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Recent advances in gestational diabetes mellitus

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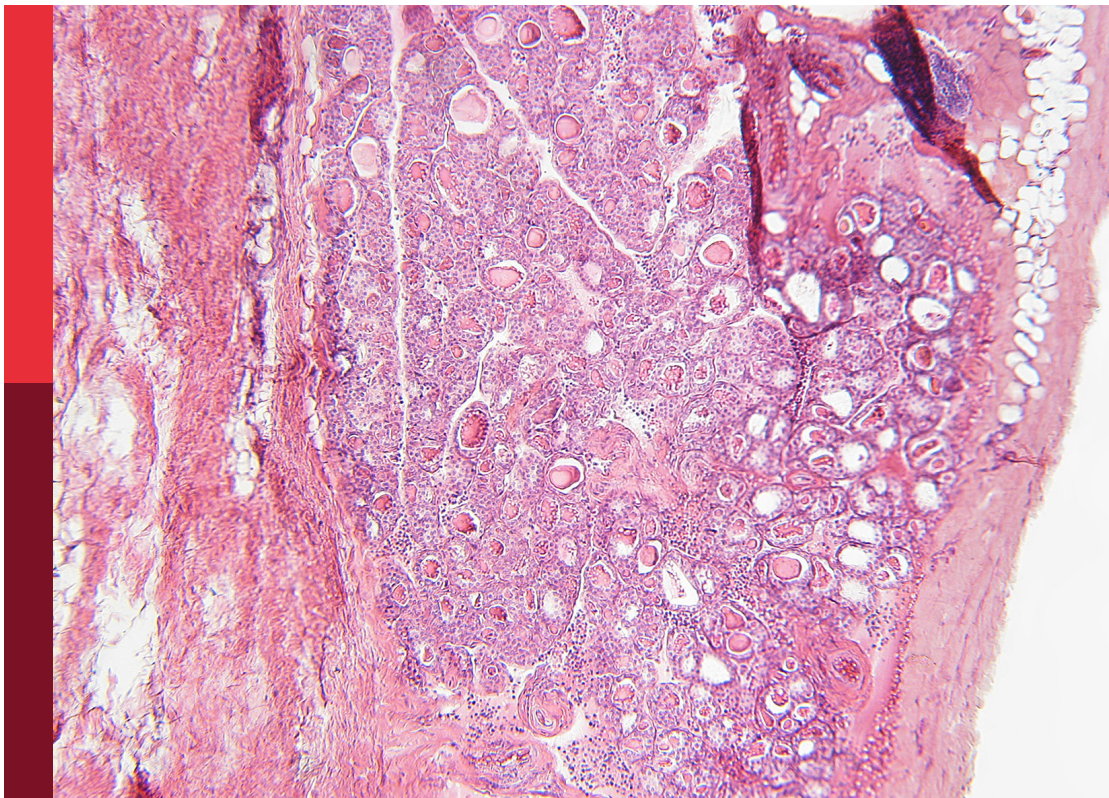

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Recent advances in gestational diabetes mellitus

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Progress and indication for use of continuous glucose monitoring in patients with diabetes in pregnancy: a review

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Gestational diabetes mellitus is one of the most common endocrine diseases that occur during pregnancy. Disorders of blood glucose metabolism during pregnancy can increase the risk of adverse pregnancy outcomes, such as pregnancy-related hypertension, preeclampsia, eclampsia, miscarriage, macrosomia, and neonatal hypoglycemia. Continuous glucose monitoring (CGM) can safely and effectively monitor blood glucose changes in patients with gestational hyperglycemia, thereby reducing adverse pregnancy outcomes. Hence, this article aimed to provide a comprehensive review of the progress and indications for using CGM in pregnant patients with diabetes. CGM can reduce blood glucose fluctuations and the occurrence of serious hypoglycemia and hyperglycemia events and can provide time in range (TIR). TIR is an important indicator of blood glucose level. Patients with a higher TIR during pregnancy have better gestational outcomes.

KEYWORDS

gestational diabetes, continuous glucose monitoring, CGM, pregnancy outcome, perinatal outcome

1 Introduction

Diabetes is a common clinical complication of pregnancy, including gestational diabetes mellitus (GDM) and preexisting diabetes. Among these, GDM is the predominant type, accounting for 80–90% of pregnancies with hyperglycemia. According to the International Association of Diabetes and Pregnancy Study Groups

Abbreviations: ADA, American Diabetes Association; AGP, ambulatory glucose profile; CGM, continuous glucose monitoring; GA, glycosylated albumin; GDM, gestational diabetes mellitus; GV, glycemic variability; HbA1C, hemoglobin A1c; IADPSG, International Association of Diabetes and Pregnancy Study Groups; IGT, impaired glucose tolerance; is-CGM, intermittently scanned continuous glucose monitoring; LGA, large-for-gestational-age; OGTT, oral glucose tolerance test; rt-CGM, real time continuous glucose monitoring; SMBG, self-monitoring of blood glucose; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TAR, time above average; TBR, time below average; TIR, time in range.

(IADPSG), the global incidence of GDM is estimated to be 17.8% (1). Recent studies have shown that maternal pre-pregnancy body mass index is a potential modifiable risk factor for GDM. Moreover, this study showed that the incidence of GDM increased significantly with age. For women under 35 years of age, the prevalence of GDM is 16.4% in normal-weight, 23.0% in overweight, and 38.5% in obese women. For women over 35 years of age, the prevalence of GDM is 20.4%, 37.2%, and 51.4%, respectively (2).

With economic development and improvement in living standards, the prevalence of GDM has increased over the years (3), leading to increased adverse pregnancy outcomes in mothers and their offspring. For mothers, the incidences of dystocia, miscarriage, and eclampsia has increased (4). In the long term, the risk of type 2 diabetes mellitus (T2DM) in women with a history of GDM is nearly 10 times higher than that in women with normal blood glucose during pregnancy (5). The risks of macrosomia, neonatal hypoglycemia, hyperbilirubinemia, and neonatal respiratory distress syndrome are significantly increased in the offspring of women with GDM (4). A prospective study in 10–14-year-old children showed that the offspring of mothers with untreated GDM are at a high risk of impaired glucose tolerance (IGT). Among mothers with GDM, 10.6% of the children had IGT, whereas only 5.0% of the children of mothers without GDM had IGT. GDM is independently associated with children's IGT (6). Therefore, monitoring and maintaining normal blood glucose levels during pregnancy is essential.

Currently, the commonly used clinical blood glucose monitoring methods include self-monitoring of blood glucose (SMBG), continuous glucose monitoring (CGM), hemoglobin A1c (HbA1c), and glycosylated albumin (GA). Many studies have recently shown that CGM is beneficial and widely used for the clinical treatment of patients with gestational diabetes. CGM can be real-time (rt-CGM) and intermittently scanned (is-CGM). It can continuously monitor glucose levels in subcutaneous tissue fluids and automatically record blood glucose levels at regular intervals to reflect blood glucose fluctuations accurately. CGM is employed for patients with diabetes during pregnancy, offering a more effective management approach in clinical settings. It enables clinicians to make better treatment selections and adjustments for patients, leading to optimal blood glucose control and improved pregnancy outcomes. This article reviews the use of CGM in pregnant women with diabetes.

2 Classification of pregnancy hyperglycemia

According to the American Diabetes Association (ADA) guidelines for 2023, pregnancy with hyperglycemia is categorized as GDM and preexisting diabetes (7).

GDM refers to a mild abnormality in glucose metabolism during pregnancy; however, the blood glucose level does not reach that of overt diabetes. During pregnancy, an increase in progesterone, cortisol, prolactin, and human placental hormone levels leads to the gradual aggravation of insulin resistance. Patients with GDM lack sufficient insulin production to combat the aggravation of insulin resistance, which leads to hyperglycemia. According to the diagnostic cut-off point established by IADPSG, GDM diagnostic criteria are: 75-g oral glucose tolerance test (OGTT) at any time during pregnancy, fasting blood glucose ≥ 5.1 mmol/L, 1-h OGTT blood glucose ≥ 10.0 mmol/L, and 2-h OGTT blood glucose ≥ 8.5 mmol/L. GDM can be diagnosed if one of the above mentioned blood glucose levels reaches the standard (8–10).

Pre-existing diabetes in pregnancy includes type 1 diabetes (T1DM), T2DM, or a special type of diabetes diagnosed before pregnancy, which is associated with the most severe hyperglycemia during pregnancy (8, 9). Pregnant women with T1DM have a higher risk of hypoglycemia and diabetic ketoacidosis than those with T2DM.The risk of hypertension and other comorbidities may be as high or higher in patients with T2DM than in those with T1DM (7).

3 Blood glucose monitoring of gestational diabetes

3.1 Hemoglobin A1c and glycosylated albumin

HbA1c reflects the average blood glucose level in the last 2–3 months (11). During pregnancy, red blood cell renewal is physiologically accelerated and the demand for iron increases exponentially (12), leading to a physiological decrease in HbA1c (13). In addition, increased vitamin C intake during pregnancy reduces HbA1c levels (14). Therefore, evaluating blood glucose control in patients with GDM using HbA1c levels is not accurate, as it can only serve as a supplementary reference for SMBG. Although several observational studies have shown that the level of HbA1c before pregnancy is associated with adverse pregnancy outcomes, such as fetal congenital malformation, premature delivery, preeclampsia, and perinatal death (15–17), the association between HbA1c level during the second trimester and adverse pregnancy outcomes has not been demonstrated (18, 19).

GA represents the blood glucose level within 2–3 weeks (20). An increase in GA levels can be observed in GDM (21), and GA can be used as a supplementary test for GDM diagnosis and blood glucose control monitoring (22). However, with increasing gestational age, GA continues to decrease, and the detection of GA has limited value in diagnosing gestational diabetes and predicting adverse pregnancy outcomes (23).

3.2 Self blood glucose monitoring

SMBG includes daily self-monitoring of fasting and postprandial blood glucose levels. The target values recommended by the ADA are as follows: fasting blood glucose < 5.3 mmol/L, 1-h postprandial blood glucose < 7.8 mmol/L, or 2-h postprandial blood glucose < 6.7 mmol/L (7). However, owing to multiple

measurements of SMBG during pregnancy, long-term compliance is poor (24); hence, the fluctuation of blood glucose levels and the time spent within the target range cannot be readily displayed or interpreted. Errors often occur during clinical treatment processes, and new indicators are urgently needed.

3.3 Continuous glucose monitoring

CGM is an effective means of evaluating the fluctuation range of daytime and nighttime blood glucose levels in patients with diabetes. In the past decade, CGM has been proven to exhibit similar accuracy to that of SMBG (25) and can provide better treatment optimization, as well as indicate the trend of blood glucose, owing to its high test frequency (26). CGM can comprehensively analyze the patients' blood glucose changes and provide information to patients and clinicians more intuitively by presenting an ambulatory glucose profile (AGP) and trend arrows. More importantly, CGM can also provide an alarm to help avoid serious hypoglycemic and hyperglycemic crises. CGM can improve the mental health and quality of life of patients by reducing the pain associated with fingertip blood sampling, thus improving compliance (27, 28). With its wide adoption in clinical practice, CGM can improve HbA1c and reduce glucose variability in patients with T1DM (29) and is more suitable for treatment monitoring than the use of SMBG in patients with T2DM (30). CGM is also widely used in patients with preexisting T1DM and T2DM during pregnancy and can improve gestational outcomes (31). Among women with GDM, CGM can provide a more comprehensive assessment of nocturnal hyperglycemia and improve the targeting of GDM interventions (32). CGM is also better than SMBG in detecting hypoglycemic episodes, which may improve maternal and fetal outcomes (26). Moreover, patient compliance is higher in CGM than in SMBG. In a prospective study, patient compliance in the CGM group was as high as 90%, which was significantly higher than that in the SMBG group (14). Therefore, CGM is recommended for patients with preexisting diabetes in pregnancy (especially T1DM complicated with pregnancy), GDM requiring insulin treatment, large blood glucose fluctuation, and potential nighttime hypoglycemia (33, 34).

In addition, a recent prospective cohort study of 73 women showed that CGM was well accepted among patients, could better demonstrate the blood glucose control of patients with GDM, and revealed the potential misdiagnosis of OGTT in GDM (35).Another pilot study conducted by the same team, involving 107 women, further validated the potential of CGM in detecting OGTT misdiagnosis. Additionally, CGM was more acceptable than OGTT to the participants (36).

4 Classification of CGM

4.1 Real-time continuous glucose monitoring

The rt-CGM system can provide a comprehensive glucose status for 3–14 days based on different needs. The device comprises a glucose-sensing device based on tiny glucose oxidase-filled electrodes and a glucose monitor connected by a cable. The system measures glucose concentration in the interstitial fluid every 5 min, continuously monitors glucose level for 24 h, and then forms an AGP. Rt-CGM has been extensively studied in patients with diabetes, and its clinical practicality has been demonstrated. It can detect postprandial hyperglycemia, nocturnal hyperglycemia, and hypoglycemia, which have not been previously reported. Rt-CGM displays not only glucose data in real time but also uses "arrows" to indicate the direction and rate of glucose changes, providing high and low blood glucose alarms and warnings. It can also provide data synchronization to enable timely intervention by the doctors and patients, thereby reducing the occurrence of serious hypoglycemia and hyperglycemia events (37–39). Moreover, CGM can improve the accuracy and effectiveness of clinical decision-making in patients with preexisting diabetes during pregnancy (40); however, the current rt-CGM system partially relies on SMBG for calibration.

4.2 Intermittently scanned continuous glucose monitoring

The current is-CGM system, also known as the instant glucose monitoring system, tracks glucose concentration in the human interstitial fluid approximately once every minute and requires scanning near the sensor placed on the skin to retrieve the data. Flash glucose monitoring is a typical example of is-CGM, which was identified by ADA in 2019 as a method that can replace SMBG for blood glucose monitoring (4). When the user scans the sensor, the current blood glucose value is recorded and retrospective reports for blood glucose data and related parameters, such as time in range (TIR), are generated (41). The is-CGM can be used for up to 14 days and does not need calibration with SMBG; however, it cannot deliver alerts (42).

Some studies have compared the two types of CGM and found that both is-CGM and rt-CGM can improve TIR, while rt-CGM has a greater percentage of TIR and can significantly reduce the incidence of hypoglycemia (43). When switching from is-CGM (FreeStyle Libre version 1) to rt-CGM (Dexcom G4) in 18 adult patients with T1DM, without changing insulin therapy management, there was an increase in TIR, a decrease in time below average (TBR), and no change in time above average (TAR) (44). Another study showed that in pregnant women with T1DM, no differences in TIR and TAR were observed, but women monitored by rt-CGM had a lower TBR compared to those monitored by is-CGM (45). Therefore, rt-CGM is more suitable for reducing the occurrence of hypoglycemia.

5 CGM indicators

In clinical practice, patients are recommended to wear CGM for 14 days. For patients with T1DM, 12–15 days of monitoring every 3 months can more accurately assess the level of blood glucose control (46, 47).

The CGM measurement value includes three key indicators: TIR (the proportion of time when the blood glucose is 3.9–10.0 mmol/L), TBR (proportion of time when blood glucose is <3.9 mmol/L), and TAR (proportion of time when blood glucose is>10.0 mmol/L). The main objective of effective and safe glucose control is to increase the TIR while reducing the TAR and TBR (48). Beck et al. found that in patients with diabetes mellitus, the probability of developing diabetic retinopathy and microalbuminuria increased by 64% and 40%, respectively, for every 10% reduction in TIR (49, 50). A study conducted among 141 pregnant women showed that among those with T2DM or GDM who utilized CGM, approximately 40% had TIR ≤ 70% and a higher likelihood of adverse neonatal and maternal outcomes compared to those with TIR > 70% (51). Murphy et al. pointed out that every 5% reduction in TIR and 5% increase in TAR in the second and third trimesters will increase the risk of being older than the gestational age, neonatal hypoglycemia, and admission to the neonatal intensive care unit (52). Therefore, it is necessary to improve the TIR levels in patients. In 2019, the TIR International Consensus recommended a TIR control target of >70% in pregnant women with T1DM. However, TIR control targets should be personalized. Patients with GDM and pregnant women with T2DM require more stringent targets and greater attention to overnight glucose (53).

In addition, common indicators of CGM include glucose management indicators, also called estimating A1C (54), blood glucose change rate [CV, target ≤ 36% (55)], and glycemic variability (GV). Patients with GDM risk factors have higher CV, and the corresponding incidence of adverse pregnancy outcomes is higher (56). GV in early pregnancy can be used as a potential predictor of subsequent GDM diagnosis. The mean amplitude of glycemic excursion, which is derived from GV, was significantly higher in patients with GDM (57).

6 CGM can better control blood glucose and improve pregnancy outcomes

Gestational diabetes increases the risk of pregnancy-related complications, such as hypertension, preeclampsia, eclampsia, premature rupture of membranes, cesarean section, postpartum hemorrhage, and intrauterine infection (58). Therefore, the management of blood glucose levels during pregnancy is very important for reducing adverse pregnancy outcomes. As shown in Table 1, many studies have reported that CGM can reduce adverse pregnancy outcomes. CGM provides patients with intuitive information on changes in blood glucose levels, enabling them to change their lifestyle and participate in treatment (59). Currently, CGM is being increasingly used in patients with gestational diabetes.

In a prospective study in Australia, 68 consecutive blood glucose monitoring examinations were conducted in 55 pregnant women. Sixty-two percent of the results provided important information for altering clinical management decisions, including postprandial and nocturnal hypoglycemia, and 77% of the participants acknowledged that CGM provided more benefits than inconvenience (60). CGM is a practical clinical tool with good compliance and is helpful in clinical decision-making.

The use of CGM is more suitable for the control of blood glucose levels, reduction of blood glucose fluctuations, and improvement of TIR in mothers with preexisting diabetes during pregnancy. Patients with T1DM have a high risk of developing severe hypoglycemia, which can have serious adverse effects on both the mother and fetus during pregnancy. Using CGM allows detection of glycemia fluctuations that might have gone unnoticed with intermittent blood glucose monitoring (61). An international study titled the CONCEPTT divided 325 women with T1DM into two groups. Only capillary blood glucose levels were monitored in one group, and CGM-assisted capillary blood glucose levels were monitored for the other group. Pregnant women who underwent CGM had a higher TIR and lower TAR and TBR. This report suggests that CGM should be administered to all pregnant women with T1DM receiving intensive insulin therapy (62). Viralnshah et al. conducted a prospective study and collected CGM data from 27 women with T1DM during pregnancy and found that TIR was significantly negatively correlated with HbA1c. For every 10% increase in TIR, HbA1c decreased by 0.3%, and the correlation between TIR and HbA1c in the second and third trimesters was stronger than that in the first trimester ($r = -0.4$) (63). Therefore, we assumed that CGM is suitable for pregnant women with T1DM, as it can help control blood glucose better.

A prospective study including 300 patients with gestational hyperglycemia found that CGM could reduce the incidence of gestational hypertension and preeclampsia in patients with T1DM and improve the level of HbA1c (64). However, although CGM can reduce the incidence of hypertensive disorders that complicate pregnancy in patients with diabetes, it does not significantly reduce the incidence of preeclampsia; the impact of CGM on preeclampsia remains to be discussed (65). Therefore, more robust evidence is required to confirm the effectiveness of CGM in improving pregnancy outcomes.

Although the blood glucose level in patients with GDM is much lower than that in patients with preexisting diabetes during pregnancy, its adverse effects on the future of the mother and fetus should not be underestimated. A follow-up study in Asia showed that women with a history of GDM had a high risk of developing T2DM in the future, and this risk increased with age (66).

García-Moreno et al. searched and screened a large number of studies and conducted a meta-analysis of 482 patients. Compared to women using traditional blood glucose monitoring methods, women with GDM using CGM may have lower average blood glucose levels, lower maternal weight gain, and lower birth weight of infants (67).

Majewska et al. recruited 100 women diagnosed with GDM and randomly assigned them to is-CGM and SMBG groups. The average blood glucose and total insulin resistance levels were determined. The average blood glucose was more stable and total insulin resistance was higher in the group using CGM, which may help to improve and treat glucose tolerance disorder during pregnancy (68).

One study found that the application of the CGM system can reduce the daily blood glucose fluctuation of patients with GDM by more than 25%, and the valley value of hyperglycemia can be significantly reduced (69, 70). This shows that CGM can better control blood glucose fluctuations and avoid excessive increases in blood glucose levels in patients with GDM. Compared to SMBG, CGM can reduce the average blood glucose level, increase the amplitude of maternal and infant birth weights, and improve pregnancy outcomes (68).

A randomized crossover study aimed to determine how the distribution of dietary carbohydrates affects blood glucose levels in women with GDM. CGM was used to monitor the blood glucose levels of 12 women with GDM undergoing diet treatment. The study concluded that "50% carbohydrate distribution in the morning is beneficial for reducing blood glucose and improving insulin sensitivity of women with GDM; however, it resulted in higher blood glucose variability." Thus, women with GDM should reasonably manage their diet (71).

