin Nutr.* (2020) 111:835–44. doi: 10.1093/ajcn/nqaa016

38. Cappel DA, Deja S, Duarte JAG, Kucejova B, Inigo M, Fletcher JA, et al. Pyruvate-Carboxylase-Mediated anaplerosis promotes antioxidant capacity by sustaining TCA cycle and redox metabolism in liver. *Cell Metab.* (2019) 129:e1298.

39. Zhou L, Zeng X, Liao J, Chen L, Ouyang D. Gut microbiota modulates the protective role of ginsenoside compound K against sodium valproate-induced hepatotoxicity in rat. *Front Microbiol.* (2022) 13:936585. doi: 10.3389/fmicb.2022.936585

40. Ouldamer L, Jourdan ML, Pinault M, Arbion F, Goupille C. Accumulation of arachidonic acid, precursor of pro-inflammatory eicosanoids, in adipose tissue of obese women: association with breast cancer aggressiveness indicators. *Biomedicines.* (2022) 10:995. doi: 10.3390/biomedicines10050995

41. Sonnweber T, Pizzini A, Nairz M, Weiss G, Tancevski I. Arachidonic acid metabolites in cardiovascular and metabolic diseases. *Int J Mol Sci.* (2018) 19:3285. doi: 10.3390/ijms19113285

42. Hanna VS, Hafez EAA. Synopsis of arachidonic acid metabolism: A review. *J Adv Res.* (2018) 11:23–32. doi: 10.1016/j.jare.2018.03.005

43. Xu SS, Zhang XL, Liu SS, Feng ST, Xiang GM, Xu CJ, et al. Multi-Omic analysis in a metabolic syndrome porcine model implicates arachidonic acid metabolism disorder as a risk factor for atherosclerosis. *Front Nutr.* (2022) 9:807118. doi: 10.3389/fnut.2022.807118

44. Zhou C, Zhang W, Lin H, Zhang L, Wu F, Wang Y, et al. Effect of theaflavin-3,3'-digallate on leptin-deficient induced nonalcoholic fatty liver disease might be related to lipid metabolism regulated by the Fads1/PPARdelta/Fabp4 axis and gut microbiota. *Front Pharmacol.* (2022) 13:925264. doi: 10.3389/fphar.2022.861319

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Screening of lipids and kidney function in children and adolescents with Type 1 Diabetes: does age matter?

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Introduction: The purpose of this study was to evaluate lipid profile and kidney function in children and adolescents with Type 1 Diabetes.

Methods: This was a retrospective study including 324 children and adolescents with Type 1 Diabetes (48% females, mean age 13.1 ± 3.2 years). For all participants, demographic and clinical information were collected. The prevalence of dyslipidemia and kidney function markers were analyzed according to age. Multivariate linear regression analyses were performed to test the association of lipids or markers of renal function with demographic and clinical information (sex, age, disease duration, BMI SDS, HbA1c).

Results: In our study the rate of dyslipidemia reached 32% in children <11 years and 18.5% in those ≥11 years. Children <11 years presented significantly higher triglyceride values. While the albumin-to-creatinine ratio was normal in all individuals, 17% had mildly reduced estimated glomerular filtration rate. Median of HbA1c was the most important determinant of lipids and kidney function, being associated with Total Cholesterol (p-value<0.001); LDL Cholesterol (p-value=0.009), HDL Cholesterol (p-value=0.045) and eGFR (p-value=0.001).

Conclusion: Dyslipidemia could be present both in children and adolescents, suggesting that screening for markers of diabetic complications should be performed regardless of age, pubertal stage, or disease duration, to optimize glycemia and medical nutrition therapy and/or to start a specific medical treatment.

KEYWORDS

type 1 diabetes, lipids profile, kidney function, age, guidelines

1 Introduction

The development of micro and macrovascular complications is rare in children and adolescents with Type 1 Diabetes (T1D) meeting recommended HbA1c value: a declining incidence of vascular complications has been reported in the developed world (1, 2), while it is still an issue in developing countries (3).

The 2023 ADA Standards of Medical Care and the 2022 ISPAD Clinical Practice in children and adolescents with T1D (4, 5) still suggest an age limit to perform screening for risk factors for diabetes-related complications, such as dyslipidemia and albuminuria. Nevertheless, several studies demonstrated that the atherosclerotic process begins in childhood and that the first subclinical indications of cardiovascular risk may occur before puberty (6). Lipids above the recommended values were previously reported in subjects with T1D from 2 years of age, confirming that risk factors for cardiovascular complications start early after diabetes diagnosis (7, 8). Similarly, decreased estimated glomerular filtration rates were reported in children with T1D between 2 and 18 years of age (9).

However, overall, these past studies did not perform a comparison of the prevalence of lipid and renal abnormalities among T1D subjects belonging to different age groups.

Therefore, in this study, we analyzed lipid profile and kidney function in children and adolescents with T1D stratified for cut-off established by ADA and ISPAD guidelines for start to screening, hypothesizing that their screening is of fundamental importance, regardless of age, pubertal status and disease duration. Moreover, we also analyzed demographic and clinical factors associated with lipids and renal markers.

2 Methods

2.1 Study participants

Three hundred twenty-four subjects with T1D and age <21 years, with at least one year of disease duration, were recruited at Diabetes Units of IRCCS Burlo Garofolo (Trieste, Italy) (n=90), Regina Margherita Children's hospital (Torino, Italy) (n=90), Santa Chiara hospital (Trento, Italy), (n=14), University Medical Center (Ljubljana, Slovenia) (n=80) and IRCCS San Raffaele (Milano, Italy) (n=50) between 2018 and 2021.

2.2 Study procedures

We retrospectively collected demographic and anthropometric information, such as age, sex, height, weight, and pubertal status. Puberty is defined as the presence of breast budding in girls and a testicular volume of 4 ml in boys. Standard deviation scores of body mass index (BMI SDS) were calculated according to WHO reference charts using the Growth Calculator 4 software (http://www.weboriented.it/gh4/).

Clinical information included age at diagnosis, disease duration, and HbA1c at the regular visits in the previous year. Based on the cut-off of HbA1c of 7% (53mmol/mol), T1D subjects were categorized in two groups (4, 5).

Laboratory data were collected from the latest performed screening.

Fasting lipids were collected, and the lipids profile was defined as acceptable, borderline, and abnormal according to the NHLBI guidelines (10). The following cut-off points for dyslipidemia definition were considered: total cholesterol (TC) ≥200 mg/dL; LDL cholesterol (LDL-C) ≥130 mg/dL; HDL cholesterol (HDL-C) <40 mg/dL; triglycerides (TG) ≥100 mg/dL up to 9 years and triglycerides ≥130 mg/dL over 9 years. Subjects with single or combined lipids alterations were considered "abnormal lipid profile."

Evaluation of albuminuria was determined as albumin to creatinine ratio (ACR), by first monitoring urine sample, and ACR value >30 mg/g were considered abnormal (11).

The estimated glomerular filtration rate (eGFR) was calculated using Schwartz's equation: 0.413 x height (cm)/serum creatinine mg/dL (12). eGFR values <90 mL/min/1.73 m^2 were considered abnormal (11).

2.3 Statistical analysis

Descriptive statistics represent percentages, means, standard deviations (SD). T1D subjects were stratified for pubertal status (pre-pubertal *vs.* pubertal), disease duration (<5 *vs.* ≥5 years) following ADA or ISPAD guidelines. Moreover, for age ISPAD guidelines (<11 *vs.* ≥11) were applied for lipids analysis, while ADA guidelines (≤10 *vs.* >10 years) for kidney function analysis.

The skewness and kurtosis were calculated for testing the normality. Differences between T1D individuals stratified for age, pubertal status or disease duration were analyzed by chi-squared tests to compare categorical data. While t-tests or Mann-Whitney test used to compare the means when the variable is normally or not-normally distributed, respectively.

Multivariate linear regression analyses were performed to test the association of lipids or markers of renal function with sex, age, disease duration, BMI SDS and median HbA1c as explanatory variables.

Statistical analyses were performed with R stats package, v 4.2.2 (www.r-project.org).

3 Results

Among the 324 T1D individuals included in this study, 156 (48%) were females. The mean age at enrollment was 13.1 ± 3.2 years, and 77% were pubertal, with a mean BMI SDS of 0.13 ± 1.1. The mean disease duration was 5.4 ± 3.6 years. Median HbA1c over the previous year was 7.69 ± 1.01% (60 mmol/mol). T1D characteristics were summarized in Table 1.

Lipid profiles and renal function markers are reported in Table 2 and Table 3, respectively.