7 CGM improves perinatal outcomes

In patients with gestational diabetes, blood glucose level increases, leading to excessive glucose passing through the placenta and stimulating the pancreatic islets. This stimulation causes the fetus to produce excess insulin, resulting in increased synthesis of protein and fat in the fetus, consequently resulting in the development of a large baby (72). In addition, owing to excessive insulin production, hypoglycemia can occur easily when the fetus separates from the mother during childbirth. If glucose is not supplemented in time, the incidence of hypoglycemia increases. Both hyperglycemia and hyperinsulinemia can reduce the surface-active substance of fetal lung type II cells, hindering the growth of the fetal lung and affecting its normal development. This condition can lead to neonatal respiratory distress syndrome (73). Poor blood glucose control during pregnancy can result in adverse perinatal outcomes. As shown in Table 1, several studies have reported that CGM reduces adverse perinatal outcomes.

In a prospective study, CGM was used to monitor blood glucose changes in 77 patients with GDM at 26–32 weeks of gestation for 6days. The pattern of hyperglycemia before, after, and at night and its correlation with maternal and fetal complications and drug treatment were analyzed. TAR was related to the occurrence of macrosomia and large-for-gestational-age (LGA) infants. Every 1% increase in TAR increased the probability of requiring drug treatment by 24%. Using CGM to monitor blood glucose changes in patients with GDM enables identification of patients who require drug treatment at an early stage. This proactive approach can help reduce the incidence of adverse pregnancy outcomes, such as macrosomia (74).

LGA infants are referred as newborns whose birth weight is above the 90th percentile of the average weight of infants at the same gestational age, which is closely related to the increase in maternal blood glucose. Long-term glucose metabolic dysfunction may increase the risk of macrosomia (75). A prospective observational study was conducted using CGM in 162 pregnant women with GDM for 7 days at 30–32 weeks of gestation. Using the blood glucose index and blood glucose variability measurements provided by CGM, functional data analysis showed that mothers who delivered LGA infants had significantly higher blood glucose levels at night. Monitoring and controlling nocturnal blood glucose levels may help further reduce the incidence rate of LGA infants in women with GDM (76).

The CONCEPTT study pointed out that compared with SMBG, patients who underwent CGM had significantly improved newborn health outcomes, including a reduced incidence of LGA infants, fewer neonatal intensive care inpatients lasting more than 24 h, a decreased occurrence of neonatal hypoglycemia, and a shortened hospitalization period by one day (62). The use of CGM during pregnancy in patients with T1DM is related to an improvement in neonatal outcomes, which may be attributed to a reduction in maternal hyperglycemia exposure.

Murphy et al. studied the effects of CGM on the offspring of pregnant women with T1DM (46 women) or T2DM (25 women). These women were randomly assigned to the CGM and standard prenatal treatment group (CGM+SMBG, 38 women) or the standard prenatal treatment group (SMBG, 33 women). Women in the CGM group, as measured by the median percentile of birth weight, eventually delivered significantly smaller babies than those in the SMBG group. However, no significant difference was observed between the two groups in terms of LGA infants, cesarean section, preeclampsia, or other indicators used to measure the incidence rate of newborns (77).

Similarly, Kristensen et al. conducted a prospective study of 186 pregnant women with T1DM in Sweden, 92 of whom underwent rt-CGM and 94 underwent is-CGM. The number of LGA infants was similar in rt-CGM and is-CGM users, and high maternal average blood glucose levels and low TIR during pregnancy were associated with an increased risk of LGA and comprehensive adverse outcomes in newborns. However, the rt-CGM group exhibited a lower TBR than the is-CGM group. Therefore, although the impact of rt-CGM on perinatal outcomes was not significantly different from that of is-CGM, rt-CGM was still more suitable for reducing the occurrence of hypoglycemia (45). However, another study showed that intermittent rt-CGM use during pregnancy did not improve blood glucose control or pregnancy outcomes in women with GDM (76).

In summary, there are still few controversial findings regarding CGM improving perinatal outcomes in patients with gestational diabetes. Therefore, a large number of prospective studies are needed to explore the effectiveness of CGM in improving perinatal outcomes in patients with gestational diabetes.

8 Summary

The prevalence of gestational diabetes is increasing with improvements in living standards. Blood glucose monitoring is

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TABLE 1 the impact of CGM on pregnancy outcomes and perinatal outcomes.

Number	Country	Reference	Period	Size			Result		Recommendation
				TID	T2D	GDM	Maternal	Offspring	
1	UK, Austria	25	2018	24	11	39	The blood glucose measured by CGM and SMBG are highly consistent, and CGM reduces the pain and burden of users.	–	CGM is safe and accurate to use by pregnant women with diabetes.
2	Australia	32	2020	–	–	90	CGM data revealed nocturnal hyperglycemia in patients who were not commenced on insulin, with 60% of subjects breaching glucose targets overnight for >10% time. SMBG is hard to get such results.	–	CGM can make a more comprehensive assessment of nocturnal hyperglycemia.
3	Australia	35	2022	–	–	40	CGM can evaluate the diurnal pattern of glucose metabolism and has the potential to identify false positive and false negative OGTT.	–	CGM was well accepted and could better demonstrate the blood glucose control of GDM patients.
4	Sweden	45	2019	186	–	–	–	High maternal average blood glucose level and low TIR during pregnancy were associated with increased risk of LGA in offspring and comprehensive adverse outcomes in newborns.	Despite the use of CGM throughout pregnancy, daily blood glucose control is not ideal, and the incidence of LGA is still high.
5	Denmark	63	2021	20	–	–	The TBR measured by is-CGM is higher than that measured by rt-CGM.	–	The type of CGM device may affect the judgment of nocturnal hypoglycemia and thus affect the adjustment of nocturnal insulin dose.
6	England	52	2019	186	–	–	–	Every 5% reduction in TIR and 5% increase in TAR in the second and third trimesters will increase the risk of older than gestational age infants, neonatal hypoglycemia and admission to the neonatal intensive care unit.	Pregnant women should monitor TIR through CGM and raise the TIR to>70% as early as possible during pregnancy.
7	Australia	60	2007	8	10	37	CGM can show undetected postprandial hyperglycemia and overnight hypoglycemia.	–	CGM is a practical clinical tool with good compliance and is helpful for clinical decision-making.
8	England	62	2017	325	–	–	Pregnant CGM users spent more time in target and less time hyperglycemic, less hypoglycemia episodes and less time spent hypoglycemic.	Lower incidence of large for gestational age, fewer neonatal intensive care admissions lasting more than 24h, fewer incidences of neonatal hypoglycemia, and 1-day	CGM should be provided to all pregnant women with type 1 diabetes who use intensive insulin therapy.

(Continued)

TABLE 1 Continued

Number	Country	Reference	Period	Size			Result		Recommendation
				TID	T2D	GDM	Maternal	Offspring	
								shorter length of hospital stay.	
9	Holland	64	2018	109	82	109	CGM can reduce the incidence of gestational hypertension and preeclampsia in patients with type 1 diabetes and improve the level of HbA1c.	the use of is-CGM did not reduce the risk of macrosomia	CGM provides detailed information concerning glycemic fluctuations but, as a treatment strategy, does not translate into improved pregnancy outcome.
10	Worldwide	67	2022	–	–	482	Women with GDM using CGM may achieve lower average blood glucose levels and lower maternal weight gain.	Compared with using SMBG, patients using CGM to monitor blood glucose birth infants with lower birth weight	CGM is good for both mother and infant.
11	England	68	2021	–	–	100	the average blood glucose was more stable and TIR was higher in the group using is-CGM.	–	CGM may help to improve and treat the glucose tolerance disorder during pregnancy
12	China	69	2011-2012	–	–	340	Subjects in the CGM group were at lower risk of preeclampsia and primary cesarean delivery	The mean infant birth weight of women in the CGM group was lower	The use of supplementary CGM combined with routine antenatal care can improve the glycemic control and pregnancy outcomes of patients with GDM
13	Spain	74	2020	–	–	77	Every 1% increase in TAR would increase the probability of requiring drug treatment by 24%.	TAR was related to the occurrence of macrosomia and large for gestational age infants.	Using CGM to monitor the blood glucose changes of GDM patients can identify those patients who need drug treatment as early as possible, and reduce the occurrence of adverse pregnancy outcomes
14	England	76	2019	–	–	162	Mothers who delivered LGA infants had significantly higher blood glucose at night.	–	Using CGM to monitor and control the nocturnal blood glucose may help reduce the incidence rate of LGA in GDM women.
15	England	77	2008	46	25	–	–	Women in the CGM group delivered significantly smaller babies than the SMBG group	CGM during pregnancy is associated with improved glycemic control in the third trimester, lower birth weight, and reduced risk of macrosomia.

the basis for GDM management. The goal of GDM treatment is to minimize maternal and fetal adverse events related to hyperglycemia or severe hypoglycemia. Several clinical studies have demonstrated that satisfactory glucose control during pregnancy effectively reduces maternal and infant complications. CGM can effectively monitor blood glucose changes in patients with diabetes during pregnancy, thereby providing better guidance for clinical treatment. Therefore, CGM is recommended for patients with preexisting diabetes in pregnancy (especially T1DM complicated with pregnancy), GDM requiring insulin treatment, large blood glucose fluctuations, and possible nighttime hypoglycemia. This article reviews the use of CGM in patients

with diabetes during pregnancy, and many studies have confirmed that CGM can improve pregnancy outcomes. However, there is still some controversy about the impact of CGM on maternal and infant health, which necessitates further discussion and clarification using big data and large samples.

Author contributions

YS wrote the first draft of the manuscript and edited it. XZ summarized the manuscript and drew the Table 1. YB and CL reviewed literature and organized them. LZ performed critical revision of the literature and editing of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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References

1. Mariotti C, Giovannini A, Reibaldi M, Nicolai M, Saitta A. 25-gauge vitrectomy combined with half-fluence photodynamic therapy for the treatment of juxtapapillary retinal capillary hemangioma: a case report. *Case Rep Ophthalmol* (2014) 5(2):162–7. doi: 10.1159/000363564

2. Mirabelli M, Tocci V, Donnici A, Giuliano S, Sarnelli P, Salatino A, et al. Maternal preconception body mass index overtakes age as a risk factor for gestational diabetes mellitus. *J Clin Med* (2023) 12(8). doi: 10.3390/jcm12082830

3. Chinese Elderly Type 2 Diabetes Prevention and Treatment of Clinical Guidelines Writing Group, Geriatric Endocrinology and Metabolism Branch of Chinese Geriatric Society, Geriatric Endocrinology and Metabolism Branch of Chinese Geriatric Health Care Society, Geriatric Professional Committee of Beijing Medical Award Foundation and National Clinical Medical Research Center for Geriatric Diseases (PLA General Hospital). [Clinical guidelines for prevention and treatment of type 2 diabetes mellitus in the elderly in China (2022 edition)]. *Zhonghua Nei Ke Za Zhi* (2022) 61(1):12–50. doi: 10.3760/cma.j.cn112138-20211027-00751

4. Association AD. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2020. *Diabetes Care* (2019) 43(Supplement_1):S14–31. doi: 10.2337/dc20-S002

5. Juan J, Yang H-X, Su R-N, Kapur A. Diagnosis of gestational diabetes mellitus in China: perspective, progress and prospects. *Maternal-Fetal Med* (2019) 1(1):31–7. doi: 10.1097/FM9.0000000000000008

6. Lowe WL Jr., Scholtens DM, Kuang A, Linder B, Lawrence JM, Lebenthal Y, et al. Hyperglycemia and adverse pregnancy outcome follow-up study (HAPO FUS): maternal gestational diabetes mellitus and childhood glucose metabolism. *Diabetes Care* (2019) 42(3):372–80. doi: 10.2337/dc18-1646

7. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. 15. Management of diabetes in pregnancy: standards of care in diabetes—2023. *Diabetes Care* (2022) 46(Supplement_1):S254–66. doi: 10.2337/dc23-S015

8. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care* (2010) 33 (3):676–82. doi: 10.2337/dc10-0719

9. Gupta Y, Kalra B, Baruah MP, Singla R, Kalra S. Updated guidelines on screening for gestational diabetes. *Int J Womens Health* (2015) 7:539–50. doi: 10.2147/IJWH.S82046

10. Committee, A.D.A.P.P. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2022. *Diabetes Care* (2021) 45(Supplement_1):S17–38. doi: 10.2337/dc22-S002

11. Nathan DM, Turgeon H, Regan S. Relationship between glycated haemoglobin levels and mean glucose levels over time. *Diabetologia* (2007) 50(11):2239–44. doi: 10.1007/s00125-007-0803-0

12. Hashimoto K, Osugi T, Noguchi S, Morimoto Y, Wasada K, Imai S, et al. A1C but not serum glycated albumin is elevated because of iron deficiency in late pregnancy in diabetic women. *Diabetes Care* (2009) 33(3):509–11. doi: 10.2337/dc09-1954

13. Mosca A, Paleari R, Dalfrà MG, Di Cianni G, Cuccuru I, Pellegrini G, et al. Reference intervals for hemoglobin A1c in pregnant women: data from an Italian multicenter study. *Clin Chem* (2006) 52(6):1138–43. doi: 10.1373/clinchem.2005.064899

14. Bolinder J, Antuna R, Geelhoed-Duijvestijn P, Kröger J, Weitgasser R. Novel glucose-sensing technology and hypoglycaemia in type 1 diabetes: a multicentre, non-masked, randomised controlled trial. *Lancet* (2016) 388(10057):2254–63. doi: 10.1016/S0140-6736(16)31535-5

15. Guerin A, Nisenbaum R, Ray JG. Use of maternal GHb concentration to estimate the risk of congenital anoMalies in the offspring of women with prepregnancy diabetes. *Diabetes Care* (2007) 30(7):1920–5. doi: 10.2337/dc07-0278

16. Jensen DM, Korsholm L, Ovesen P, Beck-Nielsen H, Moelsted-Pedersen L, Westergaard JG, et al. Peri-conceptional A1C and risk of serious adverse pregnancy outcome in 933 women with type 1 diabetes. *Diabetes Care* (2009) 32(6):1046–8. doi: 10.2337/dc08-2061

17. Nielsen GL, Møller M, Sørensen HT. HbA1c in early diabetic pregnancy and pregnancy outcomes: a Danish population-based cohort study of 573 pregnancies in women with type 1 diabetes. *Diabetes Care* (2006) 29(12):2612–6. doi: 10.2337/dc06-0914

18. Hong JGS, Fadzleeyanna MYN, Omar SZ, Tan PC. HbA1c at term delivery and adverse pregnancy outcome. *BMC Pregnancy Childbirth* (2022) 22(1):679. doi: 10.1186/s12884-022-05000-7

19. Yamamoto JM, Kallas-Koeman MM, Butalia S, Lodha AK, Donovan LE. Large-for-gestational-age (LGA) neonate predicts a 2.5-fold increased odds of neonatal hypoglycaemia in women with type 1 diabetes. *Diabetes Metab Res Rev* (2017) 33(1). doi: 10.1002/dmrr.2824

20. Koga M. Glycated albumin; clinical usefulness. *Clin Chim Acta* (2014) 433:96–104. doi: 10.1016/j.cca.2014.03.001

21. Mendes N, Alves M, Andrade R, Ribeiro RT, Papoila AL, Serrano F. Association between glycated albumin, fructosamine, and HbA1c with neonatal outcomes in a prospective cohort of women with gestational diabetes mellitus. *Int J Gynaecol Obstet* (2019) 146(3):326–32. doi: 10.1002/ijgo.12897

22. Toft JH, Bleskestad IH, Skadberg Ø, Gøransson LG, Økland I. Glycated albumin in pregnancy: LC-MS/MS-based reference interval in healthy, nulliparous Scandinavian women and its diagnostic accuracy in gestational diabetes mellitus. *Scand J Clin Lab Invest* (2022) 82(2):123–31. doi: 10.1080/00365513.2022.2033827

23. Dong Y, Zhai Y, Wang J, Chen Y, Xie X, Zhang C, et al. Glycated albumin in pregnancy: reference intervals establishment and its predictive value in adverse

pregnancy outcomes. *BMC Pregnancy Childbirth* (2020) 20(1):12. doi: 10.1186/s12884-019-2704-x

24. Hsu CR, Chen YT, Sheu WH. Glycemic variability and diabetes retinopathy: a missing link. *J Diabetes Complications* (2015) 29(2):302–6. doi: 10.1016/j.jdiacomp.2014.11.013

25. Scott EM, Bilous RW, Kautzky-Willer A. Accuracy, user acceptability, and safety evaluation for the freeStyle libre flash glucose monitoring system when used by pregnant women with diabetes. *Diabetes Technol Ther* (2018) 20(3):180–8. doi: 10.1089/dia.2017.0386

26. Yu Q, Aris IM, Tan KH, Li LJ. Application and utility of continuous glucose monitoring in pregnancy: A systematic review. *Front Endocrinol* (2019) 10. doi: 10.3389/fendo.2019.00697

27. Runge AS, Kennedy L, Brown AS, Dove AE, Levine BJ, Koontz SP, et al. Does time-in-range matter? Perspectives from people with diabetes on the success of current therapies and the drivers of improved outcomes. *Clin Diabetes* (2018) 36(2):112–9. doi: 10.2337/cd17-0094

28. Gavin JR, Bailey CJ. Real-world studies support use of continuous glucose monitoring in type 1 and type 2 diabetes independently of treatment regimen. *Diabetes Technol Ther* (2021) 23(S3):S19–s27. doi: 10.1089/dia.2021.0211

29. Slattery D, Choudhary P. Clinical use of continuous glucose monitoring in adults with type 1 diabetes. *Diabetes Technol Ther* (2017) 19(S2):S55–s61. doi: 10.1089/dia.2017.0051

30. Carlson AL, Mullen DM, Bergenstal RM. Clinical use of continuous glucose monitoring in adults with type 2 diabetes. *Diabetes Technol Ther* (2017) 19(S2):S4–s11. doi: 10.1089/dia.2017.0024

31. Carreiro MP, Nogueira AI, Ribeiro-Oliveira A. Controversies and advances in gestational diabetes-an update in the era of continuous glucose monitoring. *J Clin Med* (2018) 7(2). doi: 10.3390/jcm7020011

32. Zaharieva DP, Teng JH, Ong ML, Lee MH, Paldus B, Jackson L, et al. Continuous glucose monitoring versus self-monitoring of blood glucose to assess glycemia in gestational diabetes. *Diabetes Technol Ther* (2020) 22(11):822–7. doi: 10.1089/dia.2020.0073

33. Hawkins JS. Glucose monitoring during pregnancy. *Curr Diabetes Rep* (2010) 10 (3):229–34. doi: 10.1007/s11892-010-0111-9

34. Nakhleh A, Shehadeh N. Hypoglycemia in diabetes: An update on pathophysiology, treatment, and prevention. *World J Diabetes* (2021) 12(12):2036–49. doi: 10.4239/wjd.v12.i12.2036

35. Di Filippo D, Ahmadzai M, Chang MHY, Horgan K, Ong RM, Darling J, et al. Continuous glucose monitoring for the diagnosis of gestational diabetes mellitus: A pilot study. *J Diabetes Res 2022* (2022) p:5142918. doi: 10.1155/2022/5142918

36. Di Filippo D, Henry A, Bell C, Haynes S, Chang MHY, Darling J, et al. A new continuous glucose monitor for the diagnosis of gestational diabetes mellitus: a pilot study. *BMC Pregnancy Childbirth* (2023) 23(1):186. doi: 10.1186/s12884-023-05496-7

37. Mian Z, Hermayer KL, Jenkins A. Continuous glucose monitoring: review of an innovation in diabetes management. *Am J Med Sci* (2019) 358(5):332–9. doi: 10.1016/j.amjms.2019.07.003

38. Kudva YC, Ahmann AJ, Bergenstal RM, Gavin JR 3rd, Kruger DF, Midyett LK, et al. Approach to using trend arrows in the freeStyle libre flash glucose monitoring systems in adults. *J Endocr Soc* (2018) 2(12):1320–37. doi: 10.1210/js.2018-00294

39. Tanenberg R, Bode B, Lane W, Levetan C, Mestman J, Harmel AP, et al. Use of the Continuous Glucose Monitoring System to guide therapy in patients with insulin-treated diabetes: a randomized controlled trial. *Mayo Clin Proc* (2004) 79(12):1521–6. doi: 10.4065/79.12.1521

40. Chen R, Yogev Y, Ben-Haroush A, Jovanovic L, Hod M, Phillip M. Continuous glucose monitoring for the evaluation and improved control of gestational diabetes mellitus. *J Matern Fetal Neonatal Med* (2003) 14(4):256–60. doi: 10.1080/jmf.14.4.256.260

41. Bruttomesso D, Laviola L, Avogaro A, Bonora E, Del Prato S, Frontoni S, et al. The use of real time continuous glucose monitoring or flash glucose monitoring in the management of diabetes: A consensus view of Italian diabetes experts using the Delphi method. *Nutr Metab Cardiovasc Dis* (2019) 29(5):421–31. doi: 10.1016/j.numecd.2019.01.018

42. Edelman SV, Argento NB, Pettus J, Hirsch IB. Clinical implications of real-time and intermittently scanned continuous glucose monitoring. *Diabetes Care* (2018) 41 (11):2265–74. doi: 10.2337/dc18-1150

43. Hásková A, Radovnická L, Petruželková L, Parkin CG, Grunberger G, Horová E, et al. Real-time CGM is superior to flash glucose monitoring for glucose control in type 1 diabetes: the CORRIDA randomized controlled trial. *Diabetes Care* (2020) 43 (11):2744–50. doi: 10.2337/dc20-0112

44. Préau Y, Galie S, Schaepelynck P, Armand M, Raccah D. Benefits of a switch from intermittently scanned continuous glucose monitoring (isCGM) to real-time (rt) CGM in diabetes type 1 suboptimal controlled patients in real-life: A one-year prospective study (§). *Sensors (Basel)* (2021) 21(18). doi: 10.3390/s21186131

45. Kristensen K, Ögge LE, Sengpiel V, Kjölhede K, Dotevall A, Elfvin A, et al. Continuous glucose monitoring in pregnant women with type 1 diabetes: an observational cohort study of 186 pregnancies. *Diabetologia* (2019) 62(7):1143–53. doi: 10.1007/s00125-019-4850-0

46. Xing D, Kollman C, Beck RW, Tamborlane WV, Laffel L, Buckingham BA, et al. Optimal sampling intervals to assess long-term glycemic control using continuous glucose monitoring. *Diabetes Technol Ther* (2011) 13(3):351–8. doi: 10.1089/dia.2010.0156

47. Riddlesworth TD, Beck RW, Gal RL, Connor CG, Bergenstal RM, Lee S, et al. Optimal sampling duration for continuous glucose monitoring to determine long-term glycemic control. *Diabetes Technol Ther* (2018) 20(4):314–6. doi: 10.1089/dia.2017.0455

48. 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.

49. Beck RW, Bergenstal RM, Cheng P, Kollman C, Carlson AL, Johnson ML, et al. The relationships between time in range, hyperglycemia metrics, and hbA1c. *J Diabetes Sci Technol* (2019) 13(4):614–26. doi: 10.1177/1932296818822496

50. Beck RW, Bergenstal RM, Riddlesworth TD, Kollman C, Li Z, Brown AS, et al. Validation of time in range as an outcome measure for diabetes clinical trials. *Diabetes Care* (2019) 42(3):400–5. doi: 10.2337/dc18-1444

51. Bitar G, Cornthwaite JA, Sadek S, Ghorayeb T, Daye N, Nazeer S, et al. Continuous glucose monitoring and time in range: association with adverse outcomes among people with type 2 or gestational diabetes mellitus. *Am J Perinatol* (2023). doi: 10.1055/s-0043-1764208

52. Murphy HR. Continuous glucose monitoring targets in type 1 diabetes pregnancy: every 5% time in range matters. *Diabetologia* (2019) 62(7):1123–8. doi: 10.1007/s00125-019-4904-3

53. 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

54. Bergenstal RM, Beck RW, Close KL, Grunberger G, Sacks DB, Kowalski A, et al. Glucose management indicator (GMI): A new term for estimating A1C from continuous glucose monitoring. *Diabetes Care* (2018) 41(11):2275–80. doi: 10.2337/dc18-1581

55. Monnier L, Colette C, Wojtusciszyn A, Dejager S, Renard E, Molinari N, et al. Toward defining the threshold between low and high glucose variability in diabetes. *Diabetes Care* (2017) 40(7):832–8. doi: 10.2337/dc16-1769

56. Gáborová M, Doničová V, Bačová I, Pallayová M, Bona M, Peregrim I, et al. Glycaemic variability and risk factors of pregnant women with and without gestational diabetes mellitus measured by continuous glucose monitoring. *Int J Environ Res Public Health* (2021) 18(7). doi: 10.3390/ijerph18073402

57. Quah PL, Tan LK, Lek N, Thain S, Tan KH. Glycemic variability in early pregnancy may predict a subsequent diagnosis of gestational diabetes. *Diabetes Metab Syndr Obes* (2022) 15:4065–74. doi: 10.2147/DMSO.S379616

58. Yang H. Current situation and countermeasures of diagnosis and treatment of gestational diabetes in China Chinese Journal of Practical Gynecology and Obstetrics. (2013).

59. Alfadhli E, Osman E, Basri T. Use of a real time continuous glucose monitoring system as an educational tool for patients with gestational diabetes. *Diabetol Metab Syndr* (2016) 8:48. doi: 10.1186/s13098-016-0161-5

60. McLachlan K, Jenkins A, O'Neal D. The role of continuous glucose monitoring in clinical decision-making in diabetes in pregnancy. *Aust N Z J Obstet Gynaecol* (2007) 47(3):186–90. doi: 10.1111/j.1479-828X.2007.00716.x

61. Lenhard MJ, Kinsley BT. Insulin therapy for the treatment of type 1 diabetes during pregnancy. *J Matern Fetal Neonatal Med* (2014) 27(12):1270–5. doi: 10.3109/14767058.2013.864631

62. Feig DS, Donovan LE, Corcoy R, Murphy KE, Amiel SA, Hunt KF, et al. Continuous glucose monitoring in pregnant women with type 1 diabetes (CONCEPTT): a multicentre international randomised controlled trial. *Lancet* (2017) 390(10110):2347–59. doi: 10.1016/S0140-6736(17)32400-5

63. Nørgaard SK, Mathiesen ER, Nørgaard K, Ringholm L. Comparison of glycemic metrics measured simultaneously by intermittently scanned continuous glucose monitoring and real-time continuous glucose monitoring in pregnant women with type 1 diabetes. *Diabetes Technol Ther* (2021) 23(10):665–72. doi: 10.1089/dia.2021.0109

64. Voormolen DN, DeVries JH, Sanson RME, Heringa MP, de Valk HW, Kok M, et al. Continuous glucose monitoring during diabetic pregnancy (GlucoMOMS): A multicentre randomized controlled trial. *Diabetes Obes Metab* (2018) 20(8):1894–902. doi: 10.1111/dom.13310

65. Jones LV, Ray A, Buckley BS, West HM. Techniques of monitoring blood glucose during pregnancy for women with pre-existing diabetes. *Cochrane Database Syst Rev* (2019) 5(5):Cd009613. doi: 10.1002/14651858.CD009613.pub4

66. Li Z, Cheng Y, Wang D, Chen H, Chen H, Ming WK, et al. Incidence rate of type 2 diabetes mellitus after gestational diabetes mellitus: A systematic review and meta-analysis of 170,139 women. *J Diabetes Res 2020* (2020), 3076463. doi: 10.1155/2020/3076463

67. García-Moreno RM, Benítez-Valderrama P, Barquiel B, González Pérez-de-Villar N, Hillman N, Lora Pablos D, et al. Efficacy of continuous glucose monitoring on maternal and neonatal outcomes in gestational diabetes mellitus: a systematic review and meta-analysis of randomized clinical trials. *Diabetes Med* (2022) 39(1):e14703. doi: 10.1111/dme.14703

68. Majewska A, Stanirowski P, Wielgoś M, Bomba-Opoń D. Flash glucose monitoring in gestational diabetes mellitus: study protocol for a randomised controlled trial. *BMJ Open* (2021) 11(3):e041486. doi: 10.1136/bmjopen-2020-041486

69. Yu F, Lv L, Liang Z, Wang Y, Wen J, Lin X, et al. Continuous glucose monitoring effects on maternal glycemic control and pregnancy outcomes in patients with gestational diabetes mellitus: a prospective cohort study. *J Clin Endocrinol Metab* (2014) 99(12):4674–82. doi: 10.1210/jc.2013-4332

70. Sugiyama T, Nagao K, Metoki H, Nishigori H, Saito M, Tokunaga H, et al. Pregnancy outcomes of gestational diabetes mellitus according to pre-gestational BMI in a retrospective multi-institutional study in Japan. *Endocr J* (2014) 61(4):373–80. doi: 10.1507/endocrj.EJ13-0541

71. Rasmussen L, Christensen ML, Poulsen CW, Rud C, Christensen AS, Andersen JR, et al. Effect of high versus low carbohydrate intake in the morning on glycemic variability and glycemic control measured by continuous blood glucose monitoring in women with gestational diabetes mellitus-A randomized crossover study. *Nutrients* (2020) 12(2). doi: 10.3390/nu12020475

72. Ornoy A, Becker M, Weinstein-Fudim L, Ergaz Z. Diabetes during pregnancy: A maternal disease complicating the course of pregnancy with long-term deleterious effects on the offspring. A clinical review. *Int J Mol Sci* (2021) 22(6):2965. doi: 10.3390/ijms22062965

73. McGillick EV, Morrison JL, McMillen IC, Orgeig S. Intrafetal glucose infusion alters glucocorticoid signaling and reduces surfactant protein mRNA expression in the lung of the late-gestation sheep fetus. *Am J Physiol Regul Integr Comp Physiol* (2014) 307(5):R538–45. doi: 10.1152/ajpregu.00053.2014

74. Márquez-Pardo R, Torres-Barea I, Córdoba-Doña JA, Cruzado-Begines C, García-García-Doncel L, Aguilar-Diosdado M, et al. Continuous glucose monitoring and glycemic patterns in pregnant women with gestational diabetes mellitus. *Diabetes Technol Ther* (2020) 22(4):271–7. doi: 10.1089/dia.2019.0319

75. Secher AL, Ringholm L, Andersen HU, Damm P, Mathiesen ER. The effect of real-time continuous glucose monitoring in pregnant women with diabetes: a randomized controlled trial. *Diabetes Care* (2013) 36(7):1877–83. doi: 10.2337/dc12-2360

76. Law GR, Alnaji A, Alrefaii L, Endersby D, Cartland SJ, Gilbey SG, et al. Suboptimal nocturnal glucose control is associated with large for gestational age in treated gestational diabetes mellitus. *Diabetes Care* (2019) 42(5):810–5. doi: 10.2337/dc18-2212

77. Murphy HR, Rayman G, Lewis K, Kelly S, Johal B, Duffield K, et al. Effectiveness of continuous glucose monitoring in pregnant women with diabetes: randomised clinical trial. *BMJ* (2008) 337:a1680. doi: 10.1136/bmj.a1680

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Association between sex steroid hormones and subsequent hyperglycemia during pregnancy

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Objective: Sex steroid hormones may play a role in insulin resistance and glucose dysregulation. However, evidence regarding associations between early-pregnancy sex steroid hormones and hyperglycemia during pregnancy is limited. The primary objective of this study was to assess the relationships between first trimester sex steroid hormones and the subsequent development of hyperglycemia during pregnancy; with secondary evaluation of sex steroid hormones levels in mid-late pregnancy, concurrent with and subsequent to diagnosis of gestational diabetes.

Methods: Retrospective analysis of a prospective pregnancy cohort study was conducted. Medically low-risk participants with no known major endocrine disorders were recruited in the first trimester of pregnancy (n=319). Sex steroid hormones in each trimester, including total testosterone, free testosterone, estrone, estradiol, and estriol, were assessed using high-performance liquid chromatography and tandem mass spectrometry. Glucose levels of the 1-hour oral glucose tolerance test and gestational diabetes diagnosis were abstracted from medical records. Multivariable linear regression models were fitted to assess the associations of individual first trimester sex steroids and glucose levels.

Results: In adjusted models, first trimester total testosterone (β=5.24, 95% CI: 0.01, 10.46, p=0.05) and free testosterone (β=5.98, 95% CI: 0.97, 10.98, p=0.02) were positively associated with subsequent glucose concentrations and gestational diabetes diagnosis (total testosterone: OR=3.63, 95% CI: 1.50, 8.78; free testosterone: OR=3.69; 95% CI: 1.56, 8.73). First trimester estrone was also positively associated with gestational diabetes (OR=3.66, 95% CI: 1.56, 8.55). In mid-late pregnancy, pregnant people with gestational diabetes had lower total testosterone levels (β=-0.19, 95% CI: -0.36, -0.02) after adjustment for first trimester total testosterone.

Conclusion: Early-pregnancy sex steroid hormones, including total testosterone, free testosterone, and estrone, were positively associated with glucose levels and gestational diabetes in mid-late pregnancy. These hormones may serve as early predictors of gestational diabetes in combination with other risk factors.

KEYWORDS

sex steroid hormone, hyperglycemia, gestational diabetes, testosterone, estrogen

Introduction

Hyperglycemia, mainly caused by gestational diabetes mellitus (GDM), is a common metabolic complication during pregnancy (1, 2). GDM is associated with an increased risk of pregnancy related and neonatal outcomes, such as cesarean delivery, macrosomia, and neonatal hypoglycemia (1, 2). Furthermore, in the longer term, people diagnosed with GDM have a higher risk of progression to type 2 diabetes (T2DM), with around 19% of people with GDM develop T2DM after 5 years or more from delivery (3, 4). Children born to people with GDM have an increased risk of obesity, metabolic diseases and neurodevelopmental disorders (5, 6). Genetic predisposition, age, race/ethnicity, and obesity have been identified as risk factors for GDM (1, 7–9). Yet, the pathogenesis of GDM still is poorly understood.

GDM and T2DM are both characterized by insulin resistance (1, 7). Evidence suggests that endogenous sex steroid hormones (SSH), such as testosterone and estradiol, play important roles in glucose intolerance, insulin resistance and the development of T2DM in non-pregnant people (10–13). Additionally, people with hyperandrogenic conditions, such as polycystic ovary syndrome (PCOS) and congenital adrenal hyperplasia, have a higher risk of insulin resistance and T2DM (14–16). Lowering androgen production in PCOS patients leads improved insulin sensitivity and reduces fasting insulin levels (17, 18). Postmenopausal hormone therapy with estrogen/progestin reduces the incidence of diabetes (19, 20). Therefore, through their impacts on insulin and glucose metabolism, endogenous SSH may be involved in the pathogenesis of T2DM.

Likewise, SSH may play a role in the development of GDM. Nevertheless, pregnancy is a unique period given the rapid hormonal changes and the substantially increased estrogen concentrations (21), which may affect the relationship between SSH and glucose regulation. Evidence from people with PCOS substantiates the link between SSH and the risk of GDM during pregnancy (22, 23). However, to date, very few prospective studies have assessed the involvement of SSH, including testosterone and estriol (E3), in the development of GDM in people without PCOS (24–27). Yet, these previous studies have only examined total testosterone (TT) rather than free testosterone (fT) which represents the biologically active fraction of testosterone. Also, these studies did not concurrently examine multiple estrogens as well as testosterone despite their interrelatedness.

Additionally, the association between SSH and GDM may be bidirectional, operating through adipose tissue and insulin regulation (28, 29). Insulin induces androgen biosynthesis in cultured human ovarian theca and stromal cells (30), which suggests that GDM could in turn alter androgen production. Several small case-control studies have assessed differences in SSH in late pregnancy, subsequent to GDM diagnosis, with inconsistent findings (31–33). Moreover, the previous studies did not consider the potential confounding effect of early-pregnancy SSH on the relationship between GDM and SSH in late pregnancy.

Here, we leverage data and biospecimens from a pregnancy cohort that was medically not greater than normal risk at baseline with no known preexisting hormonal conditions to assess testosterone (fT and TT) and estrogens (estrone, estradiol, E3) in early pregnancy in relation to glucose concentrations and GDM diagnosis assessed in mid-late pregnancy. Secondarily, we evaluated associations between GDM diagnosis and the same set of SSH assessed later in pregnancy with and without adjusting for early-pregnancy SSH levels.

Materials and methods

Study overview

The current study is a retrospective analysis of a prospective pregnancy cohort, the Understanding Pregnancy Signals and Infant Development (UPSIDE) study that is a part of the Environmental Influences on Child Health Outcomes (ECHO) program (34). From 2015 to 2019, the UPSIDE study recruited pregnant people (n=326) in their first trimester receiving prenatal care through the University of Rochester Medical Center affiliated obstetric clinics (35). Briefly, the inclusion criteria for the UPSIDE study were (1) <14 weeks of gestation, (2) age 18 or older, (3) a singleton pregnancy, (4) able to communicate in English, (5) no known substance abuse problems or history of psychotic illness, and (6) no greater than normal medical risk. Additionally, women with diagnosed PCOS and T2DM were excluded from the cohort. The study was approved by the institutional review boards at the University of Rochester and

Rutgers University. All participants provided written informed consent prior to participation. The current analysis included participants with SSH measured during pregnancy and a 1-hour oral glucose tolerance test (OGTT) or GDM diagnosis (n=319; Figure 1).

Sex steroid hormone assays

Blood samples were collected in each trimester (1st trimester: 12.2 ± 1.3 weeks; 2nd trimester: 21.2 ± 1.8 weeks; 3rd trimester: 31.4 ± 2 weeks) and after processing, serum was stored in a -80°C freezer until overnight shipment to the Endocrine and Metabolic Research Laboratory at Harbor-UCLA Medical Center. SSH, including TT, fT, estrone(E1), estradiol(E2), and E3, were quantified using validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) methods (36). Briefly, LC–MS/MS was used to assess testosterone concentrations using a Shimadzu HPLC system (Columbia, MD) and an Applied Biosystems API5500 LC–MS/MS (Foster City, CA) equipped with a Turbo-Ion-Spray source that used positive mode. Quality control was performed on each assay run using spiked samples. The limit of quantification (LOQ) for TT was 2 ng/dL. Equilibrium dialysis using labeled testosterone was used to measure fT% which is used to calculate fT levels (fT=TT x fT%). fT% was not detected in one sample collected in the 1st trimester. The Shimadzu HPLC system (Columbia, MD) and a triple quadrupole mass spectrometer (API5000 LC–MS/MS, Foster City, CA) were used to measure estrogen concentrations. The LOQ was 2 pg/mL for E1 and E2, and 50 pg/mL for E3. E3 was not detected in 32 samples collected in the 1st trimester LOQ/$\sqrt{2}$ was used to replace missing E3 values (n=32) and E3 values less than LOQ/$\sqrt{2}$. We additionally calculated the ratio of TT to E2 as a measure of hormone balance.

Glucose measures

As part of routine obstetric care, participants were screened for GDM with 1-hour 50g OGTT at an average gestational age of 27.7 weeks (± 2.9 weeks). Participants with a 1-hour OGTT value of more than 135 mg/dL underwent a further diagnostic test with 3-hour 100g OGTT. Per clinical protocols, GDM was diagnosed according to the National Diabetes Data Group (NDDG) criteria: if the 3-hour OGTT values met more than two of the following values: fasting, 105 mg/dL; 1 hour, 190 mg/dL; 2 hours, 165 mg/dL; and 3 hours, 145 mg/dL. Several participants (n=5) were diagnosed with GDM without completing the 3-hour OGTT by either (1) 1-hour OGTT >200 mg/dL, (2) fasting glucose levels >125 mg/dL, or (3) by paneled blood glucose levels due to inability to complete 3-hour OGTT because of intolerance or history of gastric bypass surgery. OGTT values and GDM diagnosis were abstracted from electronic medical records by trained study staff.

For the purpose of this study, we additionally considered the Carpenter-Coustan (CC) criteria which may identify more GDM cases (37, 38). CC criteria use lower threshold values: if the 3-hour OGTT values met more than two of the following values: fasting, 95 mg/dL; 1 hour, 180 mg/dL; 2 hours, 155 mg/dL; and 3 hours, 140 mg/dL. Six additional participants were classified as having GDM based on the CC criteria.

Body weight measures and other covariates

Adipose tissue may be involved in the metabolism of SSH (39–41) and glucose dysregulation (28, 42, 43). We, therefore, included early-pregnancy body mass index (BMI) as a key confounder in the

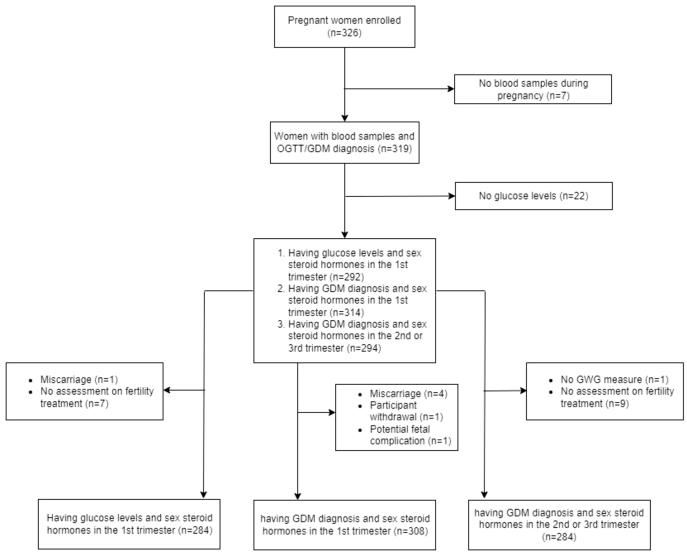

FIGURE 1
Flow chart displaying inclusion and exclusion of this study. GDM, gestational diabetes.

analyses. Early-pregnancy BMI, used as a proxy for pre-pregnancy BMI, was calculated based on weight and height abstracted from medical records from the first clinical visit prior to 14 weeks gestation and the formula $BMI = \frac{Weight(kg)}{Height(m)^2}$ (44).

SSH have been linked to adiposity (45) and early excess gestational weight gain (GWG) has been associated with GDM (46). Therefore, early GWG through the end of 2nd trimester was explored as a potential mediator between the associations of first trimester SSH and GDM. GWG through the end of 2nd trimester was calculated as weight at the end of the 2nd trimester minus early-pregnancy weight. Additionally, GWG through the end of 2nd trimester and total GWG until delivery were included as confounders in our secondary analyses of associations between GDM diagnosis and SSH assessed in mid-late pregnancy.