Overall, 21% (n=65) of the participants had abnormal plasma lipid values; the percentage of dyslipidemia reached 32% (n=25) in children <11 and 18.5% (n=40) in those ≥11 years. Moreover, TG values were higher in individuals <11 (n=14) than in those ≥11 years (n=12) (16% *vs.* 6%, p-value=0.01) (Table 2). No other

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TABLE 1 Demographic, anthropometric and clinical information in T1D subjects stratified by age.

	All (n=324)	<11 years (n=93)	≥11 years (n=231)	p-value
Age mean ± sd	13.1 ± 3.2	9.03 ± 1.5	14.7 ± 2.1	**<0.001**
Sex				**0.03**
females (%)	48%	58%	44%	
males (%)	52%	42%	56%	
Standardized BMI mean ± sd	0.14 ± 1.1	-0.06 ± 1.1	0.21 ± 1.1	**0.04**
HbA1c mean ± sd	7.69 ± 1.01	7.59 ± 0.95	7.72 ± 1.03	0.26
HbA1c ≥7% (≥53 mmol/mol)	75%	69%	77%	**0.15**
Age at onset mean ± sd	7.7 ± 3.9	5.2 ± 2.4	8.7 ± 3.8	**<0.001**
Years of disease mean ± sd	5.4 ± 3.6	3.9 ± 2.4	6.0 ± .3.8	**<0.001**
Puberty yes (%)	76%	29.5%	99%	**<0.001**

Significant results were indicated in bold (p-value<0.05).
T1D subjects are stratified according to the ADA or ISPAD guidelines.
Differences by age were computed by t-test and chi-square test.

significant differences in lipid levels emerged, except for HDL-C values, which were slightly higher in children <11 than in children ≥11 years (p-value=0.047).

No significant differences emerged for eGFR. ACR levels are statistically different between non-pubertal and pubertal T1D subjects (p value=0.02), although the ACR values are in the normal range in both groups. Furthermore, around 17% of T1D subjects presented an eGFR <90 mL/min/1.73 m^2, and this percentage reached 25% in children aged ≤10 years (Table 3).

At multivariate analysis, higher TC levels were associated with female sex (p-value=0.001) and high median HbA1c over the previous year (p-value<0.001); higher LDL-C levels were associated with increasing age (p-value=0.007), female sex (p-value=0.009), high median HbA1c (p-value=0.009) and slightly with increased BMI SDS (p-value=0.046); higher HDL-C value was associated with younger age (p-value=0.001) and high median HbA1c (p-value=0.045); finally, higher TG levels were directly associated with increased median HbA1c (p-value=0.033) (Table 4).

Moreover, greater ACR levels were associated with female sex (p-value=0.01), whereas higher eGFR levels were associated with female sex (p-value=0.02) and higher HbA1c value (p-value=0.001) (Table 4). Finally, eGFR<90 was associated with male sex (p-value=0.009) and with slightly increased BMI (p-value=0.045).

4 Discussion

Despite improvements in management, kidney and cardiovascular diseases are still significant causes of mortality among people with T1D (13, 14). Although they rarely occur during childhood, changes in cardiac function and lipid levels may indicate a disease process that begins early in the course of the disease (15, 16); moreover, disease onset before the age of 15 has been associated with early onset albuminuria with a more rapid decrease in eGFR (17). Intensive education and treatment at a young age may prevent or delay the onset and progression of complications (18), with a positive 'legacy effect' even after 30 years (19). To prevent the development of such complications, diabetes management requires multi-level risk reduction strategies that include also screening for risk factors (20).

Concerning dyslipidemia screening, ADA recommended that "if initial LDL-C is <100 mg/dL, subsequent testing should be performed at 9–11 years of age" (4). Similarly, ISPAD recommends that "screening for dyslipidemia is recommended soon after diagnosis (when glycemia is stabilized) in all young people with T1D from age 11 years", in the absence of a family history of hypercholesterolemia or early cardiovascular death (5).

In the present study, we showed that not only there are no significant differences in lipids profile in children below and over 11 years, but also that rate of dyslipidemia (defined as single or combined lipids alterations) reached 32% in children <11 years compared to 18.5% in those ≥11 years.

We also found that elevated lipid levels were associated with higher HbA1c, confirming HbA1c over the previous year as one of the most important determinants of lipid profile. Moreover, LDL-C and HDL-C levels were associated with age, with a direct and inverse relationship, respectively. With regards to HDL-C (known as the "good" cholesterol for the well-documented inverse relationship with adverse cardiovascular outcomes), it has been shown that, in the presence of chronic inflammation or renal dysfunction, it might reverse its effect and become detrimental to endothelial function (21). Interestingly, children <11 years presented higher triglyceride values than adolescents (p-value=0.01), although this age group had

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TABLE 2 Lipid profile in T1D subjects stratified by age.

	All (n=324)	<11 years (n=93)	≥11 years (n=231)	p-value
TC (mg/dL) mean ± sd	164.7 ± 28.8	165.9 ± 26.4	164.2 ± 29.8	0.62
Acceptable	60%	58%	61%	0.68
Borderline	30%	33%	28%	
Abnormal	10%	9%	11%	
LDL-C (mg/dL) mean ± sd	87.9 ± 25.5	85.9 ± 23.3	88.8 ± 26.4	0.35
Acceptable	83%	86%	82%	0.30
Borderline	10%	10.5%	10%	
Abnormal	7%	3.5%	8%	
HDL-C (mg/dL) mean ± sd	62.4 ± 15.3	65.4 ± 17.2	61.2 ± 14.3	**0.047**
Acceptable	89%	92%	87%	0.10
Borderline	7%	2%	9%	
Abnormal	4%	6%	4%	
TG (mg/dL) mean ± sd	72.8 ± 37.0	76.05 ± 38.7	71.4 ± 36.4	0.33
Acceptable	77%	72%	79%	**0.01**
Borderline	14%	12%	15%	
Abnormal	9%	16%	6%	
Lipid profile				
Acceptable	43%	42%	45%	0.15
Borderline	36%	26%	36.5%	
Abnormal	21%	32%	18.5%	

Significant results were indicated in bold (p-value<0.05).
T1D subjects are stratified according to the ADA or ISPAD guidelines.
Differences by age were computed by t-test, Mann-Whitney U test and chi-square test.
HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TC, total cholesterol; TG, triglycerides.
Lipid profile was defined as acceptable, borderline, and abnormal according to the NHLBI guidelines (7).

fewer non-modifiable risk factors for diabetes mismanagement, such as puberty and long disease duration.

With regards to nephropathy screening, ADA recommended that "annual screening for albuminuria with a random spot urine sample for ACR should be considered at puberty or at age >10 years, whichever is earlier, once the child has had diabetes for five years" and that "eGFR should be considered at baseline and repeated as indicated based on clinical status, age, diabetes duration, and therapies" (4). Similar recommendations are given in the ISPAD guidelines that recommend starting screening for ACR and eGFR at puberty or age 11 years with 2-5 years of diabetes duration (5). In our sample, ACR was normal in all individuals. However, it has

TABLE 3 Kidney function markers in T1D subjects stratified by age, pubertal status and disease duration.

	All (n=324)	Age ≤10y (n=63)	Age >10y (n=261)	p-value	Pre-pubertal (n=64)	Pubertal (n=210)	p-value	Disease duration <5y (n=175)	Disease duration ≥5y (n=149)	p-value
ACR (mg/gr) mean ± sd	4.0 ± 5.7	4.6 ± 6.4	3.9 ± 5.6	0.56	6.3 ± 8.0	3.4 ± 5.1	0.02	4.5 ± 5.9	3.5 ± 5.6	0.18
eGFR (mL/min/1.73 m^2) mean ± sd	114.2 ± 33.7	113.2 ± 38.1	114.4 ± 32.8	0.59	113.4 ± 34.9	115.7 ± 33.2	0.94	113.6 ± 33.9	114.8 ± 33.6	0.89
eGFR <90 (mL/min/1.73 m^2) yes (%)	17%	25%	15%	0.26	23%	14%	0.25	19.5%	14%	0.42

Differences were computed by Mann-Whitney U test and chi-square test.
T1D subjects are stratified according to the ADA or ISPAD guidelines.
ACR, urinary albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate.

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TABLE 4 Factors associated with lipids and renal function as assessed by multivariate regression models.