Age, race/ethnicity, parity, gestational age at the time of blood sample collection, fertility treatment, and infant sex, have been associated with SSH levels during pregnancy and in some cases, GDM as well, and were thus included as covariates (8, 47). Race/ethnicity was categorized as non-Hispanic White, non-Hispanic Black, Hispanic, and others. Parity was characterized as nulliparous and parous. Gestational dating was based on crown-rump length at the earliest available ultrasound and last menstrual period was used when an early ultrasound was not available (7%). Fertility treatment (any/none) was classified based on participant self-report. Although participants diagnosed with PCOS were excluded from the UPSIDE study, to address the possibility of undiagnosed cases, participants were evaluated with several questions to address relevant symptoms, including regularity of periods, hirsutism and acne (see Supplementary Materials) (48). Participants (n=13) categorized as potentially undiagnosed PCOS cases and were excluded in the sensitivity analyses. Additionally, four participants reported having a history of GDM in previous pregnancies and were excluded in the sensitivity analyses.

Statistical analysis

Descriptive statistics were calculated for all variables of interest. SSH were not normally distributed and were thus log-transformed. Early-pregnancy BMI was right skewed and was inverse-transformed. In the primary analyses, a multivariable linear regression model was fitted to assess the association of each first trimester SSH and glucose levels (continuous variable) based on routine 1-hour OGTT. A logistic regression model was fitted to assess the association of each first trimester SSH and GDM diagnosis. Age, race/ethnicity, parity, gestational age at the time of blood sample collection, fertility treatment, early-pregnancy BMI, and infant sex were included as covariates. Fertility treatment was not included in logistic regression models as no positive GDM cases were diagnosed in people reporting fertility treatment for the current pregnancy. GWG through the end of 2nd trimester was further assessed as a potential mediator of the associations between first trimester SSH and GDM diagnosis (Supplementary Figure 1) with bootstrap to estimate bias-corrected confidence intervals (CI). In secondary analyses, linear mixed effects models were fitted to assess the associations of GDM diagnosis and individual SSH in the 2nd and 3rd trimesters. Age, race/ethnicity, parity, gestational age at the time of blood sample collection, fertility treatment, infant sex, early-pregnancy BMI and GWG were included as covariates. First trimester SSH was additionally included as a key confounder. All analyses were conducted using STATA 17.0 (College Station, TX: StataCorp LLC).

Results

Characteristics of the study cohort

The majority of participants (n=319) were non-Hispanic White (55.5%), had at least one prior birth (65.2%), had a college education or more (62.0%), and were overweight or obese in early pregnancy (57.6%). Twenty-two participants (6.9%) were classified as having GDM in this study. The characteristics of the participants grouped by GDM diagnosis are described in Table 1. Participants with GDM were slightly older that those without GDM (30.95 ± 0.71 vs 28.66 ± 0.27 years, p=0.005). SSH varied significantly across trimesters except for fT (Supplementary Table 1). Trend tests indicated that E1, E2, and E3 levels increased and TT/E2 ratios decreased across pregnancy (p<0.001). The correlations among first trimester SSH were weak to moderate (r=0.17-0.35) except for the high correlations between TT and fT (r=0.91) and between E1 and E2 (r=0.81). The correlation between TT and E3 was not significant (Supplementary Table 2).

Associations of first trimester sex steroid hormones with mid-late pregnancy glucose levels and GDM diagnosis

In the primary multivariable regression models, first trimester TT and fT were positively associated with glucose levels measured in mid-late pregnancy after adjusting for maternal age, race/ethnicity, parity, gestational age of blood draw, early-pregnancy BMI, fertility treatment, and infant sex (Table 2). One natural-log unit increases in TT and fT were associated with 5.24 mg/dL (TT: 95% CI: 0.01, 10.46, p=0.05) and 5.98 mg/dL (fT: 95% CI: 0.97, 10.98, p=0.02) higher glucose levels, respectively. Associations between first trimester estrogens and glucose levels were also positive but slightly weaker. Higher first trimester TT and fT was also associated with increased odds of GDM diagnosis (TT: OR=3.63, 95% CI: 1.50, 8.78, p=0.004, Figure 2A; fT: OR=3.69, 95% CI: 1.56, 8.73, p=0.003, Figure 2B). Higher first trimester E1 (OR=3.66, 95% CI: 1.56, 8.55, p=0.003, Figure 2C) and E2 (OR=2.92, 95% CI: 1.00, 8.55, p=0.05), but not E3, were also associated with higher odds of GDM diagnosis. Exclusion of potentially undiagnosed PCOS cases in sensitivity analyses slightly strengthened associations between testosterone and E1 concentrations and glucose levels/GDM diagnosis (Supplementary Table 3). Exclusion of participants with a history of GDM during previous pregnancies had similar results on the associations of fT and E1 with glucose levels and GDM diagnosis (Supplementary Table 4). Associations of TT, fT, and E1 with clinical GDM

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TABLE 1 Characteristics of UPSIDE Participants (n=319).

Variable[a]	All Participants (n=319)[b]	Participants with GDM (n=22)	Participants without GDM (n=297)[c]
Age (years)	28.82 ± 4.68	30.95 ± 3.33	28.66 ± 4.73
Race/Ethnicity			
White, Non-Hispanic	177 (55.5%)	14 (63.6%)	163 (54.9%)
Black, Non-Hispanic	82 (25.7%)	3 (13.6%)	79 (26.6%)
Hispanic	34 (10.7%)	3 (13.6%)	31 (10.4%)
Others	26 (8.2%)	2 (9.1%)	24 (8.1%)
Nulliparous	110 (34.8%)	10 (45.5%)	100 (34%)
Education			
High school or less	120 (38.0%)	8 (36.4%)	112 (38.1%)
Fetal sex_male	158 (50.5%)	10 (45.5%)	148 (50.9%)
Early-pregnancy BMI (kg/m^2)	28.27 ± 7.04	30.65 ± 8.34	28.09 ± 6.92
Glucose levels[d] (mg/dL)	113.66 ± 26.32	157.82 ± 16.58	110.13 ± 23.63

[a]Continuous variables are summarized using mean and standard deviation; Categorical variables are summarized using count and percentage. [b]Sample size for parity, education and early-pregnancy BMI is 316; sample size for infant sex is 313; sample size for glucose levels is 297. [c]Sample size for parity, education and early-pregnancy body mass index (BMI) is 294; sample size for infant sex is 291; sample size for glucose level is 275. [d]Glucose levels were derived from 1-hour glucose tolerance test results.

diagnosis (solely by clinical criteria, not CC criteria) remained significant (Supplementary Table 5). Given the relatively weak correlations between testosterone and estrogens, we explored models including both fT and E1 simultaneously, fT and E1 were still associated with higher odds of GDM diagnosis (fT: OR=3.33, 95% CI: 1.35, 8.23, p=0.009; E1: OR=3.32, 95% CI: 1.38, 8.03 p=0.008); associations with glucose levels were positive but attenuated compared to models assessing the hormones individually (fT: β =5.12, 95% CI: -0.02, 10.26, p=0.05; E1: β =3.31, 95% CI: -1.35, 7.97, p=0.16).

Evaluation of confounding and mediation by adiposity

Early-pregnancy BMI was a key confounding variable in the associations between sex steroids and glucose levels. Early-pregnancy BMI was positively associated with glucose levels (β=0.65, 95% CI: 0.21, 1.08, p=0.004) and first trimester fT and TT/E2 ratio, but was negatively associated with E1 and E2 (Supplementary Table 6). Regression models including early-pregnancy BMI as a covariate (Table 2) showed similar but slightly weakened significant positive associations between testosterone and glucose levels/GDM compared to regression models excluding early-pregnancy BMI (Supplementary Table 7). The relationships among early-pregnancy fT, early-pregnancy BMI and GDM are also illustrated in Supplementary Figure 2. The association between E1 and GDM was attenuated (OR=2.95, 95% CI: 1.31, 6.64, p=0.01) by excluding early-pregnancy BMI in the models (Supplementary Table 7).

GWG might mediate the effect of sex steroids on glucose levels. But GWG through the end of the 2nd trimester was not significantly associated with GDM diagnosis (OR=0.96, p=0.15) and only showed a borderline association with first trimester TT (β=1.61,

TABLE 2 Associations of Log-transformed First Trimester Sex Steroid Hormones with Glucose Levels and Gestational Diabetes Diagnosis in Mid-late Pregnancy.

Sex Steroid Hormones	Glucose Levels (mg/dL) (n=284)			GDM Diagnosis (n=308)		
	Coefficient	95% CI	*P*	OR	95% CI	*P*
TT (ng/dL)	5.24	0.01, 10.46	0.05	3.63	1.50, 8.78	0.004
fT (ng/dL)	5.98	0.97, 10.98	0.02	3.69	1.56, 8.73	0.003
E1 (pg/mL)	4.39	-0.15, 8.94	0.06	3.66	1.56, 8.55	0.003
E2 (pg/mL)	5.65	-1.02, 12.31	0.10	2.92	1.00, 8.55	0.05
E3 (pg/mL)	2.99	-0.17, 6.14	0.06	1.06	0.66, 1.71	0.82
TT/E2	1.68	-3.46, 6.83	0.52	1.62	0.77, 3.44	0.21

Maternal age, race/ethnicity, parity, gestational age of blood draw, early-pregnancy BMI, and infant sex were adjusted in all models. Fertility treatment was adjusted in the models with glucose levels as the outcome. All sex steroids were log-transformed. GDM, gestational diabetes; TT, total testosterone; fT, free testosterone; E1, estrone; E2, estradiol; E3, estriol.

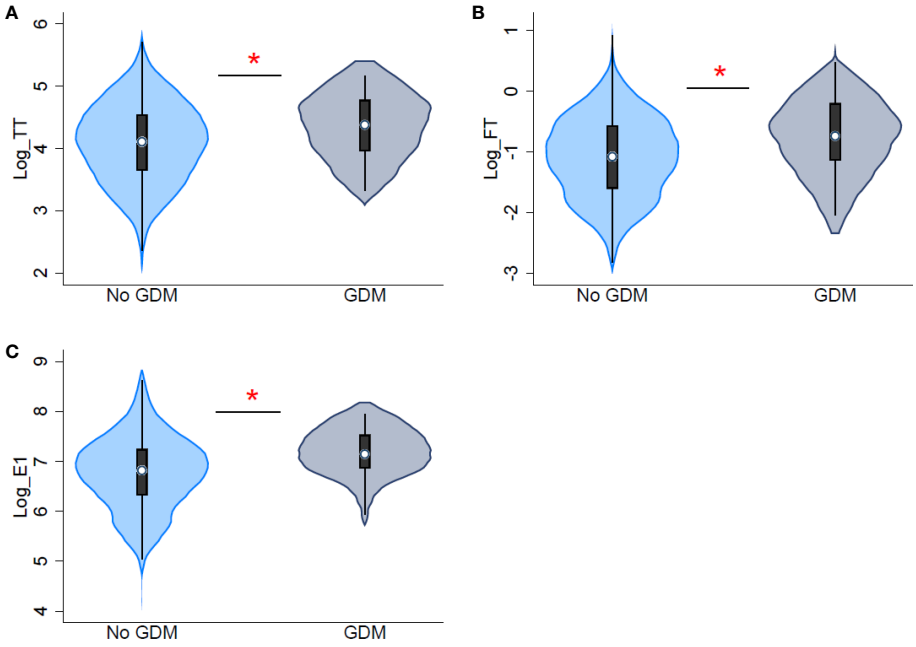

FIGURE 2
Distribution of first trimester log-transformed sex steroid hormones by gestational diabetes diagnosis. **(A)** distribution of total testosterone (TT) **(B)** distribution of free testosterone (FT) **(C)** distribution of estrone (E1). * indicates significant differences between participants with and without gestational diabetes (GDM) diagnosis.

95% CI: -0.08, 3.30, p=0.06). The mediation effect of GWG on the relationship between TT and GDM was not significant (indirect effect: β=-0.05, 95% $CI_{bootstrap}$: -0.22, 0.03).

Associations of GDM diagnosis with sex steroid hormones in the 2nd and 3rd trimesters

GDM diagnosis was positively associated with E1 levels (β=0.29, 95% CI: 0.02, 0.56, p=0.03) in the 2nd and 3rd trimesters (Supplementary Table 8). Further adjusting for E1 levels in the 1st trimester, the association between GDM and E1 levels in the 2nd and 3rd trimesters was not significant (β=0.01, 95% CI: -0.18, 0.19, p=0.95). However, GDM diagnosis was inversely associated with TT in the 2nd and 3rd trimesters (β=-0.19, 95% CI: -0.36, -0.02, p=0.03), after adjustment for first trimester TT. But no associations between GDM and fT in the 2nd and 3rd trimesters were observed.

Discussion

In this prospective pregnancy cohort including pregnant people who were medically not greater than normal risk at enrollment, first trimester TT, fT, and E1 were positively associated with glucose levels and GDM diagnosis in mid-late pregnancy, with similar trends observed for E2. fT and E1 were independently associated with increased odds of subsequent GDM diagnosis, when both were included in the same model. Results were robust to the exclusion of participants with potentially undiagnosed PCOS. GDM diagnosis was associated with lower TT but not fT levels in the 2nd and 3rd trimesters, when first trimester SSH was adjusted, respectively.

In females, androgens are mainly produced by the ovaries, adrenal glands, and adipose tissue (49). The placenta may also contribute to androgen synthesis during pregnancy (50). Prior studies that assessed associations between first trimester androgen levels and subsequent GDM diagnosis are limited. Two studies found a positive relationship between total testosterone levels in early pregnancy and GDM diagnosis in White pregnant people (25, 26), consistent with the results of this study. However, Gözükara, et al. (2015) and Mustaniemi, et al. (2023) measured TT levels using immunoassays and did not directly measure fT, the biologically active form of testosterone (25, 26). Improving upon the limitations of immunoassays, this study used LC-MS/MS, a gold standard method with greater sensitivity and specificity for steroid measurement (51). Similar to TT, first trimester fT showed positive and slightly stronger associations with glucose levels and GDM diagnosis.

Although evidence of the associations of first trimester TT and fT with GDM diagnosis is scarce, in prospective studies of non-pregnant people, TT and/or fT have been positively associated with development of T2DM in pre- and post-menopausal people (10, 12, 52, 53); but other studies have observed either no or attenuated associations after adjusting for adiposity (54–56). Generally, concentrations of TT and fT are higher in pregnant people compared to non-pregnant people (49), so to the extent that androgens play a causal role in glucose dysregulation, pregnancy may be a period of particular vulnerability.

We observed little evidence that adiposity was a confounder or mediator of the relationship between early-pregnancy testosterone

and the development of GDM. Gözükara, et al. (2015) and Mustaniemi, et al. (2023) also identified that early pregnancy TT levels were higher among participants who subsequently developed GDM after adjusting for BMI (25, 26), which was consistent with our findings. However, evidence suggests that androgens exert direct and indirect effects on insulin sensitivity in adipose tissue and skeletal muscle (28, 42, 43). In female animal models, testosterone administration increased insulin resistance with or without western diet (57, 58). In subcutaneous adipocytes harvested from healthy non-pregnant people, testosterone treatment induced insulin resistance *in vitro* and inhibited insulin-stimulated glucose uptake (59). Administration of testosterone to oophorectomized female rodents impaired whole-body insulin-mediated glucose uptake potentially by lessening glycogen synthase expression and GLUT4 transporter expression in skeletal muscle (60–62). Also, anti-androgen treatments improved glucose tolerance in pregnant rat models (63). Given the findings in this study and the research in animal models and human adipocytes, it is postulated that androgens may contribute to the development of GDM by inducing insulin resistance not only in adipose tissue but also in other tissues, such as skeletal muscle.

In pregnant people, estrogens are mainly produced by the ovaries and placenta, with smaller contributions from other tissues, such as adipose tissue and adrenal glands (24). In this study, first trimester E1 levels were positively associated with subsequent GDM diagnosis. Although excessive testosterone could be converted into E1 in adipose tissue (43), in this study E1 was found to be a predictor of GDM independent of fT. We know of no other study that has addressed this association previously, but in a study of non-pregnant premenopausal people, estrone sulfate levels were positively correlated with postprandial glucose levels (56). In non-pregnant premenopausal people with PCOS, higher E1/E2 ratio was associated with increased fasting and postprandial glucose levels and insulin resistance (64). Therefore, E1 is potentially involved in glucose intolerance and GDM.

Research on the mechanisms linking estrogens to glucose regulation has primarily focused on E2 and evidence on E1 is sparse (65). In this study, while all estrogens showed positive associations with glucose levels and GDM, associations were strongest for E1. Borthwick et al. (2001) found that estrone sulfate could normalize hyperglycemia in obese-diabetic mice (both male and female) via the reduction of hepatic glucose-6-phosphatase (66). Although this finding conflicts with our results and findings in premenopausal women (56, 64), it is consistent with findings on E2, which may protect pancreatic β cell functions (67–69), reduce adipocyte hypertrophy and insulin resistance (68, 70), and improve hepatic glucose utilization (71). On the other hand, high concentrations of endogenous E2, particularly seen during pregnancy (21), may reduce insulin sensitivity (72) via decreased GLUT4 transporter expression in skeletal muscle (73) and interfere with insulin binding to insulin receptors (74). Therefore, the effect of endogenous estrogens on glucose regulation may vary in a non-linear manner and high concentrations of E1, similar to E2, potentially induce insulin resistance during pregnancy.

Because GDM may affect the production of SSH via insulin (30, 75), we further assessed the associations of GDM with SSH in mid-late pregnancy. When first-trimester SSH was not considered, the associations between GDM and SSH levels in mid-late pregnancy were consistent with the directions of associations between early-pregnancy SSH and GDM. These results were also similar to previous findings (31–33). When first-trimester estrogen was considered, the associations between GDM and estrogen were greatly attenuated, which indicates that the positive associations in mid-late pregnancy could be accounted by or driven by early-pregnancy estrogen levels. When first-trimester testosterone was considered, the directions of associations between GDM and testosterone were reversed, although the association between GDM and fT was not significant. These findings indicate that other factors changing during mid-late pregnancy, such as insulin levels which may be affected by GDM treatment, sex hormone binding globulin (SHBG) levels which is bound to fT to form TT, placental aromatase, and increasing gestational weight, may affect mid-late pregnancy testosterone levels and thus the relationship between GDM and mid-late pregnancy testosterone levels (30, 75–77).

A strength of this study is the measurement of SSH using the gold standard LC-MS/MS method, which is an advance over prior studies in this field. Furthermore, the prospective design of the study cohort established the temporal relationships between SSH in the 1st trimester and glucose levels and GDM in mid-late pregnancy. In addition, repeat measures of SSH throughout pregnancy enabled us to assess hormone levels both prior to and after GDM diagnosis, while taking early-pregnancy SSH levels into consideration. Several limitations should be considered when interpreting the results of the current analyses. We did not assess insulin resistance or visceral adiposity in our cohort, which are potential key mechanisms linking SSH to GDM (1, 7, 24, 42). Further investigations of the relationship among SSH, adiposity, and insulin resistance during pregnancy are warranted. Also, future studies could assess the effect of insulin and SHGB levels during mid-late pregnancy on the relationship between GDM and mid-late pregnancy testosterone levels. Another limitation is that the limited GDM cases in this study could not provide reliable estimations of the cutoff values of first trimester TT, fT or E1 to predict GDM. Additionally, we did not assess SHBG, which was negatively associated with GDM in a recent meta-analysis (78). SHBG binds both testosterone and E2 during pregnancy (42, 79) and thus, low SHBG levels indicate high serum concentrations of fT and free E2. Therefore, the previous findings of the negative association between SHBG and GDM are consistent with the positive associations between fT and GDM found in this study (78). We assessed the potential undiagnosed PCOS cases by self report using a two-question response to oligomenorrhoea and hirsutism. This self-report approach has been found in longitudinal studies to be associated with clinical biomarkers and measures (48, 80), although additional assessments could confirm the diagnosis.

Conclusion

In this prospective study of pregnant people, higher levels of first-trimester TT, fT and estrone were positively associated with

glucose levels and GDM diagnosis in mid-late pregnancy. Our findings suggest that the early-pregnancy hormonal milieu may contribute to and/or predict gestational hyperglycemia. Studies such as the current study that identify early-pregnancy biomarkers may inform future targeted screening and interventions (lifestyle modifications, etc.) aimed at preventing GDM in pregnant people who are at risk.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://dash.nichd.nih.gov/study/417122.

Ethics statement

The studies involving humans were approved by The University of Rochester Research Subjects Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

All authors contribute to the generation of hypotheses, statistical analyses, manuscript preparation and final approval of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1213402/full#supplementary-material

References

1. Baz B, Riveline J-P, Gautier J-F. ENDOCRINOLOGY OF PREGNANCY: Gestational diabetes mellitus: definition, aetiological and clinical aspects. *Eur J Endocrinol* (2016) 174(2):R43–51. doi: 10.1530/eje-15-0378

2. Hod M, Kapur A, Sacks DA, Hadar E, Agarwal M, Di Renzo GC, et al. The International Federation of Gynecology and Obstetrics (FIGO) Initiative on gestational diabetes mellitus: A pragmatic guide for diagnosis, management, and care. *Int J Gynecology Obstetrics* (2015) 131:S173–211. doi: 10.1016/S0020-7292(15)30033-3

3. Juan J, Sun Y, Wei Y, Wang S, Song G, Yan J, et al. Progression to type 2 diabetes mellitus after gestational diabetes mellitus diagnosed by IADPSG criteria: Systematic review and meta-analysis. *Front Endocrinol (Lausanne).* (2022) 13:1012244. doi: 10.3389/fendo.2022.1012244

4. Bellamy L, Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet* (2009) 373 (9677):1773–9. doi: 10.1016/s0140-6736(09)60731-5

5. Bianco ME, Josefson JL. Hyperglycemia during pregnancy and long-term offspring outcomes. *Curr Diabetes Rep* (2019) 19(12):143. doi: 10.1007/s11892-019-1267-6

6. Rowland J, Wilson CA. The association between gestational diabetes and ASD and ADHD: a systematic review and meta-analysis. *Sci Rep* (2021) 11(1):5136. doi: 10.1038/s41598-021-84573-3

7. Dalfrà MG, Burlina S, Del Vescovo GG, Lapolla A. Genetics and epigenetics: new insight on gestational diabetes mellitus. *Front Endocrinol (Lausanne).* (2020) 11:602477. doi: 10.3389/fendo.2020.602477

8. Farrar D, Simmonds M, Bryant M, Lawlor DA, Dunne F, Tuffnell D, et al. Risk factor screening to identify women requiring oral glucose tolerance testing to diagnose gestational diabetes: a systematic review and meta-analysis and analysis of two pregnancy cohorts. *PloS One* (2017) 12(4):e0175288. doi: 10.1371/journal.pone.0175288

9. Bower JK, Butler BN, Bose-Brill S, Kue J, Wassel CL. Racial/ethnic differences in diabetes screening and hyperglycemia among US women after gestational diabetes. *Prev Chronic Dis* (2019) 16:E145. doi: 10.5888/pcd16.190144

10. Rasmussen JJ, Selmer C, Frøssing S, Schou M, Faber J, Torp-Pedersen C, et al. Endogenous testosterone levels are associated with risk of type 2 diabetes in women without established comorbidity. *J Endocr Soc* (2020) 4(6):bvaa050. doi: 10.1210/jendso/bvaa050

11. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. *Jama* (2006) 295 (11):1288–99. doi: 10.1001/jama.295.11.1288

12. Ding EL, Song Y, Manson JE, Rifai N, Buring JE, Liu S. Plasma sex steroid hormones and risk of developing type 2 diabetes in women: a prospective study. *Diabetologia* (2007) 50(10):2076–84. doi: 10.1007/s00125-007-0785-y

13. Golden SH, Dobs AS, Vaidya D, Szklo M, Gapstur S, Kopp P, et al. Endogenous sex hormones and glucose tolerance status in postmenopausal women. *J Clin Endocrinol Metab* (2007) 92(4):1289–95. doi: 10.1210/jc.2006-1895

14. Moran LJ, Misso ML, Wild RA, Norman RJ. Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod Update* (2010) 16(4):347–63. doi: 10.1093/humupd/dmq001

15. Macut D, Zdravković V, Bjekić-Macut J, Mastorakos G, Pignatelli D. Metabolic perspectives for non-classical congenital adrenal hyperplasia with relation to the classical form of the disease. *Front Endocrinol (Lausanne).* (2019) 10:681. doi: 10.3389/fendo.2019.00681

16. O'Reilly MW, Taylor AE, Crabtree NJ, Hughes BA, Capper F, Crowley RK, et al. Hyperandrogenemia predicts metabolic phenotype in polycystic ovary syndrome: the utility of serum androstenedione. *J Clin Endocrinol Metab* (2014) 99(3):1027–36. doi: 10.1210/jc.2013-3399

17. Banaszewska B, Wrotyńska-Barczyńska J, Spaczynski RZ, Pawelczyk L, Duleba AJ. Effects of resveratrol on polycystic ovary syndrome: A double-blind, randomized, placebo-controlled trial. *J Clin Endocrinol Metab* (2016) 101(11):4322–8. doi: 10.1210/jc.2016-1858

18. Gambineri A, Patton L, Vaccina A, Cacciari M, Morselli-Labate AM, Cavazza C, et al. Treatment with flutamide, metformin, and their combination added to a hypocaloric diet in overweight-obese women with polycystic ovary syndrome: a randomized, 12-month, placebo-controlled study. *J Clin Endocrinol Metab* (2006) 91 (10):3970–80. doi: 10.1210/jc.2005-2250

19. Margolis KL, Bonds DE, Rodabough RJ, Tinker L, Phillips LS, Allen C, et al. Effect of oestrogen plus progestin on the incidence of diabetes in postmenopausal women: results from the Women's Health Initiative Hormone Trial. *Diabetologia* (2004) 47(7):1175–87. doi: 10.1007/s00125-004-1448-x

20. Kanaya AM, Herrington D, Vittinghoff E, Lin F, Grady D, Bittner V, et al. Glycemic effects of postmenopausal hormone therapy: the heart and estrogen/progestin replacement study: A randomized, double-blind, placebo-controlled trial. *Ann Internal Med* (2003) 138(1):1–9. doi: 10.7326/0003-4819-138-1-200301070-00005%m12513038

21. Schock H, Zeleniuch-Jacquotte A, Lundin E, Grankvist K, Lakso H, Idahl A, et al. Hormone concentrations throughout uncomplicated pregnancies: a longitudinal study. *BMC Pregnancy Childbirth* (2016) 16(1):146. doi: 10.1186/s12884-016-0937-5

22. Toulis KA, Goulis DG, Kolibianakis EM, Venetis CA, Tarlatzis BC, Papadimas I. Risk of gestational diabetes mellitus in women with polycystic ovary syndrome: a systematic review and a meta-analysis. *Fertil Steril* (2009) 92(2):667–77. doi: 10.1016/j.fertnstert.2008.06.045

23. Joham AE, Ranasinha S, Zoungas S, Moran L, Teede HJ. Gestational diabetes and type 2 diabetes in reproductive-aged women with polycystic ovary syndrome. *J Clin Endocrinol Metab* (2014) 99(3):E447–52. doi: 10.1210/jc.2013-2007

24. Vejrazkova D, Vcelak J, Vankova M, Lukasova P, Bradnova O, Halkova T, et al. Steroids and insulin resistance in pregnancy. *J Steroid Biochem Mol Biol* (2014) 139:122–9. doi: 10.1016/j.jsbmb.2012.11.007

25. Gözükara YM, Aytan H, Ertunc D, Tok EC, Demirtürk F, Şahin Ş, et al. Role of first trimester total testosterone in prediction of subsequent gestational diabetes mellitus. *J Obstet Gynaecol Res* (2015) 41(2):193–8. doi: 10.1111/jog.12525

26. Mustaniemi S, Morin-Papunen L, Keikkala E, Öhman H, Surcel HM, Kaaja R, et al. Associations of low sex hormone-binding globulin and androgen excess in early pregnancy with fasting and post-prandial hyperglycaemia, gestational diabetes, and its severity. *Diabetes Metab Res Rev* (2023) 39(2):e3599. doi: 10.1002/dmrr.3599

27. Hur J, Cho EH, Baek KH, Lee KJ. Prediction of gestational diabetes mellitus by unconjugated estriol levels in maternal serum. *Int J Med Sci* (2017) 14(2):123–7. doi: 10.7150/ijms.17321

28. Schiffer L, Kempegowda P, Arlt W, O'Reilly MW. MECHANISMS IN ENDOCRINOLOGY: The sexually dimorphic role of androgens in human metabolic disease. *Eur J Endocrinol* (2017) 177(3):R125–r143. doi: 10.1530/eje-17-0124

29. Brüning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, et al. Role of brain insulin receptor in control of body weight and reproduction. *Science* (2000) 289 (5487):2122–5. doi: 10.1126/science.289.5487.2122

30. Barbieri RL, Makris A, Ryan KJ. Insulin stimulates androgen accumulation in inCubations of human ovarian stroma and theca. *Obstet Gynecol* (1984) 64(3 Suppl):73s–80s. doi: 10.1097/00006250-198409001-00019

31. Yuan T, Li Y. Steroid profiling and genetic variants in Chinese women with gestational diabetes mellitus. *J Steroid Biochem Mol Biol* (2021) 214:105999. doi: 10.1016/j.jsbmb.2021.105999

32. Villarroel C, Salinas A, López P, Kohen P, Rencoret G, Devoto L, et al. Pregestational type 2 diabetes and gestational diabetes exhibit different sexual steroid profiles during pregnancy. *Gynecol Endocrinol* (2017) 33(3):212–7. doi: 10.1080/09513590.2016.1248933

33. Morisset AS, Dubé MC, Drolet R, Pelletier M, Labrie F, Luu-The V, et al. Androgens in the maternal and fetal circulation: association with insulin resistance. *J Matern Fetal Neonatal Med* (2013) 26(5):513–9. doi: 10.3109/14767058.2012.735725

34. Knapp EA, Kress AM, Parker CB, Page GP, McArthur K, Gachigi KK, et al. The environmental influences on child health outcomes (ECHO)-wide cohort. *Am J Epidemiol* (2023) 192(8):1249–63. doi: 10.1093/aje/kwad071

35. O'Connor T, Best M, Brunner J, Ciesla AA, Cunning A, Kapula N, et al. Cohort profile: Understanding Pregnancy Signals and Infant Development (UPSIDE): a pregnancy cohort study on prenatal exposure mechanisms for child health. *BMJ Open* (2021) 11(4):e044798. doi: 10.1136/bmjopen-2020-044798

36. Sathyanarayana S, Butts S, Wang C, Barrett E, Nguyen R, Schwartz SM, et al. Early prenatal phthalate exposure, sex steroid hormones, and birth outcomes. *J Clin Endocrinol Metab* (2017) 102(6):1870–8. doi: 10.1210/jc.2016-3837

37. Berggren EK, Boggess KA, Stuebe AM, Jonsson Funk M. National Diabetes Data Group vs Carpenter-Coustan criteria to diagnose gestational diabetes. *Am J Obstet Gynecol* (2011) 205(3):253.e1–7. doi: 10.1016/j.ajog.2011.06.026

38. Harper LM, Mele L, Landon MB, Carpenter MW, Ramin SM, Reddy UM, et al. Carpenter-coustan compared with national diabetes data group criteria for diagnosing gestational diabetes. *Obstet Gynecol. May* (2016) 127(5):893–8. doi: 10.1097/aog.0000000000001383

39. Quinkler M, Sinha B, Tomlinson JW, Bujalska IJ, Stewart PM, Arlt W. Androgen generation in adipose tissue in women with simple obesity–a site-specific role for 17beta-hydroxysteroid dehydrogenase type 5. *J Endocrinol Nov* (2004) 183(2):331–42. doi: 10.1677/joe.1.05762

40. Hetemäki N, Mikkola TS, Tikkanen MJ, Wang F, Hämäläinen E, Turpeinen U, et al. Adipose tissue estrogen production and metabolism in premenopausal women. *J Steroid Biochem Mol Biol* (2021) 209:105849. doi: 10.1016/j.jsbmb.2021.105849

41. Maliqueo M, Cruz G, Espina C, Contreras I, García M, Echiburú B, et al. Obesity during pregnancy affects sex steroid concentrations depending on fetal gender. *Int J Obes (Lond)* (2017) 41(11):1636–45. doi: 10.1038/ijo.2017.159

42. Gambineri A, Pelusi C. Sex hormones, obesity and type 2 diabetes: is there a link? *Endocr Connect* (2019) 8(1):R1–r9. doi: 10.1530/ec-18-0450

43. Li Y, Chen C, Ma Y, Xiao J, Luo G, Li Y, et al. Multi-system reproductive metabolic disorder: significance for the pathogenesis and therapy of polycystic ovary syndrome (PCOS). *Life Sci* (2019) 228:167–75. doi: 10.1016/j.lfs.2019.04.046

44. Groth SW, Fernandez ID, Block RC, Thurston SW, Wong E, Brunner J, et al. Biological changes in the pregnancy-postpartum period and subsequent cardiometabolic risk-UPSIDE MOMS: A research protocol. *Res Nurs Health* (2021) 44(4):608–19. doi: 10.1002/nur.22141

45. Pasquali R, Oriolo C. Obesity and androgens in women. *Front Horm Res* (2019) 53:120–34. doi: 10.1159/000494908

46. Robitaille J. Excessive gestational weight gain and gestational diabetes: importance of the first weeks of pregnancy. *Diabetologia* (2015) 58(10):2203–5. doi: 10.1007/s00125-015-3725-2

47. Barrett ES, Mbowe O, Thurston SW, Butts S, Wang C, Nguyen R, et al. Predictors of steroid hormone concentrations in early pregnancy: results from a multi-center cohort. *Matern Child Health J* (2019) 23(3):397–407. doi: 10.1007/s10995-018-02705-0

48. Taponen S, Martikainen H, Järvelin MR, Laitinen J, Pouta A, Hartikainen AL, et al. Hormonal profile of women with self-reported symptoms of oligomenorrhea and/or hirsutism: Northern Finland birth cohort 1966 study. *J Clin Endocrinol Metab* (2003) 88(1):141–7. doi: 10.1210/jc.2002-020982

49. Makieva S, Saunders PT, Norman JE. Androgens in pregnancy: roles in parturition. *Hum Reprod Update* (2014) 20(4):542–59. doi: 10.1093/humupd/dmu008

50. Escobar JC, Patel SS, Beshay VE, Suzuki T, Carr BR. The human placenta expresses CYP17 and generates androgens de novo. *J Clin Endocrinol Metab* (2011) 96 (5):1385–92. doi: 10.1210/jc.2010-2504

51. French D. Advances in bioanalytical techniques to measure steroid hormones in serum. *Bioanalysis* (2016) 8(11):1203–19. doi: 10.4155/bio-2015-0025

52. Oh JY, Barrett-Connor E, Wedick NM, Wingard DL. Endogenous sex hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo study. *Diabetes Care* (2002) 25(1):55–60. doi: 10.2337/diacare.25.1.55

53. Kalyani RR, Franco M, Dobs AS, Ouyang P, Vaidya D, Bertoni A, et al. The association of endogenous sex hormones, adiposity, and insulin resistance with incident diabetes in postmenopausal women. *J Clin Endocrinol Metab* (2009) 94 (11):4127–35. doi: 10.1210/jc.2009-0910

54. Muka T, Nano J, Jaspers L, Meun C, Bramer WM, Hofman A, et al. Associations of steroid sex hormones and sex hormone-binding globulin with the risk of type 2 diabetes in women: A population-based cohort study and meta-analysis. *Diabetes* (2017) 66(3):577–86. doi: 10.2337/db16-0473

55. Fenske B, Kische H, Gross S, Wallaschofski H, Völzke H, Dörr M, et al. Endogenous androgens and sex hormone-binding globulin in women and risk of metabolic syndrome and type 2 diabetes. *J Clin Endocrinol Metab* (2015) 100(12):4595–603. doi: 10.1210/jc.2015-2546

56. Mather KJ, Kim C, Christophi CA, Aroda VR, Knowler WC, Edelstein SE, et al. Steroid sex hormones, sex hormone-binding globulin, and diabetes incidence in the diabetes prevention program. *J Clin Endocrinol Metab* (2015) 100(10):3778–86. doi: 10.1210/jc.2015-2328

57. Beloosesky R, Gold R, Almog B, Sasson R, Dantes A, Land-Bracha A, et al. Induction of polycystic ovary by testosterone in immature female rats: Modulation of apoptosis and attenuation of glucose/insulin ratio. *Int J Mol Med* (2004) 14(2):207–15. doi: 10.3892/ijmm.14.2.207

58. Bishop CV, Takahashi D, Mishler E, Slayden OD, Roberts CT, Hennebold J, et al. Individual and combined effects of 5-year exposure to hyperandrogenemia and Western-style diet on metabolism and reproduction in female rhesus macaques. *Hum Reprod* (2021) 36(2):444–54. doi: 10.1093/humrep/deaa321

59. Corbould A. Chronic testosterone treatment induces selective insulin resistance in subcutaneous adipocytes of women. *J Endocrinol* (2007) 192(3):585–94. doi: 10.1677/joe.1.07070

60. Rincon J, Holmäng A, Wahlström EO, Lönnroth P, Björntorp P, Zierath JR, et al. Mechanisms behind insulin resistance in rat skeletal muscle after oophorectomy and additional testosterone treatment. *Diabetes* (1996) 45(5):615–21. doi: 10.2337/diab.45.5.615

61. Holmäng A, Larsson BM, Brzezinska Z, Björntorp P. Effects of short-term testosterone exposure on insulin sensitivity of muscles in female rats. *Am J Physiol* (1992) 262(6 Pt 1):E851–5. doi: 10.1152/ajpendo.1992.262.6.E851

62. Inada A, Fujii NL, Inada O, Higaki Y, Furuichi Y, Nabeshima YI. Effects of 17β-estradiol and androgen on glucose metabolism in skeletal muscle. *Endocrinology* (2016) 157(12):4691–705. doi: 10.1210/en.2016-1261

63. Zhang Y, Hu M, Yang F, Zhang Y, Ma S, Zhang D, et al. Increased uterine androgen receptor protein abundance results in implantation and mitochondrial defects in pregnant rats with hyperandrogenism and insulin resistance. *J Mol Med (Berl)* (2021) 99(10):1427–46. doi: 10.1007/s00109-021-02104-z

64. Kim N, Chun S. Association between the serum estrone-to-estradiol ratio and parameters related to glucose metabolism and insulin resistance in women with polycystic ovary syndrome. *Clin Exp Reprod Med* (2021) 48(4):374–9. doi: 10.5653/cerm.2021.04553

65. Alemany M. Estrogens and the regulation of glucose metabolism. *World J Diabetes* (2021) 12(10):1622–54. doi: 10.4239/wjd.v12.i10.1622

66. Borthwick EB, Houston MP, Coughtrie MW, Burchell A. The antihyperglycemic effect of estrone sulfate in genetically obese-diabetic (ob/ob) mice is associated with reduced hepatic glucose-6-phosphatase. *Horm Metab Res* (2001) 33(12):721–6. doi: 10.1055/s-2001-19136

67. Liu S, Mauvais-Jarvis F. Rapid, nongenomic estrogen actions protect pancreatic islet survival. *Islets* (2009) 1(3):273–5. doi: 10.4161/isl.1.3.9781

68. Mauvais-Jarvis F, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. *Endocr Rev* (2013) 34(3):309–38. doi: 10.1210/er.2012-1055

69. Nadal A, Alonso-Magdalena P, Soriano S, Ropero AB, Quesada I. The role of oestrogens in the adaptation of islets to insulin resistance. *J Physiol* (2009) 587(Pt 21):5031–7. doi: 10.1113/jphysiol.2009.177188

70. Stubbins RE, Najjar K, Holcomb VB, Hong J, Núñez NP. Oestrogen alters adipocyte biology and protects female mice from adipocyte inflammation and insulin resistance. *Diabetes Obes Metab Jan* (2012) 14(1):58–66. doi: 10.1111/j.1463-1326.2011.01488.x

71. Liebmann M, Asuaje Pfeifer M, Grupe K, Scherneck S. Estradiol (E2) improves glucose-stimulated insulin secretion and stabilizes GDM progression in a prediabetic mouse model. *Int J Mol Sci* (2022) 23(12):6693. doi: 10.3390/ijms23126693

72. González C, Alonso A, Alvarez N, Díaz F, Martínez M, Fernández S, et al. Role of 17beta-estradiol and/or progesterone on insulin sensitivity in the rat: implications during pregnancy. *J Endocrinol* (2000) 166(2):283–91. doi: 10.1677/joe.0.1660283

73. Barros RP, Morani A, Moriscot A, MaChado UF. Insulin resistance of pregnancy involves estrogen-induced repression of muscle GLUT4. *Mol Cell Endocrinol* (2008) 295(1-2):24–31. doi: 10.1016/j.mce.2008.07.008

74. Root-Bernstein R, Podufaly A, Dillon PF. Estradiol binds to insulin and insulin receptor decreasing insulin binding in *vitro*. Original research. *Front Endocrinol* (2014) 5:118. doi: 10.3389/fendo.2014.00118

75. Uzelac PS, Li X, Lin J, Neese LD, Lin L, Nakajima ST, et al. Dysregulation of leptin and testosterone production and their receptor expression in the human placenta with gestational diabetes mellitus. *Placenta* (2010) 31(7):581–8. doi: 10.1016/j.placenta.2010.04.002

76. Kallak TK, Hellgren C, Skalkidou A, Sandelin-Francke L, Ubhayasekhera K, Bergquist J, et al. Maternal and female fetal testosterone levels are associated with maternal age and gestational weight gain. *Eur J Endocrinol* (2017) 177(4):379–88. doi: 10.1530/eje-17-0207

77. O'Leary P, Boyne P, Flett P, Beilby J, James I. Longitudinal assessment of changes in reproductive hormones during normal pregnancy. *Clin Chem* (1991) 37(5):667–72. doi: 10.1093/clinchem/37.5.667

78. Li MY, Rawal S, Hinkle SN, Zhu YY, Tekola-Ayele F, Tsai MY, et al. Sex hormone-binding globulin, cardiometabolic biomarkers, and gestational diabetes: A longitudinal study and meta-analysis. *Matern Fetal Med* (2020) 2(1):2–9. doi: 10.1097/fm9.0000000000000037

79. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* (1999) 84(10):3666–72. doi: 10.1210/jcem.84.10.6079

80. Taponen S, Ahonkallio S, Martikainen H, Koivunen R, Ruokonen A, Sovio U, et al. Prevalence of polycystic ovaries in women with self-reported symptoms of oligomenorrhoea and/or hirsutism: Northern Finland Birth Cohort 1966 Study. *Hum Reprod* (2004) 19(5):1083–8. doi: 10.1093/humrep/deh214

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Pre-pregnancy body mass index and glycated-hemoglobin with the risk of metabolic diseases in gestational diabetes: a prospective cohort study

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Background: Metabolic diseases during pregnancy result in negative consequences for mothers. Pre-pregnancy body mass index (BMI) and late-pregnancy glycated-hemoglobin (HbA1c) are most important factors independently affecting the risk of gestational diabetes mellitus (GDM). However how both affect the combined risk of other metabolic diseases in women with GDM is unclear. The study aims to investigate the influence of pre-pregnancy BMI and pregnancy glycemic levels on other gestational metabolic diseases in women with GDM.