	Lipids				Renal function		
Explanatory variables	**Total Cholesterol** (mg/dL)	**LDL Cholesterol** (mg/dL)	**HDL Cholesterol** (mg/dL)	**Triglycerides** (mg/dL)	**ACR** (mg/gr)	**eGFR** (mL/min/1.73 m^2)	**eGFR** <90 (mL/min/1.73 m^2) % yes
Age	0.41 (0.44)	**0.007 (1.29)**	**0.001 (-0.95)**	0.72 (-0.25)	0.69 (0.06)	0.46 (-0.59)	0.59 (-0.94)
Sex	**0.001 (-10.5)**	**0.009 (-7.61)**	0.46 (-1.30)	0.22 (-5.27)	**0.017 (-2.4)**	**0.02 (-11.1)**	**0.009 (0.06)**
Years of disease	0.61 (-0.25)	0.36 (-0.40)	0.33 (0.26)	0.44 (0.50)	0.62 (-0.07)	0.79 (-0.19)	0.56 (-0.03)
Standardized BMI	0.11 (2.34)	**0.046 (2.65)**	0.64 (-0.38)	0.22 (2.44)	0.26 (-0.49)	0.21 (-2.70)	**0.045 (0.35)**
Median HbA1c	**<0.001 (6.8)**	**0.009 (3.82)**	**0.045 (1.80)**	**0.033 (4.71)**	0.18 (-0.65)	**0.001 (7.42)**	0.14 (-0.30)

The values are p-value and beta in brackets.
Significant results were indicated in bold (p-values<0.05).
For the non-quantitative Sex variable, the reference category is female.

been proposed that non-albuminuric patients with diabetes may progress in any case toward chronic kidney disease; as a matter of fact, in a previous study also pediatric individuals with normal albuminuria but mildly reduced eGFR (60-89 mL/min/1.73 m^2) showed a worst cardiometabolic risk profile with higher levels of insulin requirement, TG/HDL-C ratio, neutrophil/lymphocytes ratio, blood pressure, uric acid, and low HDL-C levels (22). The rate of subjects with an eGFR value below 90 mL/min/1.73 m^2 in the present study was 17%, and this figure reached 25% in children under the age of 10 and 23% in the prepubertal stage, although there were no statistically significant differences between age, pubertal status, or disease duration. On the contrary, higher eGFR was associated with higher HbA1c, that directly correlates with hyperfiltration, also in individuals with short duration of T1D (23).

5 Limitations of the study

A potential limitation of this study is the lack of information on LDL-C levels at onset and family history. In addition, we have no information on other markers for the screening of renal function, such as Cystatin C. Lastly, the recruitment of only European samples, makes our findings not generalizable to other ethnic groups. Despite these limitations, the accuracy of collected clinical and demographic information is the key strength of the present study.

6 Conclusions

The findings of our study show that diabetes-associated risk factors, such as abnormal lipid profiles and mildly reduced eGFR, are already present in childhood, suggesting that screening for markers of diabetic complications should be performed regardless of age, pubertal stage, or disease duration, to optimize glycemia and medical nutrition therapy and/or to start a specific medical treatment. This should be considered in clinical management and the following standards of medical care.

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 ethics committee approved the study [CEUR-2018-Em-323-Burlo (Italy) and KME-0120-65/2019/4]. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

EC, AR, and GTo designed and performed the research. EC, KD, DT, GTa, RB, RF acquired data. EC and AR analyzed and interpreted data. EC, AR, and GTo drafted the manuscript. EC, AR, KD, DT, GTa, RB, RF, IR, TB, and GTo revised the manuscript. GTo is the guarantor of this work and, as such, has full access to all the data in the study and takes 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

References

1. Bojestig M, Arnqvist HJ, Hermansson G, Karlberg BE, Ludvigsson J. Declining incidence of nephropathy in insulin-dependent diabetes mellitus. *N Engl J Med* (1994) 330(1):15–8. doi: 10.1056/nejm199401063300103

2. Downie E, Craig ME, Hing S, Cusumano J, Chan AK, Donaghue KC. Continued reduction in the prevalence of retinopathy in adolescents with type 1 diabetes: role of insulin therapy and glycemic control. *Diabetes Care* (2011) 34(11):2368–73. doi: 10.2337/dc11-0102

3. Majaliwa ES, Munubhi E, Ramaiya K, Mpembeni R, Sanyiwa A, Mohn A, et al. Survey on acute and chronic complications in children and adolescents with type 1 diabetes at muhimbili national hospital in dar es salaam, Tanzania. *Diabetes Care* (2007) 30(9):2187–92. doi: 10.2337/dc07-0594

4. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. 14. children and adolescent: standard of care in diabetes-2023. *Diabetes Care* (2023) 146(Suppl 1):S230–53. doi: 10.2337/dc23-S014

5. Bjornstad P, Dart A, Donaghue KC, Dost A, Feldman EL, Tan GS, et al. ISPAD clinical practice consensus guidelines 2022: microvascular and macrovascular complications in children and adolescents with diabetes. *Pediatr Diabetes* (2022) 23(8):1432–50. doi: 10.1111/pedi.13444

6. Haller MJ, Stein J, Shuster J, Theriaque D, Silverstein J, Schatz DA, et al. Peripheral artery tonometry demonstrates altered endothelial function in children with type 1 diabetes. *Pediatr Diabetes* (2007) 8(4):193–8. doi: 10.1111/j.1399-5448.2007.00246.x

7. Fornari E, Piona C, Rabbone I, Cardella F, Mozzillo E, Predieri B, et al. Cardiovascular risk factors in children and adolescents with type 1 diabetes in Italy: a multicentric observational study. *Pediatr Diabetes* (2020) 21(8):1546–55. doi: 10.1111/pedi.13123

8. Schwab KO, Doerfer J, Hecker W, Grulich-Henn J, Wiemann D, Kordonouri O, et al. Spectrum and prevalence of atherogenic risk factors in 27,358 children, adolescents, and young adults with type 1 diabetes: cross-sectional data from the German diabetes documentation and quality management system (DPV). *Diabetes Care* (2006) 29(2):218–25. doi: 10.2337/diacare.29.02.06.dc05-0724

9. Favel K, Irvine M, Ronsley R, Panagiotopoulos C, Mammen C. Glomerular filtration rate abnormalities in children with type 1 diabetes. *Can J Diabetes* (2022) 46 (5):457–63.e1. doi: 10.1016/j.jcjd.2022.01.007

10. Expert Panel of Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescent; National Heart, Lung and Blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. *Pediatrics* (2011) 128(5):S213–56. doi: 10.1542/peds.2009-2107C

11. Levey AS, Eckardt K-U, Dorman NM, Christiansen SL, Hoorn EJ, Ingelfinger JR, et al. Nomenclature for kidney function and disease: report of a kidney disease: improving global outcomes (KDIGO) consensus conference. *Kidney Int* (2020) 97 (6):1117–29. doi: 10.1016/j.kint.2020.02.010

12. Schwartz GJ, Muñoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol* (2009) 20 (3):629–37. doi: 10.1681/ASN.2008030287

13. Groop PH, Thomas MC, Moran JL, Wadèn J, Thorn LM, Mäkinen VP, et al. The presence and severity of chronic kidney disease predicts all-cause mortality in type 1 diabetes. *Diabetes* (2009) 58(7):1651–8. doi: 10.2337/db08-1543

14. Sharma H, Lencioni M, Narendran P. Cardiovascular disease in type 1 diabetes. *Cardiovasc Endocrinol Metab* (2019) 8(1):28–34. doi: 10.1097/XCE.0000000000000167

15. Hensel KO, Grimmer F, Roskopf M, Jenke AC, Wirth S, Heusch A. Subclinical alterations of cardiac mechanics present early in the course of pediatric type 1 diabetes mellitus: a prospective blinded speckle tracking stress echocardiography study. *J Diabetes Res* (2016) 2016:2583747. doi: 10.1155/2016/2583747

16. Maahs DM, Dabelea D, D'Agostino RBJr, Andrews JS, Shah AS, Crimmins N, et al. Glucose control predicts 2-year change in lipid profile in youth with type 1 diabetes. *J Pediatr* (2013) 162(1):101–7.e1. doi: 10.1016/j.jpeds.2012.06.006

17. Edqvist J, Rawshani A, Rawshani A, Adiels M, Franzén S, Bjorck L, et al. Trajectories in HbA1c and other risk factors among adults with type 1 diabetes by age at onset. *BMJ Open Diabetes Res Care* (2021) 9(1):e002187. doi: 10.1136/bmjdrc-2021-002187

18. Diabetes Control and Complications Trial Research Group. Effect of intensive diabetes treatment on developing and progressing long-term complications in adolescents with insulin-dependent diabetes mellitus: diabetes control and complications trial. *J Pediatr* (1994) 125(2):177–88. doi: 10.1016/s0022-3476(94)70190-3

19. Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular outcomes in type 1 diabetes: the DCCT/EDIC study 30-year follow-up. *Diabetes Care* (2016) 39(5):686–93. doi: 10.2337/dc15-1990

20. Lithovius R, Harjutsalo V, Forsblom C, Saraheimo M, Groop PH. The consequences of failure to achieve targets of guidelines for prevention and treatment of diabetic complications in patients with type 1 diabetes. *Acta Diabetol* (2015) 52 (1):31–8. doi: 10.1007/s00592-014-0595-x

21. Chiesa ST, Charakida M, McLoughlin E, Nguyen HC, Georgiopoulos G, Motran L, et al. Elevated high-density lipoprotein in adolescents with type 1 diabetes is associated with endothelial dysfunction in the presence of systemic inflammation. *Eur Heart J* (2019) 40(43):3559–66. doi: 10.1093/eurheartj/ehz114

22. Di Bonito P, Mozzillo E, Rosanio FM, Maltoni G, Piona CA, Franceschi R, et al. Albuminuric and non-albuminuric reduced eGFR phenotypes in youth with type 1 diabetes: factors associated with cardiometabolic risk. *Nutr Metab Cardiovasc Dis* (2021) 31(7):2033–41. doi: 10.1016/j.numecd.2021.03.019

23. Soper CP, Barron JL, Hyer SL. Long-term glycaemic control directly correlates with glomerular filtration rate in early type 1 diabetes mellitus before the onset of microalbuminuria. *Diabetes Med* (1998) 15(12):1010–4. doi: 10.1002/(SICI)1096-9136(1998120)15:12<1010:AID-DIA703>3.0.CO;2-H

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Development and implementation of a workshop for young adults with diabetes entering college and the workforce

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The process of transitioning from pediatric to adult diabetes care for adolescents and young adults is challenging. This transition period may include many life changes, and can be fraught with worsening glycemic control leading to increased risk for diabetes-related hospitalizations and complications. Research has demonstrated that increased support during this period can help maintain engagement in diabetes care. Transition guidelines highlight the importance of preparation and readiness for transition. In this article, we discuss the development, implementation and content of a workshop for patients and parents/caregivers preparing for the transition to college, the workforce and adult diabetes care.

KEYWORDS

type 1 diabetes, continuous glucose monitors, prescriptions, transition, young adult

Background

The process of transitioning from pediatric to adult health care for adolescents and young adults with chronic conditions is challenging. This period is a time of major changes in school, work and living environment. Young adults with type 1 diabetes face even greater challenges during this transition period with the additional stress of managing their disease and maintaining glycemic control. Almost 50% of young adults report difficulties in transitioning from pediatric to adult diabetes care, and almost 30% disengage from medical care during this period (1–3). Attendance at follow-up appointments declines and there is often an associated rise in hemoglobin A1c levels (4, 5). In general, hemoglobin A1c levels during this age group are at their peak, and thus there is increased risk for hospitalization and diabetes-related complications (5, 6).

Studies have identified the need for more support during the transition period for young adults with type 1 diabetes (7). Trials implementing care navigators and intensive transition coordinator support have shown promising results in facilitating engagement and clinic attendance in this age group (8, 9). The American Diabetes Association (ADA) and others have developed transition guidelines to help guide providers during this period (10–12). The importance of transition preparation through education and counseling promotes transition readiness for both patients and their caregivers (11).

Development of the diabetes transition/off to college event

In 2018, we developed a workshop for young adults and their parents/caregivers to help prepare them for transitioning into the workforce, moving out of their parents' home, or going to college. We hosted this event in collaboration with key stakeholders including the JDRF and The DiabetesLink (formerly College Diabetes Network (CDN)). (Appendix A: Event flyer). Workshop invitations were directed to high school juniors and seniors, as well as young adults who recently graduated from high school or were entering the workforce, with both type 1 and type 2 diabetes. At our institution, patients are usually transitioned to adult diabetes care one-year after high school, but can remain in the pediatric practice at the discretion of the provider based upon transition readiness.

Event structure

The workshop was structured as a 2-hour event where the first hour included a series of brief presentations hosted by faculty, followed by a panel discussion featuring students who already transitioned to college or the workforce, and some with their parents/caregivers as dyads. (Appendix B: Agenda) The platform for this event was initially an in-person workshop, but due to the COVID-19 pandemic beginning in 2020, the workshop was switched to a virtual (zoom) platform. Both formats have been successful and engaging, but an in-person workshop allows for either small group discussions (one for the students and one for the parents), or panel discussions; whereas on the zoom platform a panel discussion has been easier to facilitate. In order to engage participants in the workshop, the hosts prepared a list of questions to get the panel started, and then the attendants were requested to submit questions to the host, which were then asked of the panel discussants. At the completion of the workshop, attendees were able to provide feedback through a brief survey of what was most helpful and what could be improved in the program.

Event timing

The first year that we hosted this event in 2018, the event was held on a Sunday in May, but feedback from parents was that it felt too close to the time the students were leaving for college or moving out of the house and felt that more preparation time would have been helpful. Based on this feedback, in subsequent years, this event has been held on a Sunday in March.

Topics covered during the workshop

Finding an adult diabetes provider

This presentation is given by an adult endocrinologist. During this portion of the presentation, we emphasize to patients and their parents that the overall goals of care are similar between adult and pediatric providers, namely, to help the patient achieve the best control of blood glucose that is possible for them, and to ensure good health now and in the future. The adult provider's perspective includes more focus on control of cardiovascular risk factors and management of microvascular complications. However, unlike tertiary care pediatric centers, many adult providers do not have onsite nutritionists, psychologists and diabetes educators so often separate appointments for these providers have to be made. To ensure good continuity of care, the participants are reminded that they should identify potential adult providers 6-9 months ahead of time since adult clinics often have long waits for new patient appointments and that a good time for such an appointment could be soon after graduation from high school, or later depending on the planned transition time. At the first appointment with their new provider it's important to identify key clinical and administrative staff in the office, as well as procedures for obtaining refills. If the patient is going away for college, we discuss that some individuals choose to have a second provider at their college, while maintaining a provider in their hometown. Regardless of diabetes provider location, we recommend setting up an appointment with the college student health center for the student to be aware of local resources for diabetes care and management.

Setting up disability accommodations at college

This presentation discusses the process of registering at their school's Disability Services office prior to the start of classes to facilitate reasonable accommodations for diabetes management in the classroom and on campus. While some students are hesitant to self-identify as having a disability, and are comfortable with informal accommodations provided in high school, they are encouraged to register with Disability Services prior to an issue arising to ensure equal opportunity to succeed in college. Students are encouraged to share a list of proposed accommodations and rationales with their medical team for submission. In this presentation, we review commonly requested accommodations such as ability to pause exams in case of hypoglycemia or hyperglycemia, continuous access to diabetes technology including cell phones for continuous glucose monitoring, and permission to keep a minifridge in the dorm room for insulin storage. As an example, the speaker will demonstrate how to navigate a local college website to identify the Disability services office and find the appropriate forms and contact information.

Establishing care and obtaining prescriptions at college

Maintaining diabetes care during college

This presentation encourages students to maintain frequent communication with their primary pediatric or adult diabetes team

while in college, and have their team's contact information readily accessible for urgent or after-hours needs. We also suggest students identify the nearest emergency room in case of an emergency, and also determine the capabilities and limitations of the student health office if unable to reach their primary endocrinology team.

Prescription planning

Students and parents should also develop a plan for obtaining prescriptions and supplies, and whether these will be picked up locally or shipped from their parents' home. Prescription plan logistics will depend on the student's insurance as well as proximity to home. We highlight the importance of developing a system for the student to identify when to begin the reordering process to avoid running out of medications or supplies. Families are encouraged to transition the responsibility for medication and supply management to the student during senior year of high school so that parents can provide oversight and guidance before the student moves away from home. This is especially important given the frequent confusion on whether supplies are coming from a pharmacy or DME company, and who to contact for refills. Patients are also encouraged to identify a local pharmacy near the college in case of an urgent medication need.

Nutrition & physical activity

This presentation is given by a dietitian (RD), who discusses navigating the dining hall in college and adjusting to a variable eating schedule typical in college or in the workforce. Nutrition choices and food intake often change when young adults leave home and live at college. Dining halls and cafeterias can be challenging to navigate for both food choices and carbohydrate counting. During this presentation, the RD discusses strategies on carbohydrate counting in dining halls and college food establishments. Some colleges have nutrition information on their dining websites, or have phone apps available so that students can see menus and associated nutrition information. The speaker demonstrates how to navigate a school nutrition website for a local college as an example, and how to contact the campus dietitian for additional information. An email/letter template is shared for contacting the school dietitian with request for carbohydrate information and possibly food additions or modifications to the cafeteria menu (e.g. sugar-free pancake syrup, high protein yogurt).

College dining halls often have many food options for main courses, sides, and desserts. The RD discusses how to make healthy food choices for balanced meals, in order to avoid large glycemic excursions, promote overall heart healthy food intake and healthy weight management. Estimating portions is also reviewed as the college student is unlikely to use measuring cups in the dining hall. Sample serving sizes are visualized with images and objects for different food types to help students estimate carbohydrate content.