Methods: Pregnancies with GDM from January 2015 to December 2018 in the Xi'an longitudinal mother-child cohort study (XAMC) were retrospectively enrolled. Those without other metabolic diseases by the time of oral glucose tolerance test (OGTT) detection were finally recruited and divided into four groups by pre-pregnancy BMI (Underweight <18.5kg/m^2; Normal weight 18.5-23.9 kg/m^2; Overweight 24.0-27.9 kg/m^2; Obesity ≥28.0 kg/m^2, respectively) or two groups by HbA1c in late pregnancy (normal HbA1c<5.7%; high HbA1c≥5.7%). Multivariate logistic regression analysis was used to identify risk factors. Interaction between pre-pregnancy BMI (reference group 18.5-23.9 kg/m^2) and HbA1c (reference group <5.7%) was determined using strata-specific analysis.

Results: A total of 8928 subjects with GDM were included, 16.2% of which had a composite of metabolic diseases. The pre-pregnancy overweight and obesity, compared with normal BMI, were linked to the elevated risk of the composite of metabolic diseases, particularly pre-eclampsia (both P <0.001) and gestational hypertension (both P <0.001). Meanwhile, patients with high HbA1c had an obvious higher risk of pre-eclampsia (P< 0.001) and gestational hypertension (P= 0.005) compared to those with normal HbA1c. In addition, there were significant interactions between pre-pregnancy BMI and HbA1c (P< 0.001). The OR of pre-pregnancy BMI≥ 28 kg/m^2 and HbA1c≥ 5.7% was 4.46 (95% CI: 2.85, 6.99; P< 0.001). The risk of other metabolic diseases, except for pre-eclampsia (P= 0.003), was comparable between the two groups of patients with different

HbA1c levels at normal pre-pregnancy BMI group. However, that was remarkably elevated in obese patients (P= 0.004), particularly the risk of gestational hypertension (P= 0.004).

Conclusion: Pre-pregnancy overweight/obesity and late-pregnancy high HbA1c increased the risk of other gestational metabolic diseases of women with GDM. Monitoring and controlling late-pregnancy HbA1c was effective in reducing metabolic diseases, particularly in those who were overweight/obese before conception.

KEYWORDS

pre-pregnancy body mass index, high glycated hemoglobin, gestational metabolic diseases, gestational diabetes mellitus, gestational hypertension

1 Introduction

Gestational diabetes mellitus (GDM), defined as poor glucose tolerance that develops or first occurs during pregnancy (1), is highly prevalent affecting around 4.4% (2) of pregnancies worldwide and 15% in China (3). It can trigger a series of adverse pregnancy outcomes like cesarean delivery, perinatal mortality and macrosomia (4). Notably, the chronic insulin resistance induced by GDM (5), combined with the physiological changes caused by pregnancy (6), puts women with GDM more likely to develop other metabolic diseases, including gestational hypertension (7) and hypothyroidism (8). Thus, studying the risk factors for other metabolic diseases in patients with GDM is essential.

Over the past decades, the prevalence of overweight and obesity has been rising steadily among all age groups (9). A research reported that of Chinese childbearing women, 25.9% were overweight and 9.2% were obese, higher than that of other Asian countries (10). These were associated with the higher incidence of GDM, gestational hypertension and a lot of adverse pregnant outcomes (11). In addition, one third of pre-pregnancy obese women had higher glycated hemoglobin (HbA1c) levels at delivery (12), which were associated with gestational hypertension, preterm birth, and low birth weight (13). Since the results of oral glucose tolerance test (OGTT) reflects the instantaneous glycemic profile, while the value of HbA1c reflects the average glycemic levels over the past 2 to 3 months, it is potential to make HbA1c a remarkable predictor of gestational complications (14).

However, few researches have focused on the effects of late-pregnancy HbA1c, even less on the combined impact of pre-pregnancy BMI and HbA1c on other metabolic diseases in patients with GDM. Here, we retrospectively enrolled singleton pregnant women with GDM from the Xi'an longitudinal mother-child cohort (XAMC) to address the problem.

2 Methods

2.1 Study population

This study was conducted among the participants of the XAMC. The cohort recruited women from the Northwest Women's and Children's Hospital for antenatal care in early pregnancy to examine the impact of early intrauterine exposure on the outcomes of mothers and their child. The specific protocol and baseline information has been described elsewhere (15). Based on the dynamic XAMC, 85211 pregnancies who delivered from January 2015 to December 2018 were enrolled. The eligible subjects were singleton and required to be diagnosed with GDM and had HbA1c data for the last trimester of pregnancy. Additionally, individuals diagnosed with artificial fertilization, other metabolic diseases upon enrollment, multiple pregnancies, abortion or induced labor, non-gestational diabetes and type 1 or 2 diabetes mellitus before pregnancy, other severe diseases like cancer and disease of immune system, or with incomplete or incorrect data were excluded. Finally, 8928 subjects were included in the data analysis. The detailed elimination process is described in Figure 1. The Ethics Committee of Xi'an Jiaotong University (XJTU 2016-053) and Northwest Women's and Children's Hospital (NWCH 2012-013) approved the study, which was performed according to Helsinki Declaration. All women provided written informed consent.

2.2 Definition of GDM

In China, based on the hospital's antenatal glucose assessment protocol (16), the OGTT was performed in the mid-pregnancy (24-28 weeks). According to the modified criteria of the International Association of Diabetes and Pregnancy Study Groups (12), GDM is diagnosed when at least one value reaches or exceeds any of the following three thresholds in a 75-g OGTT: 5.1 mmol/L for fasting plasma glucose (PG), 10.0 mmol/L for 1-hour PG and 8.5 mmol/L for 2-hour PG.

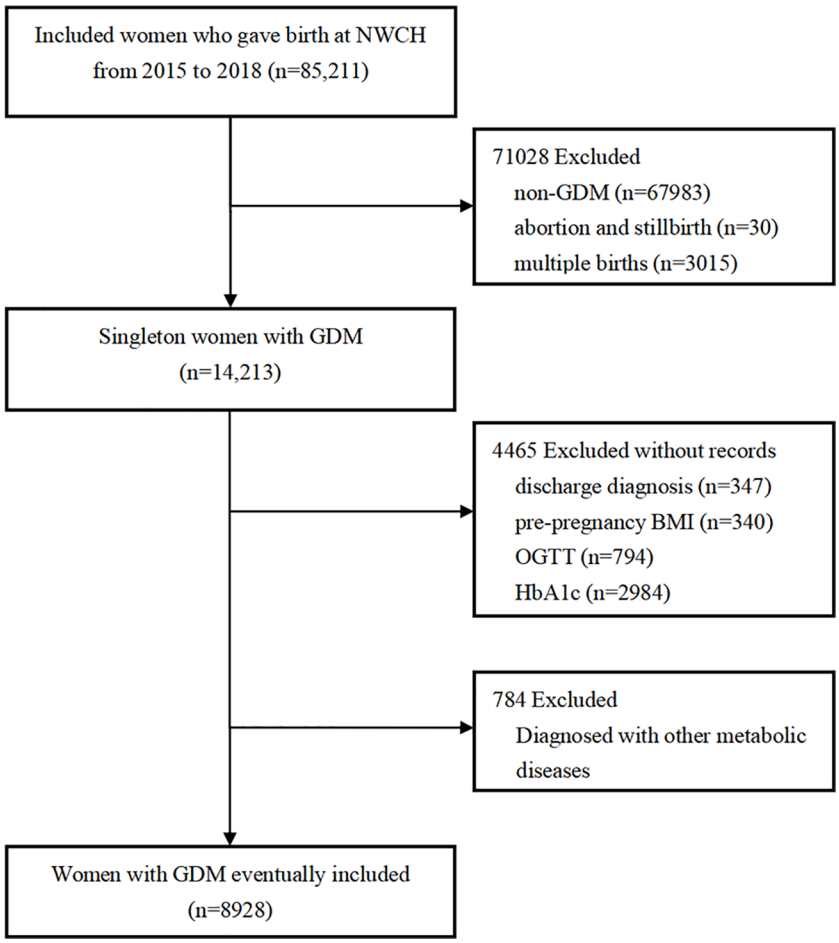

FIGURE 1
Flow chart of the participants. NWCH, the Northwest Women and Children's Hospital; BMI, body mass index; OGTT, oral glucose tolerance test; GDM, gestational diabetes mellitus; HbA1c, glycated hemoglobin. Non-GDM included diabetes mellitus complicated pregnancies, and was excluded. Subjects with other gestational metabolic diseases at enrolment were excluded. Other gestational metabolic diseases included: gestational hypertension, pre-eclampsia, subclinical hypothyroidism, hypothyroidism, hyperthyroidism, intrahepatic cholestasis and hashimoto's thyroiditis.

2.3 Definition of pre-pregnancy BMI and late-pregnancy HbA1c

The weight and height information before pregnancy was measured and recorded by a doctor at the first time of antenatal care, usually before 6 weeks of pregnancy (17). Pre-pregnancy BMI was calculated by the pre-pregnancy weight in kilograms divided by the height squared in meters. According to the criteria of the Chinese National Health Commission, subjects were categorized into four weight groups: Underweight, <18.5 kg/m^2; Normal weight, 18.5-23.9 kg/m^2; Overweight, 24.0-27.9 kg/m^2 and Obesity, ≥28.0 kg/m^2 (18).

Late pregnancy was defined as the last trimester of pregnancy, which starts from the 28th week of pregnancy until delivery. In this study, data on HbA1c were available from the hospital's Medical Case System. When the late-pregnancy HbA1c value was at or greater than the threshold value of 5.7%, the term "late-pregnancy dysglycemia" was used, as defined previously (16).

2.4 Outcomes variables

The primary outcome was the total prevalence of metabolic diseases occurring after the diagnosis of GDM, defined as the presence of at least one of the following outcomes: gestational hypertension, pre-eclampsia, subclinical hypothyroidism, hypothyroidism, hyperthyroidism, intrahepatic cholestasis and Hashimoto's thyroiditis, termed the "composite of metabolic diseases". The secondary outcome was the prevalence of each component of the primary outcome described above. Data on disease diagnoses were obtained from the hospital discharge records of subjects. Gestational hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg and required at least two blood pressure measurements in the same arm before diagnosis (19). Pre-eclampsia was defined as gestational hypertension with proteinuria (20). Hypothyroidism was defined as increased thyroid stlmulating hormone (TSH) (2.5-10 mIU/L) in conjunction with a decreased

free tetraiodothyronine (FT4) or TSH level of more than 10 mIU/L. Subclinical hypothyroidism was defined as elevated TSH (2.5-10 mIU/L) and normal FT4 concentration (21). Hyperthyroidism manifests as TSH< 0.1mIU/L and normal FT4 with laboratory confirmation of the diagnosis (22).

2.5 Statistical analysis

The normality of the continuous data distribution was assessed by the Shapiro-Wilk test. As all continuous variables in this study did not conform to normal distributions, they were expressed as medians and quartiles and analyzed by applying the Kruskal-Wallis H test. Categorical variables were described by counts and percentages. When categorical variables met the Cochran hypothesis, the analysis was conducted using a *Chi*-square test, otherwise *Fisher's* exact test was used. The associations of pre-pregnancy BMI and late HbA1c with specific metabolic diseases were determined by logistic regression of odds ratios (*ORs*) and 95% confidence intervals (*CI*). Based on the results of previous studies, we adjusted for potential confounding, including maternal age, education level, parity, previous cesarean delivery, family history of hypertension and family history of diabetes, and calculated adjusted odds ratios (*aORs*) and 95% *CI*. The interaction term between the pre-pregnancy BMI categories and HbA1c was used to explore the effect of their interaction on metabolic diseases. If the interaction was of statistical significance, strata-specific analysis was then performed. All data were analyzed by SPSS26.0 (Chicago, IL, USA). The figures were generated using GraphPad Prism 8 and R version 4.2.1. All *P* values were two-tailed, with a significance level set at 0.05.

3 Results

3.1 Baseline characteristics

Participants were at a median and quartile 31 (29, 34) years of age, 24.2% of them were over 35 years old. A majority (89.0%) had high school and above education; over 16% were overweight and 2.6% were obese before conception. Almost 61.4% of mothers were multiparities and 12.6% had a history of cesarean section. As shown in Table 1, all the maternal demographic characteristics in different pre-pregnancy BMI categories were varied significantly. Those who were overweight or obese reported having higher prevalence of family history of diabetes or hypertension. In addition, the biochemical indicators of glycolipid metabolism at the late pregnancy showed statistically significant differences in the

TABLE 1 Maternal demographic and pregnancy characteristics by pre-pregnancy BMI and late-pregnancy HbA1c categories.

Characteristic	Pre-pregnancy Body Mass Index					HbA1c		
	Underweight	Normal weight	Overweight	Obesity	*P-value*	Normal	High	*P-value*
Case (%)	857 (9.6)	6406 (71.8)	1436 (16.1)	229 (2.6)		7067 (79.2)	1861 (20.8)	
Age (%)					<0.001			0.910
<35	736 (85.9)	4809 (75.1)	1039 (72.4)	181 (79.0)		5353 (75.7)	1412 (75.9)	
≥35	121 (14.1)	1597 (24.9)	397 (27.6)	48 (21.0)		1714 (24.3)	449 (24.1)	
Education level (%)					<0.001			0.180
8th grade or less	100 (11.7)	853 (13.3)	245 (17.1)	48 (21.0)		951 (13.8)	271 (14.6)	
High school	612 (71.4)	4517 (70.5)	1018 (73.0)	167 (72.9)		4986 (70.6)	1328 (71.4)	
College and above	140 (16.3)	999 (15.6)	165 (11.5)	11 (4.8)		1067 (15.1)	248 (13.3)	
Parity (%)					<0.001			0.003
Nultiparity	422 (49.2)	2458 (38.4)	480 (33.4)	84 (36.7)		2671(37.8)	773(41.5)	
Multiparities	435 (50.8)	3948 (61.6)	956 (66.6)	145 (63.3)		4396 (62.2)	1088 (58.5)	
Previous cesarean delivery (%)	132 (15.4)	802 (12.5)	169 (11.8)	24 (10.5)	0.047	812 (11.5)	315 (16.9)	<0.001
Family history of diabetes (%)	69 (8.1)	630 (9.8)	166 (11.6)	38 (16.6)	<0.001	665 (9.4)	238 (12.8)	<0.001
Family history of hypertension (%)	107 (12.5)	986 (15.4)	256 (17.8)	54 (23.6)	<0.001	1092 (15.5)	311 (16.7)	0.184
Total GWG (kg)	15 (12.0, 18.0)	14 (10.5, 16.5)	11.5 (9.0, 15.0)	10 (6.3, 14.0)	<0.001	13.0 (10.0, 16.0)	14.5 (11.0, 17.5)	<0.001
Gestational age (weeks)	39 (38, 40)	39 (38, 40)	39 (38, 40)	39 (38, 40)	0.002	39 (38, 40)	39 (38, 40)	0.925

(Continued)

TABLE 1 Continued

Characteristic	Pre-pregnancy Body Mass Index					HbA1c		
	Underweight	Normal weight	Overweight	Obesity	*P-value*	Normal	High	*P-value*
Biochemical Indicators at the late pregnancy								
HbA1c (%)	5.3 (5.0, 5.5)	5.3 (5.1 ,5.6)	5.4 (5.1, 5.7)	5.5 (5.2, 5.8)	<0.001	5.2 (5.0, 5.4)	5.9 (5.7, 6.0)	<0.001
Total cholesterol (mmol/l)	5.8 (5.1, 6.6)	5.7 (4.9, 6.5)	5.4 (4.8, 6.2)	5.3 (4.5, 6.0)	<0.001	5.6 (4.9, 6.4)	5.6 (4.8, 6.3)	0.010
HDL (mmol/L)	1.7 (1.5, 1.9)	1.7 (1.5, 1.9)	1.6 (1.4, 1.8)	1.6 (1.4, 1.8)	<0.001	1.7 (1.4, 1.9)	1.6 (1.4, 1.9)	0.176
LDL (mmol/L)	3.0 (2.5, 3.5)	2.8 (2.3, 3.3)	2.7 (2.2, 3.2)	2.6 (2.1, 3.0)	<0.001	2.8 (2.3, 3.3)	2.8 (2.3, 3.3)	0.031
Triglycerides (mmol/L)	2.7 (2.1, 3.4)	3.0 (2.4, 3.9)	3.1 (2.5, 4.1)	3.1 (2.5, 3.8)	<0.001	3.0 (2.4, 3.9)	3.0 (2.4, 4.0)	0.018
Oral glucose tolerance test (mmol/L)								
Fasting PG	5.1 (4.7, 5.3)	5.2 (4.9, 5.4)	5.3 (5.1, 5.6)	5.34 (5.14, 5.6)	<0.001	5.2 (4.8, 5.4)	5.3 (5.1, 5.6)	<0.001
1h PG	9.4 (7.9, 10.4)	9.4 (8.1, 10.5)	9.8 (8.4, 10.8)	10.0 (8.9, 10.8)	<0.001	9.4 (6.9, 8.9)	10.0 (8.6, 11.0)	<0.001
2h PG	7.9 (6.8, 9.0)	8.0 (6.9, 9.0)	7.9 (6.9, 9.0)	7.73 (6.8, 8.7)	0.048	7.9 (6.9, 8.9)	8.2 (7.1, 9.2)	<0.001

BMI, body mass index; GWG, gestational weight gain; HDL, high density lipoprotein; LDL, low density lipoprotein; PG, plasma glucose.
Continuous variables are expressed as the median (quartile). Categorical variables are expressed as n (%).

distribution of pre-pregnancy BMI (all P <0.001). When categorized by late-pregnancy HbA1c, one in five of the patients had a higher median HbA1c levels [5.9% (5.7%, 6.0%)] and higher gestational weight gain (GWG) [14.5 (11.0, 17.5) kg] than patients with normal HbA1c levels. The total cholesterol, LDL and triglyceride of the high HbA1c group were markedly higher than those of normal HbA1c group in late pregnancy (P= 0.010, 0.031 and 0.018, respectively).

3.2 Prevalence of metabolic diseases by pre-pregnancy BMI and late-pregnancy HbA1c

Totally, 16.6% (n= 1484) of GDM women developed metabolic diseases during pregnancy, of which the prevalence was higher in the overweight (20.5%) and obesity groups (33.6%) compared to the normal BMI group (15.3%), respectively. No statistically significant differences were found between the four types group for subclinical hypothyroidism and hypothyroidism (P= 0.376, 0.256, respectively). The prevalence of pre-eclampsia and gestational hypertension differed among the BMI groups, with the prevalence increasing progressively with increasing BMI (both P <0.001) (Figure 2A; Supplementary Table S1). Meanwhile, the prevalence of composite of metabolic diseases in the high HbA1c group was significantly higher than in the normal HbA1c group (18.5% & 16.1%, P= 0.013), as well as gestational hypertension (3.3% & 1.9%, P<0.001), and pre-eclampsia (5.3% & 2.9%, P<0.001) (Figure 2B; Supplementary Table S2).

3.3 The influence of pre-pregnancy BMI and late-pregnancy HbA1c on various metabolic diseases

Participants with high HbA1c levels were at higher risk of hypertension and pre-eclampsia, which is demonstrated in Figure 3 (P= 0.004, P <0.001, respectively). According to the pre-pregnancy BMI, women with GDM who were overweight or obese had a notable risk of the composite of metabolic diseases, especially gestational hypertension and pre-eclampsia, and the risk of pre-eclampsia was significantly reduced in the underweight group (P= 0.023). Meanwhile, we found that the interaction term between pre-pregnancy BMI and late-pregnancy HbA1c had an effect on the primary outcome (P< 0.001). The OR of pre-pregnancy BMI≥ 28 kg/m^2 and HbA1c≥ 5.7% was 4.46 (95% CI: 2.85, 6.99; P< 0.001) (Supplementary Table S3).