The speaker also discusses managing meals with a variable schedule of classes and activities in college, and managing late-night eating. The dietitian reviews tips such as planning meals and snacks, which may include packing food to go, or learning about different dining halls on campus and their hours of operation. The RD provides suggestions for complex carbohydrates and protein sources to keep in their dorm room, which can be eaten as a quick meal or on-the-go.

In the last part of the nutrition presentation, the dietitian discusses the benefits of physical activity and ways to stay physically active on campus while also managing blood glucose with exercise. The speaker reviews exercise management with multiple daily injection verses insulin pumps (including hybrid closed loop systems). The discussion includes utilizing exercise functions on pumps, modifying insulin doses at meals prior to exercise, and food choices to help maintain blood glucose levels during activity. The RD provides guidance on complex carbohydrate and protein consumption to help optimize athletic performance, with examples such as peanut butter sandwich on whole wheat bread, or protein granola bar. The speaker also discusses differing carbohydrate needs for blood glucose maintenance and treatment of hypoglycemia depending on type of insulin delivery (injections, pumps, and hybrid-closed loop systems).

Managing sick days, hyperglycemia and hypoglycemia

This presentation reviews hyperglycemia, sick day management and hypoglycemia management. This includes a review of when to check for ketones, managing hyperglycemia when sick, and the signs and symptoms of DKA. A list of scenarios of when to seek help are reviewed. Hypoglycemia management is also reviewed. Importantly, if students have roommates, we remind them to demonstrate to the roommate where the glucagon is stored and how to give glucagon in an emergency. The list of contents for an "emergency diabetes kit" are outlined. In this discussion, wearing a medical alert bracelet is reinforced, as well as setting up a Medical ID on the phone for emergency responders to access if needed.

Consumption of alcohol and other legal substances for young adults over age 21

This presentation reviews responsible behavior, and effects of alcohol and other substances on diabetes. While consumption of alcohol is not condoned, it is a likely exposure after high school or in college, and thus it is important to discuss the effects of alcohol on diabetes. This presentation reviews that alcohol is predominantly metabolized by the liver, and with alcohol consumption, the liver's ability to perform gluconeogenesis and glycogenolysis is reduced and can lead to hypoglycemia. Thus, it is recommended to drink alcohol responsibly and limit to 1-2 drinks, always eat carbohydrates while drinking alcohol, and monitor blood sugars closely for potential delayed hypoglycemia (13).

In some states, recreational cannabis is legal for young adults 18 years of age and over. Recreational cannabis use can result in acute adverse events with increased risk for DKA and higher hemoglobin A1c. In this presentation, we review the effects of marijuana, and the increased food consumption typically associated with cannabis use that can lead to hyperglycemia.

Communication with colleagues, roommates and parents

Communication with peers and instructors

This presentation is usually given by a psychologist at our center, but can be given by anyone on the diabetes team. Upon arrival to college, students should share their diabetes diagnosis with their roommate, resident assistant, and other important adults such as coaches. For those joining the workforce, it is important to inform co-workers and bosses. While these individuals are not expected to participate in the student's diabetes management, they should know how to identify an emergency and respond when the student with diabetes needs assistance. In this presentation, the students are encouraged to practice having these conversations, as these initial conversations can be a source of anxiety. As an example, the speaker may role-play how to open this conversation with a new roommate, or start a conversation about diabetes with their athletic coach. Further, advice on seeking mental health resources and peer support on campus is discussed.

Communication with parents/caregivers

Parents and students are also encouraged to discuss expectations regarding communication, and set up a schedule for touching base about diabetes management successes or issues. With the advent of continuous glucose monitoring, families must establish boundaries around outreach to their college students regarding blood glucose management. Some families identify blood sugar thresholds above or below which parents will contact the student and/or other individuals such as roommate or resident assistants, while some families opt not to use glucose sharing technology. Students are also encouraged to utilize other resources and apps that connect to their continuous glucose monitors and notify them of an untreated low. The speaker also reminds parents to avoid focusing every conversation around diabetes when speaking with their children at college.

Partner organization resources

The series of presentations (Table 1) concludes with an overview of resources available from diabetes organizations, including JDRF and DiabetesLink (formerly College Diabetes Network, CDN). These resources are shared with the participants by email after the event.

Discussion

Transition preparation and readiness are important to ensure a smooth transition process from pediatric to adult diabetes care that occurs during a time of many life-transitions. Readiness should apply to both adolescents and young adults, as well as their parents and caregivers (9). The implementation of a workshop to prepare and ready patients and caregivers for the upcoming transition to college or into the workforce, and leaving the home has been a successful addition to our transition program for all young adults with diabetes. Feedback from patients and caregivers has reflected important knowledge acquisition, and insight on how to set-up accommodations at college or work, eating on a variable schedule, and ideal communication patterns among all

TABLE 1 Diabetes transition and off to college workshop content.

Presentation Title	Content covered in each presentation
Welcome & Introduction	-Transition period from adolescence to adulthood -Challenges in the diabetes transition period -Importance of transition preparation and readiness
Finding an Adult Provider	-Differences in adult and pediatric diabetes care -Scheduling with an adult provider in advance
Nutrition & Exercise	-Navigating meals and eating on an erratic schedule -Considerations for carbohydrate counting in the dining hall -Planning for exercise and physical activity
Disability Services & Accessing Prescriptions	-Setting up accommodations with the Disability office -Considerations for accommodations at school and work -Transitioning management of prescriptions from caregiver to child -Prescription planning at college and identifying local pharmacies -Identifying local care for urgent issues and emergencies
Managing Sick Days, Hyper- and Hypoglycemia	-Review management for sick days and checking for ketones -Acute management of hypoglycemia review -Emergency medications and training roommates
Alcohol and Drugs with Diabetes*	-Responsible intake of alcohol and adverse effects on diabetes management -Marijuana use and effects on diabetes (in states where it is legal) *if applicable to students >21years of age
Healthy Communication	-Communications about diabetes with peers, professors and coaches -Managing communication expectations between students and parents/caregivers
Partner Organization Resources	-Invite local diabetes organizations to present and share resources (example: JDRF, The Diabetes Link)

stakeholders. We hope this article provides a framework for other diabetes programs to establish and host a similar program for their young adults and patients transitioning to college or the work-force.

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

RW: Conceptualization, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. MW: Conceptualization, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing. AS: Conceptualization, Project administration, Resources, Writing – original draft, Writing – review & editing. MT: Conceptualization, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing.

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.2023.1288215/full#supplementary-material

References

1. Insabella G, Grey M, Knafl G, Tamborlane W. The transition to young adulthood in youth with type 1 diabetes on intensive treatment. *Pediatr Diabetes* (2007) 8:228–34. doi: 10.1111/j.1399-5448.2007.00266.x

2. Sparud-Lundin C, Ohrn I, Danielson E, Forsander G. Glycaemic control and diabetes care utilization in young adults with Type 1 diabetes. *Diabetes Med* (2008) 25:968–73. doi: 10.1111/j.1464-5491.2008.02521.x

3. Kipps S, Bahu T, Ong K, Ackland FM, Brown RS, Fox CT, et al. Current methods of transfer of young people with Type 1 diabetes to adult services. *Diabetes Med* (2002) 19:649–54. doi: 10.1046/j.1464-5491.2002.00757.x

4. Cadario F, Prodam F, Bellone S, Trada M, Binotti M, Trada M, et al. Transition process of patients with type 1 diabetes (T1DM) from paediatric to the adult health care service: a hospital-based approach. *Clin Endocrinol (Oxf)* (2009) 71:346–50. doi: 10.1111/j.1365-2265.2008.03467.x

5. Foster NC, Beck RW, Miller KM, Clements MA, Rickels MR, DiMeglio LA, et al. State of type 1 diabetes management and outcomes from the T1D exchange in 2016-2018. *Diabetes Technol Ther* (2019) 21:66–72. doi: 10.1089/dia.2018.0384

6. Bryden KS, Dunger DB, Mayou RA, Peveler RC, Neil HA. Poor prognosis of young adults with type 1 diabetes: a longitudinal study. *Diabetes Care* (2003) 26:1052–7. doi: 10.2337/diacare.26.4.1052

7. Hynes L, Byrne M, Dinneen SF, McGuire BE, O'Donnell M, Mc Sharry J., et al. Barriers and facilitators associated with attendance at hospital diabetes clinics among young adults (15-30 years) with type 1 diabetes mellitus: a systematic review. *Pediatr Diabetes* (2016) 17:509–18. doi: 10.1111/pedi.12198

8. Spaic T, Robinson T, Goldbloom E, Gallego P, Hramiak I, Lawson ML, et al. Closing the gap: results of the multicenter canadian randomized controlled trial of structured transition in young adults with type 1 diabetes. *Diabetes Care* (2019) 42:1018–26. doi: 10.2337/dc18-2187

9. White M, O'Connell MA, Cameron FJ. Clinic attendance and disengagement of young adults with type 1 diabetes after transition of care from paediatric to adult services (TrACeD): a randomised, open-label, controlled trial. *Lancet Child Adolesc Health* (2017) 1:274–83. doi: 10.1016/S2352-4642(17)30089-5

10. Association AD. Standards of medical care in diabetes—2019 abridged for primary care providers. *Clin Diabetes* (2019) 37:11–34. doi: 10.2337/cd18-0105

11. Lindholm Olinder A, DeAbreu M, Greene S, Haugstvedt A, Lange K, Majaliwa ES, et al. ISPAD Clinical Practice Consensus Guidelines 2022: Diabetes education in children and adolescents. *Pediatr Diabetes* (2022) 23:1229–42. doi: 10.1111/pedi.13418

12. Peters A, Laffel LAmerican Diabetes Association Transitions Working, Diabetes care for emerging adults: recommendations for transition from pediatric to adult diabetes care systems: a position statement of the American Diabetes Association, with representation by the American College of Osteopathic Family Physicians, the American Academy of Pediatrics, the American Association of Clinical Endocrinologists, the American Osteopathic Association, the Centers for Disease Control and Prevention, Children with Diabetes, The Endocrine Society, the International Society for Pediatric and Adolescent Diabetes, Juvenile Diabetes Research Foundation International, the National Diabetes Education Program, and the Pediatric Endocrine Society (formerly Lawson Wilkins Pediatric Endocrine Society). *Diabetes Care* (2011) 34:2477–85. doi: 10.2337/dc11-1723

13. White ND. Alcohol use in young adults with type 1 diabetes mellitus. *Am J Lifestyle Med* (2017) 11:433–5. doi: 10.1177/1559827617722137

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Semaglutide as a potential therapeutic alternative for HNF1B-MODY: a case study

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Maturity-onset diabetes of the young (MODY) is a grouping of monogenic disorders. It is characterized by dominantly inherited, non-insulin-dependent diabetes. MODY is relatively rare, encompassing up to 3.5% in those diagnosed under 30 years of age. Specific types are most commonly treated with sulfonylurea, particularly those identified as HNF4A-MODY and HNF1A-MODY. HNF1B-MODY is another type that is most frequently managed with insulin therapy but lacks a defined precision treatment. We present an 18-year-old, non-obese female patient diagnosed with HNF1B-MODY. She displays complete gene deletion, a renal cyst, and hypomagnesemia. Her treatment plan includes both long- and short-acting insulin, though she frequently encountered hypoglycemia and hyperglycemia. Semaglutide, a GLP-1RA, was administered weekly over 4 months. The patient's glucose level was continuously tracked using Dexcom's Continuous Glucose Monitoring system. The data suggested a notable improvement in her condition: time-in-range (TIR) increased from 70% to 88%, with some days achieving 100%, and the frequency of hypoglycemic episodes, indicated by time-below-range values, fell from 5% to 1%. The time-above-range values also dropped from 25% to 10%, and her HbA1c levels declined from 7% to 5.6%. During the semaglutide therapy, we were able to discontinue her insulin treatment. Additionally, her body mass index (BMI) was reduced from 24.1 to 20.1 kg/m^2. However, the semaglutide treatment was halted after 4 months due to side effects such as nausea, vomiting, and reduced appetite. Other contributing factors included exam stress and a COVID-19 infection, which forced a switch back to insulin. Her last recorded HbA1c level under exclusive insulin therapy rose to 7.1%, and her BMI increased to 24.9 kg/m^2. In conclusion, semaglutide could potentially replace insulin to improve glucose variability, TIR, and HbA1c in patients with HNF1B-MODY. However, more extensive studies are required to confirm its long-term safety and efficacy.

KEYWORDS

HNF1B-MODY, semaglutide, successful, MODY, GLP-1RA

Introduction

Maturity-onset diabetes of the young (MODY) is a genetically inherited disease caused by specific mutations that affect the development of pancreatic beta cells (1). HNF1B-MODY is one of more than 40 known subtypes of monogenic diabetes, each possessing its own unique inheritance pattern and phenotype (2). There have been at least 14 MODY types identified, representing 1%–5% of all diabetes mellitus cases, with an estimated prevalence of 1–5 per 10,000 people, according to European studies (3).

The most common types of MODY are GCK-MODY and HNF1A-MODY. HNF1B-MODY, however, is not as prevalent in the UK population (4). Most people with GCK-MODY do not require treatment. HNF4A-MODY and HNF1A-MODY, in contrast, can be effectively treated with sulfonylurea. However, patients with HNF1B-MODY are typically not responsive to sulfonylurea treatment and often require insulin injections multiple times a day (5).

The HNF1B gene is essential for the embryonic development of several tissues, such as the liver, kidney, gut, and pancreatic islet cells. It also contributes to the exact regulation of gene expression in these tissues (6), explaining the varying symptoms of the associated disease. Multiple phenotypes linked to HNF1B-MODY are observed. These include cystic kidneys, unexplained renal dysfunction, genitourinary abnormalities, gout, hypomagnesemia, primary hyperparathyroidism, abnormalities in the liver and intestines, and a rare type of kidney cancer (7).

The insufficient response to sulfonylurea and the early need for insulin can be attributed to a certain level of hepatic insulin resistance and insulin deficiency resulting from pancreatic hypoplasia (8). This case report describes an 18-year-old female patient with HNF1B-MODY, who was discovered to have a complete gene deletion (heterozygous for a 1.82-Mb deletion encompassing the entire HNF1B gene) and underwent testing for semaglutide (GLP-1RA).

A female patient, 18 years of age, first arrived at the emergency department at age 12 with symptoms of polyuria, polydipsia, and weight loss ongoing for the past 2 months. Her initial random blood glucose level was found to be 414 mg/dL, leading to a diagnosis of diabetes. She was then put on a regimen of daily doses of long-acting and ultra-short-acting insulin, amounting to 1 unit/kg/day. No insulin-related autoantibodies, including insulin autoantibodies, anti-glutamic acid decarboxylase antibodies, anti-insulin-associated protein two antibodies, or anti-zinc transporter eight antibodies, were found in her blood. Her initial HbA1c level was more than 15%, indicating high blood glucose levels over the past few months, but with no ketosis or ketonuria present and normal blood gases.

Soon after her discharge, her insulin requirements dropped to less than 0.4 units/kg/day due to frequent episodes of low blood sugar. However, her HbA1c levels, indicative of blood glucose control, consistently remained between 6% and 7.7% over the first year. This led to a suspicion of MODY, prompting whole-exome sequencing. The initial results were negative, but a follow-up deletion duplication test revealed that she was a heterozygous carrier of a 1.82-Mb deletion encompassing the entire HNF1B gene (9). This genetic variant was a de novo (parent's genetic test were negative) and is recognized as a pathogenic confirmation of HNF1B-MODY, a specific subtype that also involves renal cysts and diabetes.

Her family history revealed consanguinity in her healthy parents, with several extended family members suffering from type 2 diabetes but without any kidney dysfunction. During her initial physical examination, she looked healthy and exhibited no unusual physical features such as a goiter or acanthosis nigricans. Her growth parameters were within normal limits; her height of 154 cm fell in the 10th–25th percentile range, her body mass index (BMI) of 26.9 kg/m^2 fell within the 75th–85th percentile range, and she was at the fifth pubertal stage for breast and pubic hair development.

Laboratory results showed a below-normal magnesium level of 0.5 mmol/L (standard range: 0.86–1.17 mmol/L), while the levels of potassium and other electrolytes were normal. An abdominal ultrasound uncovered a simple cyst on the upper left lobe of the kidney, an unaffected pancreas, and incidental spleen calcification.

She was treated with magnesium supplements and a daily dose of 0.6–0.7 units/kg/day of both long- and short-acting insulin for several years. After obtaining the necessary ethical approval (IRB/0317/23) and informed consent, an initial dosage of 0.25 mg of semaglutide was administered weekly to the 18-year-old patient for 1 month. This was gradually increased to a maximum dose of 0.5 mg weekly to ensure tolerance. Dexcom Continuous Glucose Monitoring (CGM) was utilized to monitor the patient's status prior to (Figure 1A) and during (Figure 1B) the semaglutide trial. This report will present 1 month's worth of CGM data.

During the trial period, she significantly improved her HbA1c drop from 7.0% to 5.6%—the lowest it has ever been. There were also remarkable improvements in her CGM metrics. Her time-in-range (TIR) went from 70% to 88% and even reached 100% on her best days. Her time-below-range and time-above-range also improved, decreasing from 5% to 1% and 25% to 10%, respectively. The coefficient of variation dropped from 34.3% to 33.4%, and on some days, it reduced further to 27% (Figures 1A, B). On top of these, her insulin therapy was discontinued, and her BMI decreased from 24.1 kg/m^2 (75th–85th percentile) to 20.1 kg/m^2 (25th–50th percentile).

The semaglutide treatment was halted after 4 months due to various factors. These included side effects like nausea, vomiting, and reduced appetite. Additionally, family issues, exam stress, and a concurrent COVID-19 infection significantly suppressed her appetite. Insulin therapy was resumed as a result. Consequently, her last recorded HbA1c value increased to 7.1% under insulin therapy alone, and her BMI rose to 24.9 kg/m^2 (Table 1).

Discussion

MODY disrupts insulin biosynthesis and destroys pancreatic beta cells (10), generally manifesting between adolescence and early adulthood (ages 13 to 25) (11, 12). It accounts for approximately 1%–3% of all pediatric and adult diabetes, stemming from

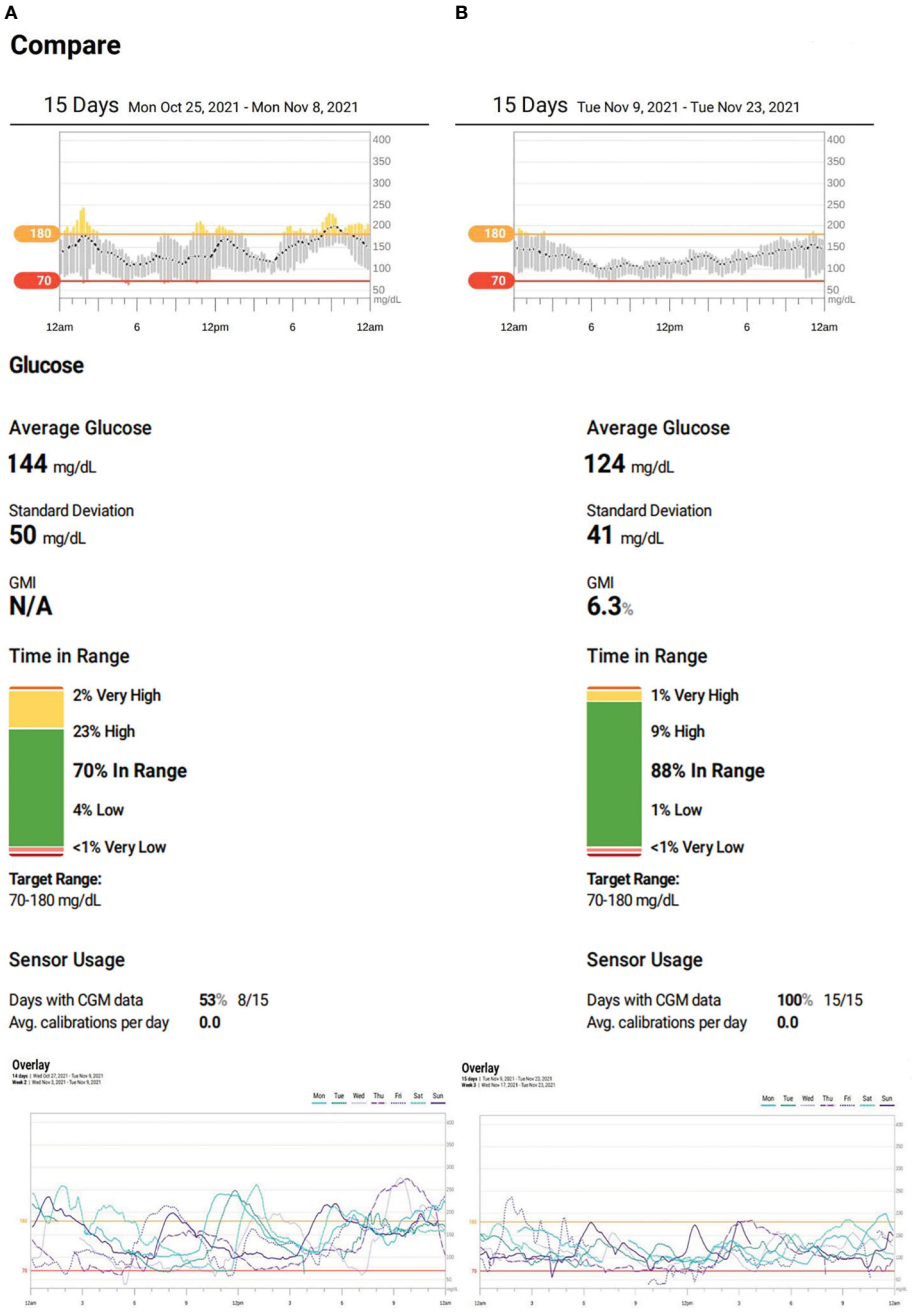

FIGURE 1
(A) Patient's continuous glucose monitoring (CGM) on insulin therapy. **(B)** Patient's CGM on semaglutide.

monogenic diabetes, a condition mainly impacting beta-cell function due to mutations. The most frequent causes of MODY are mutations in HNF1A and GCK, while HNF4A and HNF1B mutations are less common (13).

The HNF1B gene, which regulates the expression of other genes, binds to specific DNA regions. Its protein is found in numerous organs and tissues, including the liver, lungs, intestines, pancreas, kidneys, reproductive system, and urinary tract. This widespread presence accounts for the disease's phenotype (14).

HNF1B-MODY can lead to a range of kidney abnormalities, including bilaterally hyperechogenic kidneys visible on prenatal ultrasound, multiple renal cysts, dysplastic multicystic kidneys, single kidneys, horseshoe kidneys, tubular dysfunction, nephropathy caused by hyperuricemia resulting in gout, renal magnesium depletion, and chromophobe renal carcinomas. Comparatively, her kidney symptoms were less severe, presenting as hypomagnesemia and renal cysts.

The pancreas can exhibit extra-renal characteristics such as early-onset diabetes, diabetes that occurs after transplantation, exocrine dysfunction, and the absence of the pancreatic body and tail. In female patients, abnormalities such as a double uterus, underdeveloped vagina, and absence of the uterus have been

TABLE 1 BMI, HbA1c, and CGM metric pre-, on, and post-semaglutide treatment.

Parameters	Before semaglutide therapy (insulin 0.7 units/kg/day)	During semaglutide therapy (O.5 mg weekly)	3 months off semaglutide therapy (insulin 0.7 units/kg/day)
BMI kg/m^2	24.1 (75th-85th percentile)	20.1 (25th-50th percentile)	24.9 (75th-85th percentile)
HbA1c	7%	5.6 %	6.9%
Insulin dose	0.8 u/k/d	Off	0.7 u/k/d
CGM Metric: Average glucose mg/dl TIR TAR1 TAR2 TBR1 TBR2 GMI CV TIR in best days	 144 (50 mg/dl SD) 70% 23% 2% 4% <1% 6.8% 34.3% 77%	 124 (41 mg/dl SD) 88% 9% 1% 1% <1% 6.3% 33.4% 100%	 172 (Via glucometer) - - - - - - -

TIR, time-in-range (glucose level 80–180 mg/dL); TAR1, time-above-range 1 (glucose level 181–250 mg/dL); TAR2, time-above-range 2 (glucose level above 250 mg/dL); TBR1, time-below-range 1 (glucose level below 70 mg/dL); TBR2, time-below-range 2 (glucose level below 54 mg/dL); CV, coefficient of variation.

reported but were not observed in this case. An increased level of liver enzymes can be seen in HNF1B-MODY, but our patient's level was normal (14). Genetic variants can result from base substitutions leading to different types of mutations, including missense, nonsense, small deletions or insertions, frameshift, and splice mutations. In some instances, like in the present case, a complete gene deletion has been identified (9).

Unlike HNF4A-MODY and HNF1A-MODY, where cell dysfunction predominates, insulin resistance also plays a part in diabetes development in nearly half of HNF1B-MODY mutation carriers. Patients with HNF1B-MODY are often managed with insulin to control their blood glucose because they lack the sulfonylurea sensitivity associated with HNF1A and HNF4A mutations (8).

GLP-1 is a gut-derived incretin hormone that operates through various measures. These include suppressing hepatic gluconeogenesis, amplifying insulin secretion (glucose-dependent), and inhibiting glucagon release (15). Additionally, it curbs appetite, delays gastric emptying, and lessens energy intake (16).

Liraglutide and semaglutide, which stimulate GLP-1 receptors, enhance the efficacy of incretin function and result in lowered fasting and postprandial glucose levels (17). Both medications improve insulin sensitivity, likely due to an overall decrease in body weight (18). These effects were clearly demonstrated by a successful use of daily liraglutide treatment (a GLP-1RA) in a 17-year-old girl with HNF1B-MODY as a main therapy (18). This significant improvement prompted us to consider semaglutide (another GLP-1RA) as another potential weekly treatment option.

The patient underwent treatment for 4 months, initially using a well-tolerated dose of 0.25 mg of semaglutide weekly for 1 month before increasing it to a maximum dose of 0.5 mg. Her glycemic metric, as indicated by her CGM, showed significant improvements. This was due to the GLP-1 agonist, which resulted in reduced hypoglycemia, decreased variability of up to 27% on some days, and a targeted glycemic control of approximately 88%, with a TIR of 100% occasionally. Additionally, her HbA1c reduced to a record low of 5.6% (Figures 1A, B; Table 1).

Unfortunately, the semaglutide treatment was halted after 4 months despite its effectiveness in enhancing her glycemic metric and establishing tight control through weekly injections. This was due to a variety of reasons, including escalating side effects such as nausea, vomiting, and a reduced appetite. She also had to deal with family problems, examination stress, and a COVID-19 infection, which exacerbated the semaglutide side effects. After discontinuing semaglutide, she reverted to a multiple daily injection regimen of basal-bolus insulin therapy.

This case report deems the response to semaglutide as successful in a short-term trial, as it improved her CGM metrics while she was off insulin therapy. The positive response is likely due to semaglutide's stimulation of GLP-1 receptors, improving her glycemic control via different mechanisms. These include reducing appetite and weight, enhancing insulin sensitivity, curbing glucagon release, and suppressing hepatic gluconeogenesis. Despite these promising results, we must conduct more extensive studies to assess the long-term safety of this treatment in patients with HNF1B-MODY. Additionally, we should consider testing patients on new GLP-1RA treatments with fewer side effects for future large-scale trials.

The limitation of our study is that it only involves a single HNF1B-MODY patient and spans a short duration.

Conclusion

This is the premier report demonstrating the effectiveness of once-weekly GLP-1RA (semaglutide) in patients with a complete HNF1B gene deletion (HNF1B-MODY) based on a brief trial. The trial showed the ability to discontinue insulin use and resulted in improved consistency in blood glucose levels, minimized hypoglycemia and hyperglycemia episodes, and a decrease in HbA1c level.

That being said, more extensive studies should be conducted to assess its effect on a larger patient population and its long-term safety for patients with HNF1B-MODY. To safely switch between insulin and other medications, CGM is advised.

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 humans were approved by King Abdullah International Medical Research Center, Riyadh, Saudi Arabia. The studies were conducted in accordance with the local legislation and institutional requirements. The 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

AA: Conceptualization, Investigation, Methodology, Project administration, Supervision, Writing – original draft. BA: Writing – original draft.

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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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References

1. Fajans SS, Bell GI. MODY. *Diabetes Care*. (2011) 34:1878–84. doi: 10.2337/dc11-0035

2. Hattersley AT, Greeley SAW, Polak M, Rubio-Cabezas O, Njølstad PR, Mlynarski W, et al. ISPAD Clinical Practice Consensus Guidelines 2018: The diagnosis and management of monogenic diabetes in children and adolescents. *Pediatr Diabetes*. (2018) 19:47–63. doi: 10.1111/pedi.2018.19.issue-S27

3. Firdous P, Nissar K, Ali S, Ganai BA, Shabir U, Hassan T, et al. Genetic testing of maturity-onset diabetes of the young current status and future perspectives. *Front Endocrinol*. (2018) 9:253. doi: 10.3389/fendo.2018.00253

4. Beards F, Frayling T, Bulman M, Horikawa Y, Allen L, Appleton M, et al. Mutations in hepatocyte nuclear factor 1beta are not a common cause of maturity-onset diabetes of the young in the U.K. *Diabetes*. (1998) 47:1152–4. doi: 10.2337/diabetes.47.7.1152

5. Delvecchio M, Pastore C, Giordano P. Treatment Options for MODY Patients: a systematic review of literature. *Diabetes Ther*. (2020) 11:1667–85. doi: 10.1007/s13300-020-00864-4

6. Ulinski T, Lescure S, Beaufils S, Guigonis V, Decramer S, Morin D, et al. Renal phenotypes related to hepatocyte nuclear factor-1beta (TCF2) mutations in a pediatric cohort. *J Am Soc Nephrol*. (2006) 17:497–503. doi: 10.1681/ASN.2005101040

7. Clissold RL, Hamilton AJ, Hattersley AT, Ellard S, Bingham C. HNF1B-associated renal and extra-renal disease-an expanding clinical spectrum. *Nat Rev Nephrol*. (2015) 11:102–12. doi: 10.1038/nrneph.2014.232

8. Pearson ER, Badman MK, Lockwood CR, Clark PM, Ellard S, Bingham C, et al. Contrasting diabetes phenotypes associated with hepatocyte nuclear factor-1α and -1β mutations. *Diabetes Care*. (2004) 27:1102–7. doi: 10.2337/diacare.27.5.1102

9. Bingham C, Bulman MP, Ellard S, Allen LIS, Lipkin GW, Van't Hoff WG, et al. Mutations in the hepatocyte nuclear factor-1β gene are associated with familial hypoplastic glomerulocystic kidney disease. *Am J Hum Genet*. (2001) 68:219–24. doi: 10.1086/316945

10. Heuvel-Borsboom H, de Valk HW, Westerink J. Maturity onset diabetes of the young: Seek and you will find. *Neth J Med*. (2016) 74:193–200.

11. Ziegler R, Neu A. Diabetes in childhood and adolescence. *Dtsch Arztebl Int*. (2018). doi: 10.3238/arztebl.2018.0146

12. Pihoker C, Gilliam LK, Ellard S, Dabelea D, Davis C, Dolan LM, et al. Prevalence, Characteristics and clinical diagnosis of maturity onset diabetes of the young due to mutations in HNF1A, HNF4A, and glucokinase: results from the SEARCH for diabetes in youth. *J Clin Endocrinol Metab*. (2013) 98:4055–62. doi: 10.1210/jc.2013-1279

13. Shields BM, Hicks S, Shepherd MH, Colclough K, Hattersley AT, Ellard S. Maturity-onset diabetes of the young (MODY): how many cases are we missing? *Diabetologia*. (2010) 53:2504–8. doi: 10.1007/s00125-010-1799-4

14. Bockenhauer D, Jaureguiberry G. HNF1B-associated clinical phenotypes: the kidney and beyond. *Pediatr Nephrol*. (2016) 31:707–14. doi: 10.1007/s00467-015-3142-2

15. Tasyurek HM, Altunbas HA, Balci MK, Sanlioglu S. Incretins: their physiology and application in the treatment of diabetes mellitus. *Diabetes Metab Res Rev*. (2014) 30:354–71. doi: 10.1002/dmrr.2501

16. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet*. (2006) 368:1696–705. doi: 10.1016/S0140-6736(06)69705-5

17. Knudsen LB, Lau J. The discovery and development of liraglutide and semaglutide. *Front Endocrinol (Lausanne)*. (2019) 10:155. doi: 10.3389/fendo.2019.00155

18. Terakawa A, Chujo D, Yasuda K, Ueno K, Nakamura T, Hamano S, et al. Maturity-onset diabetes of the young type 5 treated with the glucagon-like peptide-1 receptor agonist. *Medicine*. (2020) 99:e21939. doi: 10.1097/MD.0000000000021939

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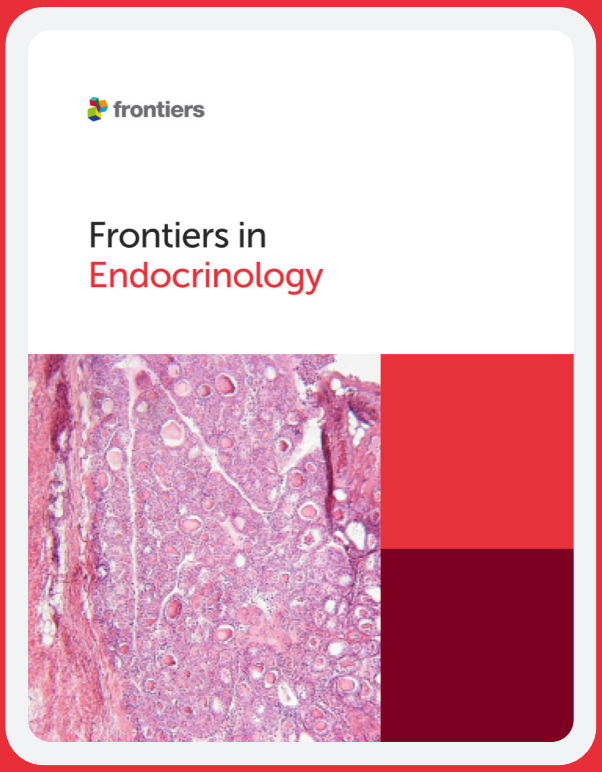

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