3.4 The effect of HbA1c on metabolic diseases in different groups of pre-pregnancy BMI

We further evaluated the effect of pre-pregnancy BMI on GDM stratified by the pre-pregnancy BMI (Table 2). In the categories of underweight and overweight, no significant differences were found in the risk of primary and secondary outcomes between normal and high HbA1c groups. However, in the category of obesity, the risk of the composite of metabolic diseases was sharply increased in the

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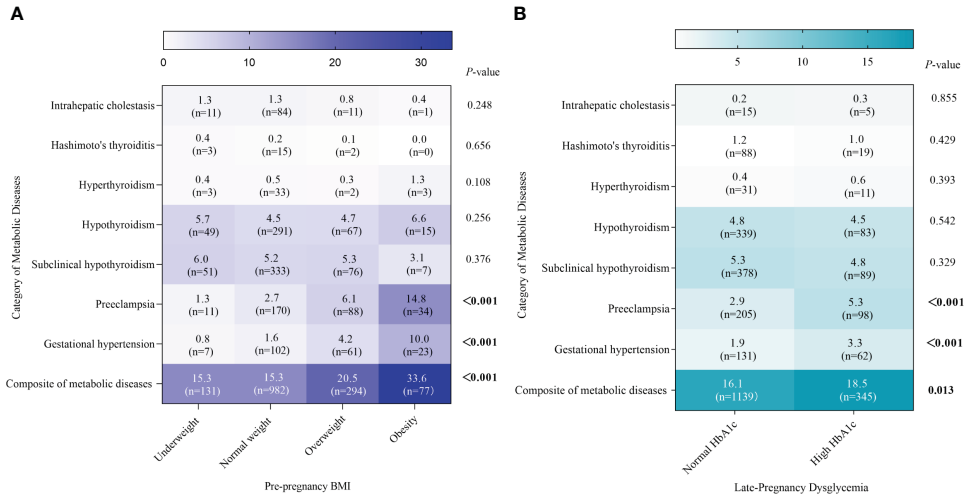

FIGURE 2
Prevalence of metabolic diseases by pre-pregnancy BMI and late-pregnancy HbA1c. Absolute prevalence of metabolic diseases are indicated by the numerals and shading within the cells, and corresponding frequencies are also shown within the cells. **(A, B)** represents the metabolic diseases by BMI groups and HbA1c groups, respectively.

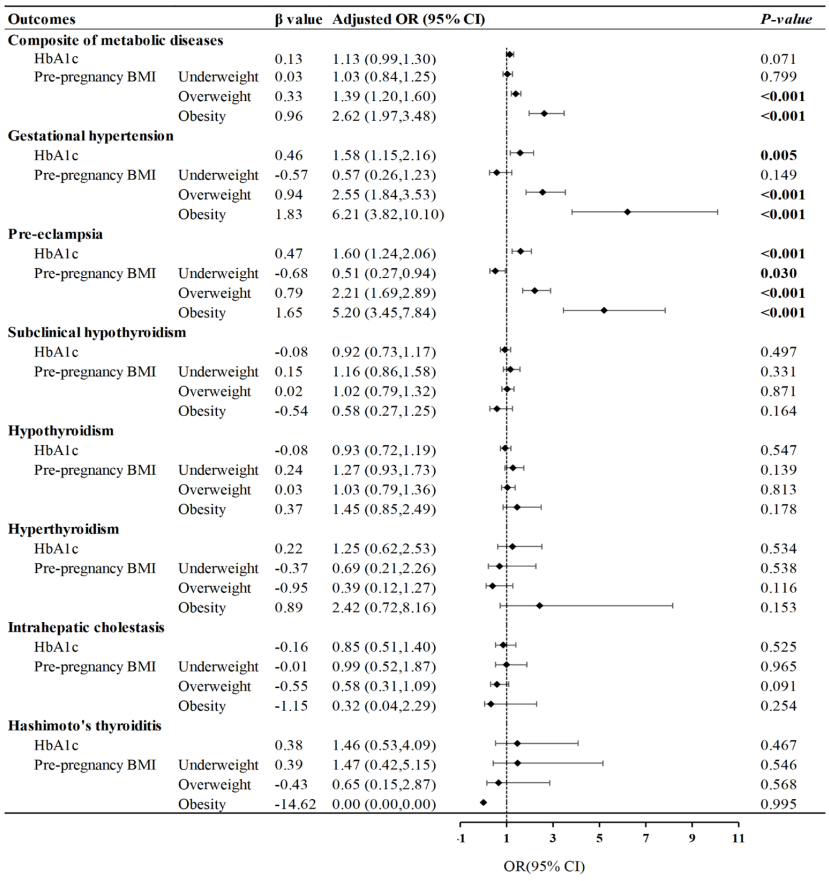

FIGURE 3
Influence of glycated hemoglobin and pre-pregnancy BMI on metabolic diseases. Forest plot of odds ratios with 95% CIs, for the risk of metabolic diseases according to pre-pregnancy BMI (<18.5; 25.0-29.9; ≥30.0), compared with pre-pregnancy normal weight (BMI: 18.5-24.9), and high HbA1c (≥5.7%), compared with normal HbA1c (<5.7%). The adjusted model was controlled for maternal age, maternal education level, parity, previous cesarean delivery, family history of diabetes and hypertension.

TABLE 2 Effect of HbA1c on metabolic diseases in different groups of pre-pregnancy BMI.

	Composite of metabolic diseases (%)	Gestational hypertension (%)	Pre-eclampsia (%)	Subclinical hypothyroidism (%)	Hypothyroidism (%)	Hyperthyroidism (%)	Intrahepatic cholestasis (%)	Hashimoto's thyroiditis (%)
Underweight (n= 857)								
Normal HbA1c	114 (15.1)	7 (1.0)	9 (1.2)	45 (6.1)	42 (5.7)	2 (0.3)	7 (0.9)	3 (0.4)
High HbA1c	17 (14.9)	0	2 (1.8)	6 (5.3)	7 (6.1)	1 (0.9)	4 (3.5)	0
P-value	0.905	/	0.974	0.739	0.835	0.349	0.690	/
Normal weight (n= 6406)								
Normal HbA1c	778 (15.1)	76 (1.5)	121 (2.4)	271 (5.3)	237 (4.6)	26 (0.5)	70 (1.4)	11 (0.2)
High HbA1c	204 (16.1)	26 (2.1)	49 (3.9)	62 (4.9)	54 (4.3)	7 (0.6)	14 (1.1)	4 (0.3)
P-value	0.387	0.143	**0.003**	0.590	0.597	0.834	0.473	0.728
Overweight (n= 1436)								
Normal HbA1c	206 (19.9)	39 (3.8)	57 (5.5)	56 (5.4)	52 (5.0)	3 (0.3)	10 (1.0)	1 (0.1)
High HbA1c	88 (21.8)	22 (5.5)	31 (7.7)	20 (5.0)	15 (3.7)	0	1 (0.2)	1 (0.2)
P-value	0.424	0.155	0.123	0.727	0.290	/	0.285	0.483
Obesity (n= 229)								
Normal HbA1c	41 (27.2)	9 (6.0)	18 (11.9)	6 (4.0)	8 (5.3)	0	1 (0.7)	0
High HbA1c	36 (46.2)	14 (17.9)	16 (20.5)	1 (1.4)	7 (9.1)	3 (1.3)	0	0
P-value	**0.004**	**0.004**	0.083	0.474	0.287	/	/	/

Statistically significant values are bolded for $p < 0.05$.

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high HbA1c group (46.2% & 27.2%, *P*= 0.004), along with gestational hypertension (17.9% & 6.0%, *P*= 0.004). In addition, in the category of normal weight, the prevalence of pre-eclampsia in the high HbA1c group was also elevated compared to the normal HbA1c group (3.9% & 2.4%, *P*= 0.003).

4 Discussion

Our present findings extended previous reports linking GDM, pre-pregnancy BMI and HbA1c with other gestational metabolic diseases. We found that both the higher pre-pregnancy BMI and late-pregnancy HbA1c increased the risk of pre-eclampsia and gestational hypertension in women with GDM. Moreover, better control of glucose metabolism in late pregnancy, which in terms of late normal HbA1c, may significantly decrease the risk of those metabolic diseases, especially in GDM women who were obese before conception.

GDM is one of the most common complications of pregnancy and is characterized by impaired glucose metabolism (23). In this study, 16.6% of women with GDM developed other metabolic diseases, with 5.5% suffering from pre-eclampsia or gestational hypertension, and 10.0% from subclinical hypothyroidism or hypothyroidism. In contrast, in the general population of women, the prevalence of gestational hypertension and pre-eclampsia were about 4.0% and 2.1%, respectively (24), and subclinical hypothyroidism or hypothyroidism was 4.7% (25), all of which were lower than the risks in this study of women with GDM. Meanwhile, the reported risk of gestational hypertension in non-GDM was significantly lower than in GDM (2.5% & 6.8%) (26). Women with GDM may be at high threat for other metabolic diseases (24, 25, 27). Gestational metabolic diseases can produce adverse short- and long-term impairments in the mother and child, such as kidney diseases and child neurodevelopmental disorders (25, 28). More importantly, possible synergistic effects between metabolic diseases may further contribute to the development of serious diseases. Evidence demonstrated that the coexistence of gestational hypertension and GDM increases the risk of cardiovascular disease (29). More researches are needed to unearth the underlying mechanisms of the interactions between GDM and other gestational metabolic diseases. Focusing on the risk of other metabolic diseases in women with GDM and targeting interventions for those at risk for GDM may be of great value.

Previously, a cohort study found that pre-pregnancy obesity was a powerful risk factor for pregnancy complications such as pre-eclampsia and gestational diabetes, to a greater extent than overweight or excessive gestational weight gain (30). Meng Li and et al. (31) further pointed out that higher values of pre-pregnancy BMI can induce GDM to complicate pre-eclampsia, gestational hypertension, preterm delivery and macrosomia. Consistently, we also observed that after adjusting for confounding factors, the prevalence of composite of metabolic diseases remained significantly higher in the overweight and obesity groups than in the normal weight group, particularly pre-eclampsia and gestational hypertension. This highlights that being overweight and obese before pregnancy is not only an independent risk factor for GDM, but also puts women with GDM at increased risk of comorbid other metabolic diseases, especially the disorders of blood pressure.

The pathophysiological mechanisms underlying the links between pre-pregnancy BMI and blood pressure during pregnancy have not been fully elucidated. Being overweight and obese before pregnancy can lead to inflammation, hyperinsulinemia and insulin resistance, further disturbing autonomic dysfunction (32). Overweight/obesity may also elicit disturbances in bioactive compounds, such as lipids, leptin function and adipokines (20). In the current analysis, there were also differences in the results for blood lipids between the BMI groups. The levels of triglyceride were generally high (median >2.7 mmol/L) and were statistically different between the groups. The overweight and obese groups had lower levels of HDL than the normal weight group. The association between hypertriglyceridemia and preeclampsia in pregnancy was previously reported that low levels of HDL were relevant to preeclampsia, but not LDL (33). Triglycerides accumulate in the lining cells of the uterine spiral arteries, resulting in decreased prostacyclin production and may lead to endothelial dysfunction and increased oxidative stress (34). Whether the prevalence of gestational metabolic diseases in pre-pregnancy obese/overweight women would be increased by some degree of lipid alteration remains to conjecture, but appropriate weight management before pregnancy is essential. For those at the high risk of GDM who were overweight/obese before pregnancy, blood pressure and lipid changes should be monitored dynamically during pregnancy to prevent the development of gestational hypertension.

Compared to OGTT for transient measurements, HbA1c represents the average glycemia level over the previous 8-12 weeks and is characterized by being easy to test and unaffected by short-term fluctuations in blood (14). Of note, despite appropriate treatments being given to patients with GDM, they may still have higher late-pregnancy HbA1c levels than pregnancies without GDM (12). Hyperglycemia positively associated with adverse pregnancy outcomes (12). Attention should be paid to the importance of HbA1c as an objective biochemical indicator of glycemic control in women with GDM (35). A recent study revealed that HbA1c ≥5.7% during pregnancy indicated impaired β-cell function and pathophysiological dysfunction of glucose disposal (36). Late-pregnancy HbA1c at or above 5.7% in obese non-GDM pregnancies posed long-term health risks to the offspring and mother (16, 37). When the cut-off value for HbA1c in this study was set at 5.7%, we also found that high HbA1c was an independent risk factor for pre-eclampsia and gestational hypertension in women with GDM. Couples of studies emphasized the important role of HbA1c in the risk of pre-eclampsia. Although there is scarce epidemiological evidence on late-pregnancy HbA1c, the available studies generally supported our results. A large population-based study indicated that the risk of pre-eclampsia increased with elevated mid-pregnancy HbA1c (14). Meanwhile, Lynn P et al. reported on the association of HbA1c measured at 28 weeks as a continuous variable with pre-eclampsia (38). Holmes et al. provided the first evidence that HbA1c <6.1% in

late pregnancy reduced the risk of pre-eclampsia in type 1 diabetic women (39). Although the mechanism of this association is not fully clear, some evidence showed that elevated HbA1c may induce endothelial dysfunction by generating superoxide anions that interfere with nitric oxide mediated response (40). Endothelial dysfunction may perturb vascular biomarkers including P-selectin, E-selectin, intercellular adhesion molecules and vascular cell adhesion molecules further impairing the vasculature (41), and thus HbA1c may be associated with hypertension. Our data reinforced previous scientific evidence. Notably, among the various risk factors and mechanisms for pre-eclampsia and gestational hypertension, poor glycemic control remains one of the most easily monitored and treated risk factors (39).

There is an important role of weight management prior to pregnancy in reducing adverse gestational metabolic diseases (42). Meanwhile, evidence suggested that the linkage between HbA1c and adverse pregnancy outcomes differed with pre-pregnancy BMI and GWG levels (43). Indicators of BMI combined with HbA1c can help to assess the prognosis of women with GDM. Given that, we further investigated whether pre-pregnancy BMI interacts with late-pregnancy HbA1c on the risk of metabolic diseases by stratifying the pre-pregnancy BMI. The results showed that the proportion of high late-pregnancy HbA1c gradually increased with elevated pre-pregnancy BMI. Notably, in the obese group, glycemia within the optimal range significantly reduced the risk of metabolic diseases, especially gestational hypertension, despite the diagnosis of GDM. In addition, the high HbA1c group was more likely to suffer from pre-eclampsia even if their pre-pregnancy BMI was normal. Therefore, we recommend that GDM women who have excessive pre-pregnancy BMI should be aware of gestational hypertension. It is advisable to use HbA1c as a clinical indicator to monitor glycemia in the last trimester of pregnancy, to assess the impact of GDM treatment timely, and to adjust the therapy to minimize the long-term hazards caused by metabolic diseases.

There are several advantages of this study. To begin with, the data derived from a large population makes the results more convincing. In addition, an accurate experimental design and data collection was conducted, with adjustments for known or potential confounders. Furthermore, we paid particular attention to the interaction of HbA1c and pre-pregnancy BMI on gestational metabolic diseases, with implications for the clinically appropriate management of women with GDM.

However, our study has some limitations. Firstly, we didn't have information on the exact gestational age at which BMI and HbA1c were recorded. Secondly, it is a regional study with all participants from Xi'an. Data from a single center may lack representation of the entire pregnancy population and selection bias is inevitable. Thirdly, although comprehensive covariates were included in this study, some potential confounders such as pregnancy lifestyle may modify the association of pre-pregnancy BMI and HbA1c with gestational metabolic diseases, inducing confounding bias. Last but not least, this study has proposed an effect of pre-pregnancy BMI and HbA1c on metabolic diseases, but has not yet explored the specific mechanisms that produce this result. Therefore, further studies will be needed to confirm this relationship.

5 Conclusions

In conclusion, our results suggested that women should be reminded to keep their BMI at an optimal range when planning for pregnancy to reduce the risk of gestational metabolic diseases. Continuous monitoring of HbA1c is necessary to manage therapeutic effects in women with GDM, especially in the last trimester of pregnancy. Tailored BMI advice, and measures to control glycemia in late pregnancy appear to be an appropriate intervention for closer preventive follow-up of metabolic diseases.

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 study was approved by both the Research Ethics Committees of NWCH (NWCH2012-013) and Xi'an Jiaotong University (XJTU2016-053). Written informed consent was obtained for all participants.

Author contributions

XW and SZ contributed equally to this work and share first authorship. XW and SZ conceived the study, analyzed the data, and drafted the original manuscript. WY and JL helped draft the manuscript. GL, JJ, and YM contributed to the review and editing manuscript. XL provided conceptualizations for research and the final revision received. All authors have approved the published version of the 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/fendo.2023.1238873/full#supplementary-material

References

1. Sweeting A, Wong J, Murphy HR, Ross GP. A clinical update on gestational diabetes mellitus. *Endocrine Rev* (2022) 43(5):763–93. doi: 10.1210/endrev/bnac003

2. Behboudi-Gandevani S, Amiri M, Yarandi RB, Tehrani FR. The impact of diagnostic criteria for gestational diabetes on its prevalence: a systematic review and meta-analysis. *Diabetol Metab Syndrome* (2019) 11(1):1–18. doi: 10.1186/s13098-019-0406-1

3. Gao C, Sun X, Lu L, Liu F, Yuan J. Prevalence of gestational diabetes mellitus in mainland China: A systematic review and meta-analysis. *J Diabetes Invest* (2018) 10 (1):154–62. doi: 10.1111/jdi.12854

4. Ye W, Luo C, Huang J, Li C, Liu Z, Liu F. Gestational diabetes mellitus and adverse pregnancy outcomes: systematic review and meta-analysis. *BMJ* (2022) 377: e067946. doi: 10.1136/bmj-2021-067946

5. Lende M, Rijhsinghani A. Gestational diabetes: overview with emphasis on medical management. *Int J Environ Res Public Health* (2020) 17(24):9573. doi: 10.3390/ijerph17249573

6. Cooper DS, Laurberg P. Hyperthyroidism in pregnancy. *Lancet Diabetes Endocrinol* (2013) 1(3):238–49. doi: 10.1016/S2213-8587(13)70086-X

7. Grieger JA, Bianco-Miotto T, Grzeskowiak LE, Leemaqz SY, Poston L, McCowan LM, et al. Metabolic syndrome in pregnancy and risk for adverse pregnancy outcomes: A prospective cohort of nulliparous women. *PloS Med* (2018) 15(12):e1002710. doi: 10.1371/journal.pmed.1002710

8. Jia M, Wu Y, Lin B, Shi Y, Zhang Q, Lin Y, et al. Meta-analysis of the association between maternal subclinical hypothyroidism and gestational diabetes mellitus. *Int J Gynecol Obstetrics.* (2019) 144(3):239–47. doi: 10.1002/ijgo.12751

9. Poston L, Caleyachetty R, Cnattingius S, Corvalán C, Uauy R, Herring S, et al. Preconceptional and maternal obesity: epidemiology and health consequences. *Lancet Diabetes Endocrinol* (2016) 4(12):1025–36. doi: 10.1016/S2213-8587(16)30217-0

10. Gao L, Bhurtyal A, Wei J, Akhtar P, Wang L, Wang Y. Double burden of malnutrition and nutrition transition in asia: A case study of 4 selected countries with different socioeconomic development. *Adv Nutr* (2020) 11(6):1663–70. doi: 10.1093/advances/nmaa064

11. Johns EC, Denison FC, Norman JE, Reynolds RM. Gestational diabetes mellitus: mechanisms, treatment, and complications. *Trends Endocrinol Metab* (2018) 29 (11):743–54. doi: 10.1016/j.tem.2018.09.004

12. International Association of Diabetes and Pregnancy Study Groups Consensus Panel, Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care* (2010) 33(3):676–82. doi: 10.2337dc09-1848

13. Ho Y-R, Wang P, Lu M-C, Tseng S-T, Yang C-P, Yan Y-H. Associations of mid-pregnancy HbA1c with gestational diabetes and risk of adverse pregnancy outcomes in high-risk Taiwanese women. *PloS One* (2017) 12(5):e0177563. doi: 10.1371/journal.pone.0177563

14. Carlsen EØ, Harmon Q, Magnus MC, Meltzer HM, Erlund I, Stene LC, et al. Glycated haemoglobin (HbA1c) in mid-pregnancy and perinatal outcomes. *Int J Epidemiol* (2022) 51(3):759–68. doi: 10.1093/ije/dyab270

15. Ji J, He Z, Qu P, Gao J, Zhang W, Wu P, et al. The Xi'an longitudinal mother–child cohort study: design, study population and methods. *Eur J Epidemiol* (2021) 36 (2):223–32. doi: 10.1007/s10654-020-00704-6

16. He Z, Mi Y, Yang H, Ji J, Gao J, Zhang W, et al. Late-pregnancy dysglycemia after negative testing for gestational diabetes and risk of the large-for-gestational-age newborns: A nest case-control study based on the xi'an longitudinal mother-child co. *Front Pediatr* (2022) 10. doi: 10.3389/fped.2022.829706

17. Luo X, Gao J, He Z, Ji J, Zhang W, Wu P, et al. What is an appropriate gestational weight gain for women with gestational diabetes mellitus: based on the adverse pregnancy outcomes of over 12 thousand participants? *Diabetol Metab Syndrome* (2022) 14(1):166. doi: 10.1186/s13098-022-00940-8

18. Song X, Shu J, Zhang S, Chen L, Diao J, Li J, et al. Pre-pregnancy body mass index and risk of macrosomia and large for gestational age births with gestational diabetes mellitus as a mediator: A prospective cohort study in central China. *Nutrients* (2022) 14(5):1072. doi: 10.3390/nu14051072

19. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. Hypertensive disorders of pregnancy. *Hypertension* (2018) 72(1):24–43. doi: 10.1161/HYPERTENSIONAHA.117.10803

20. Savitri AI, Zuithoff P, Browne JL, Amelia D, Baharuddin M, Grobbee DE, et al. Does pre-pregnancy BMI determine blood pressure during pregnancy? A prospective cohort study. *BMJ Open* (2016) 6(8):e011626. doi: 10.1136/bmjopen-2016-011626

21. Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, et al. 2017 Guidelines of the american thyroid association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. *Thyroid* (2017) 27(3):315–89. doi: 10.1089/thy.2016.0457

22. Han Y, Wang J, Wang X, Ouyang L, Li Y. Relationship between subclinical hypothyroidism in pregnancy and hypertensive disorder of pregnancy: A systematic review and meta-analysis. *Front Endocrinol* (2022) 13. doi: 10.3389/fendo.2022.823710

23. Vounzoulaki E, Khunti K, Abner SC, Tan BK, Davies MJ, Gillies CL. Progression to type 2 diabetes in women with a known history of gestational diabetes: systematic review and meta-analysis. *BMJ* (2020) 369:m1361. doi: 10.1136/bmj.m1361

24. Cosson E, Cussac-Pillegand C, Benbara A, Pharisien I, Nguyen MT, Chiheb S, et al. Pregnancy adverse outcomes related to pregravid body mass index and gestational weight gain, according to the presence or not of gestational diabetes mellitus: A retrospective observational study. *Diabetes Metab* (2016) 42(1):38–46. doi: 10.1016/j.diabet.2015.06.001

25. Garovic VD, White WM, Vaughan L, Saiki M, Parashuram S, Garcia-Valencia O, et al. Incidence and long-term outcomes of hypertensive disorders of pregnancy. *J Am Coll Cardiol* (2020) 75(18):2323–34. doi: 10.1016/j.jacc.2020.03.028

26. Saito Y, Kobayashi S, Ikeda-Araki A, Ito S, Miyashita C, Kimura T, et al. Association between pre-pregnancy body mass index and gestational weight gain and perinatal outcomes in pregnant women diagnosed with gestational diabetes mellitus: The Japan Environment and Children's Study. *J Diabetes Invest* (2021) 13(5):889–99. doi: 10.1111/jdi.13723

27. Toloza FJK, Derakhshan A, Männistö T, Bliddal S, Popova PV, Carty DM, et al. Association between maternal thyroid function and risk of gestational hypertension and pre-eclampsia: a systematic review and intdidual-participant data meta-analysis. *Lancet Diabetes Endocrinol* (2022) 10(4):243–52. doi: 10.1016/S2213-8587(22)00007-9

28. Lee SY, Pearce EN. Assessment and treatment of thyroid disorders in pregnancy and the postpartum period. *Nat Rev Endocrinol* (2022) 18(3):158–71. doi: 10.1038/s41574-021-00604-z

29. Jiang L, Tang K, Magee LA, von Dadelszen P, Ekeroma A, Li X, et al. A global view of hypertensive disorders and diabetes mellitus during pregnancy. *Nat Rev Endocrinol* (2022) 18(12):760–75. doi: 10.1038/s41574-022-00734-y

30. Lewandowska M, Więckowska B, Sajdak S. Pre-pregnancy obesity, excessive gestational weight gain, and the risk of pregnancy-induced hypertension and gestational diabetes mellitus. *J Clin Med* (2020) 9(6):1980. doi: 10.3390/jcm9061980

31. Li M, Zhang C-Y, Yue C-Y. Effects of pre-pregnancy BMI and gestational weight gain on adverse pregnancy outcomes and complications of GDM. *J Obstetrics Gynaecol* (2022) 42(4):630–5. doi: 10.1080/01443615.2021.1945009

32. Lewandowska M, Więckowska B, Sajdak S, Lubiński J. Pre-pregnancy obesity vs. Other risk factors in probability models of preeclampsia and gestational hypertension. *Nutrients* (2020) 12(9):2681. doi: 10.3390/nu12092681

33. Serrano NC, Guio-Mahecha E, Quintero-Lesmes DC, Bayona S–, Paez MC, Beltran M, et al. Lipid profile, plasma apolipoproteins, and pre-eclampsia risk in the GenPE case-control study. *Atherosclerosis* (2018) 276:189–94. doi: 10.1016/j.atherosclerosis.2018.05.051

34. Poornima IG, Indaram M, Ross JD, Agarwala A, Wild RA. Hyperlipidemia and risk for preclampsia. *J Clin Lipidol* (2022) 16(3):253–60. doi: 10.1016/j.jacl.2022.02.005

35. Sacks DB, John WG. Interpretation of hemoglobin A_{1c}Values. *JAMA* (2014) 311 (22):2271. doi: 10.1001/jama.2014.6342

36. Bozkurt L, Göbl CS, Leitner K, Pacini G, Kautzky-Willer A. HbA1c during early pregnancy reflects beta-cell dysfunction in women developing GDM. *BMJ Open Diabetes Res Care* (2020) 8(2):e001751. doi: 10.1136/bmjdrc-2020-001751

37. Gomes D, von Kries R, Delius M, Mansmann U, Nast M, Stubert M, et al. Late-pregnancy dysglycemia in obese pregnancies after negative testing for gestational diabetes and risk of future childhood overweight: An interim analysis from a longitudinal mother–child cohort stu. *PloS Med* (2018) 15(10):e1002681. doi: 10.1371/journal.pmed.1002681

38. Lowe LP, Metzger BE, Dyer AR, Lowe J, McCance DR, Lappin TR, et al. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study: associations of maternal A1C and glucose with pregnancy outcomes. *Diabetes Care* (2012) 35 (3):574–80. doi: 10.2337/dc11-1687

39. Holmes VA, Young IS, Patterson CC, Pearson DWM, Walker JD, Maresh MJA, et al. Optimal glycemic control, pre-eclampsia, and gestational hypertension in women with type 1 diabetes in the diabetes and pre-eclampsia intervention trial. *Diabetes Care* (2011) 34(8):1683–8. doi: 10.2337/dc11-0244

40. Matheus ASM, da Matta MFB, Clemente ELS, Rodrigues MLG, Valença DCT, Drummond KRG, et al. Biochemical and clinical markers of endothelial dysfunction do not outweigh traditional risk factors for the presence of diabetic retinopathy in patients with type 1 diabetes. *Diabetol Metab Syndrome* (2022) 14(1):141. doi: 10.1186/s13098-022-00912-y

41. Soleimani M. Insulin resistance and hypertension: new insights. *Kidney Int* (2015) 87(3):497–9. doi: 10.1038/ki.2014.392

42. Li H, Miao C, Xu L, Gao H, Bai M, Liu W, et al. Maternal pre-pregnancy body mass index, gestational weight gain trajectory, and risk of adverse perinatal outcomes. *Int J Gynecol Obstetrics.* (2021) 157(3):723–32. doi: 10.1002/ijgo.13922

43. Muhuza MPU, Zhang L, Wu Q, Qi L, Chen D, Liang Z. The association between maternal HbA1c and adverse outcomes in gestational diabetes. *Front Endocrinol* (2023) 14. doi: 10.3389/fendo.2023.1105899

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Predictive value of first-trimester GPR120 levels in gestational diabetes mellitus

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Background: Early diagnosis of gestational diabetes mellitus (GDM) reduces the risk of unfavorable perinatal and maternal consequences. Currently, there are no recognized biomarkers or clinical prediction models for use in clinical practice to diagnosing GDM during early pregnancy. The purpose of this research is to detect the serum G-protein coupled receptor 120 (GPR120) levels during early pregnancy and construct a model for predicting GDM.

Methods: This prospective cohort study was implemented at the Women's Hospital of Jiangnan University between November 2019 and November 2022. All clinical indicators were assessed at the Hospital Laboratory. GPR120 expression was measured in white blood cells through quantitative PCR. Thereafter, the least absolute shrinkage and selection operator (LASSO) regression analysis technique was employed for optimizing the selection of the variables, while the multivariate logistic regression technique was implemented for constructing the nomogram model to anticipate the risk of GDM. The calibration curve analysis, area under the receiver operating characteristic curve (AUC) analysis, and the decision curve analysis (DCA) were conducted for assessing the performance of the constructed nomogram.

Results: Herein, we included a total of 250 pregnant women (125 with GDM). The results showed that the GDM group showed significantly higher GPR120 expression levels in their first trimester compared to the normal pregnancy group ($p < 0.05$). LASSO and multivariate regression analyses were carried out to construct a GDM nomogram during the first trimester. The indicators used in the nomogram included fasting plasma glucose, total cholesterol, lipoproteins, and GPR120 levels. The nomogram exhibited good performance in the training (AUC 0.996, 95% confidence interval [CI] = 0.989-0.999) and validation sets (AUC=0.992) for predicting GDM. The Akaike Information Criterion of the nomogram was 37.961. The nomogram showed a cutoff value of 0.714 (sensitivity = 0.989; specificity = 0.977). The nomogram displayed good calibration and discrimination, while the DCA was conducted for validating the clinical applicability of the nomogram.

Conclusions: The patients in the GDM group showed a high GPR120 expression level during the first trimester. Therefore, GPR120 expression could be used as an effective biomarker for predicting the onset of GDM. The nomogram incorporating GPR120 levels in early pregnancy showed good predictive ability for the onset of GDM.

KEYWORDS

gestational diabetes mellitus, biomarker, GPR120, nomogram, LASSO

1 Introduction

Gestational diabetes mellitus (GDM), a common gestational disorder, is a growing public health problem worldwide (1). GDM could cause detrimental short- and long-term consequences for the newborn and mother (2–4). In recent years, with improvements in the living standard, changes in diet and lifestyle, and implementation of the "Comprehensive Three Child" policy, there has been an increase in the prevalence of GDM (5). The occurrence of diabetes during the pregnancy period has become an epidemic (4), increasing the health and economic burden in China (6). GDM may not only reflect but also promote the type 2 diabetes mellitus (T2DM) epidemic (7, 8). Women with GDM show a higher probability of developing postpartum T2DM and cardiovascular diseases. Previous studies have shown that early detection of GDM is important for its prevention and treatment (9–12).

Multiple traditional risk factors affect the onset of GDM, such as age, lifestyle, body mass index (BMI) before pregnancy, environmental and psychosocial factors, disorders of lipid metabolism (13, 14), placental hormones (15), fasting plasma glucose (FPG) levels (16), and thyroid functions (17, 18). However, these risk factors have limited diagnostic accuracy. The values of area under the curve (AUC) displayed by the traditional clinical variables was <0.8, while a majority of the models showed a poor agreement between the predicted probability and observed risk (i.e., calibration) (19, 20). The existing predictive model for GDM did not display a considerable or high predictive ability. Therefore, a standard predictive model for the diagnosis of GDM during early pregnancy is necessary (21).

Several researchers have highlighted the correlation between abnormal glucose levels, GDM, and blood lipid metabolism disorders (5, 22). The specific receptor for long-chain fatty acids includes the G-protein-coupled receptor 120 (GPR120), also called the free fatty acid receptor 4 (23). GPR120 is involved in energy metabolism and adipogenesis in adipose tissues and is involved in the onset and progression of several diseases. Our earlier study indicated that the participants in the GDM group exhibited significantly higher GPR120 expression levels compared to the normal healthy controls at 32 and 37 weeks of pregnancy, however, these variations were absent by the second day after delivery (24). Additional lipidomic studies have highlighted the positive correlation between the GPR120 expression levels and total lipid amount in GDM patients (24). Activation of GPR120 reportedly shows a potential therapeutic effect on metabolic syndrome and improves systemic insulin sensitivity in T2DM (25–28). Da et al. noted that GPR120 agonist treatment of the high-fat diet-fed obese mice led to decreased hepatic steatosis, decreased hyperinsulinemia, enhanced glucose tolerance, and increased insulin sensitivity (26). Owing to the similarity between the pathogeneses of GDM and T2DM, GPR120 expression may be correlated with the risk of GDM in the first trimester.

While our previous study has revealed that the expression of GPR120 was significantly higher in the GDM than in the control (24), all these previous studies were based on univariate analyses, and the complicated interactions among multiple male factors were not considered, which may cause biases. Therefore, this study aimed to examine GPR120 levels in patients with GDM in the first trimester and establish an effective predictive model for GDM during the early months of pregnancy.

2 Materials and methods

2.1 Data collection

This prospective cohort study recruited 1735 women in the first trimester of pregnancy at Women's Hospital of Jiangnan University between January 2020 and January 2022. Blood samples were collected from the first-trimester participants. The women at 24-28 weeks of pregnancy were classified into the GDM or control groups depending on the findings of the 75-g oral glucose tolerance test. Figure 1 presents the inclusion and exclusion criteria used in the study. Herein, 180 pregnant women were enrolled in the training dataset, while 70 women were enrolled in the validation dataset. Thereafter, their laboratory and clinical data, during the 14^{th} –16^{th} gestational week, were collected. The following maternal laboratory and clinical data, which included their systolic blood pressure, age, diastolic blood pressure, gestational week, maternity history, pre-pregnancy BMI, nulliparous, pregnancy BMI, total bilirubin, direct bilirubin, total protein (TP), globin, albumin (ALB), alanine aminotransferase, aspartate aminotransferase, fasting plasma glucose (FPG), creatine kinase, creatinine, uric acid (UA), β2-microglobulin, total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL),

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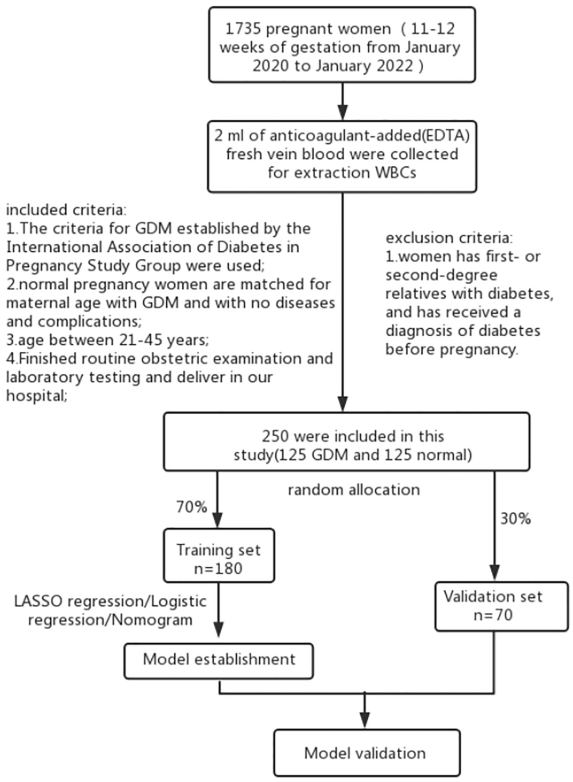

FIGURE 1
Study flow chart. The nomogram was evaluated based on the AUC-ROC values, calibration curve, C-index, and DCA.

apolipoprotein A1, lipoprotein, apolipoprotein B, *in vitro* fertilization (IVF), and GPR120. Skilled nurses collected the blood samples from the patients, and all blood tests were conducted and management in the laboratory of the Women's Hospital of Jiangnan University (24). The expression levels of the laboratory factors, except GPR120, were obtained from patient medical records. While the GDM criteria that were defined by the International Association of Diabetes in Pregnancy Study Group were employed in this study (29). The Ethics Committee of the Women's Hospital of Jiangnan University approved all the experiments conducted in this prospective cohort study (No. 2022-01-1103-15).

2.2 Determination of GPR120 mRNA levels in white blood cells

GPR120 mRNA expression levels were determined using white blood cells (WBCs). Firstly, fresh anticoagulant-containing venous blood samples (2 mL) were centrifuged at 2500× g for 10 mins, and the cell-free plasma supernatant layer was removed. Then, red blood cell lysis buffer (10 mL) was gently added to the cell pellet with a pipette, mixed, and gently shaken for 5 mins. This mixture was centrifuged at 2500× g for 5 mins. This pyrolysis step was carried out twice. The cell pellet was rinsed twice with a phosphate-buffered saline solution (3 mL). TRIzol reagent was used for extracting the total RNA content in the WBCs (Tianwei, Beijing, China) following the manufacturer's recommendations. The Primer Premier 5.0 Software (PREMIER Biosoft International, Palo Alto, CA) was used for designing the GPR120 primers, with the following primer sequences: GPR120: forward 50 -TGG AGC CCC ATC ATC ATC AC-30, reverse 50 - TGC ACA GTG TCA TGT TGT AGA G-3'; The QuantiTect SYBR Green PCR Kit (QIAGEN, Shanghai, China) was utilized for conducting the quantitative polymerase chain reaction (PCR) using the iCycler iQ (Bio-Rad) PCR instrument.

2.3 Statistical analysis

The data were statistically analyzed with the use of the R statistical software ver. 4.1.3 (R Statistical Computing Foundation, Vienna, Austria; glmnet, rms, foreign, pROC, regplot, and Nricens packages). The data that conformed to a normal distribution are expressed as mean ± standard deviation, while the nonnormal distributed data are presented as median (interquartile range). Additionally, the categorical data are described as counts and percentages. The summary statistics between the two groups were compared by the Mann–Whitney U test or unpaired Student's t-tests for continuous data, and chi-square tests for categorical data. The least absolute shrinkage and selection operator (LASSO) regression analysis was conducted for identifying the optimal predictive factors (30). Finally, a nomogram was constructed with the help of the binary logistic regression model with 5-fold cross-validation. The predictive model's accuracy was determined using the calibration curve (the Hosmer–Lemeshow test was employed for evaluating goodness of fit). Furthermore, the AUC-based receiver operating characteristic (ROC) curves were utilized for evaluating the model's discriminative ability. Also, the ROC was employed for generating the decision curve analysis (DCA) curves for determining the clinical application and benefit of the nomogram, while the best diagnostic model was chosen depending on the minimal Akaike Information Criterion (AIC). Statistical significance was established at $p < 0.05$.

3 Results

3.1 Clinical and laboratory characteristics

This study recruited 125 women with GDM and 125 healthy controls. Among these, the training set included 180 (70%) randomly assigned participants, while the validation set included 70 (30%) randomly assigned participants. Table 1 presents the basic characteristics and clinical parameters employed in the study cohort. Although the GDM and control groups were matched in terms of age, significant differences were noted between both the groups with regards to their systolic blood pressure, gestational age, pre-pregnancy BMI, pregnancy BMI, and TP, ALB, globin, UA, β2-microglobulin, FPG, TC, HDL, LDL, apolipoprotein B, apolipoprotein A1, lipoprotein, IVF, and GPR120 levels. Participants in the GDM group showed a significantly higher GPR120 expression level compared to the control individuals. The other factors exhibited no statistically significant variation (Table 1).

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TABLE 1 Comparison clinical and laboratory variables between the two groups.

Variables	GDM($\bar{x}$ ± S/ M(IQR)) (N=125)	Control($\bar{x}$ ± S/ M(IQR)) (N=125)	Z/t/χ2	*P*
age	31.00(29.00,34.00)	31.00(29.00,34.00)	-1.430	0.153
Gestational weeks (n (%))			1.224	0.542
10	19(15.20)	25(20.00)		
11	83(66.40)	81(64.80)		
12	23(18.40)	19(15.20)		
Systolic blood pressure (mmHg)	117.9±11.44	114.30±10.07	2.642	0.009
Diastolic blood pressure (mmHg)	69.01±9.45	67.82±8.30	1.059	0.290
Maternity history (n (%))			3.120	0.210
0	79(63.20)	91(72.80)		
1	38(30.40)	30(24.00)		
2	8(6.40)	4(3.20)		
Pre-pregnancy BMI(Kg/m2)	22.49(20.42,24.89)	21.05(19.37,22.66)	-4.175	<0.001
Pregnancy BMI (Kg/m2)	24.39(22.15,26.96)	21.71(19.35,23.34)	-7.047	<0.001
TBIL(umol/L)	7.60(6.60,9.55)	8.10(6.70,9.75)	-1.461	0.144
Bilirubin direct(umol/L)	2.12(1.75,2.65)	2.23(1.79,2.62)	-0.397	0.691
TP(g/L)	68.31±4.24	69.79±4.30	-2.741	0.007
ALB(g/L)	37.60(36.00,39.95)	40.70(38.9,43.25)	-6.257	<0.001
Globin(g/L)	30.10(28.00,32.60)	28.90(26.90,30.85)	-3.098	0.002
ALT (mmol/L)	12.70(9.55,17.40)	14.20(10.00,23.35)	-1.314	0.189
AST (mmol/L)	17.80(14.90,22.50)	18.40(16.00,23.85)	-1.639	0.101
CK (mmol/L)	32.7(24.4,45.35)	34.60(26.90,42.70)	-0.66	0.509
UA (mmol/L)	243.9(209.00,299.75)	218.10(185.70,245.95)	-4.466	<0.001
Cr(mmol/L)	45.9(41.40,51.05)	46.80(42.95,50.30)	-0.789	0.43
β2-microglobulin (mg/L)	1.88±0.41	1.65±0.36	4.628	<0.001
FPG(mmol/L)	6.39(6.12,6.95)	4.58(4.35,4.86)	-11.445	<0.001
TC(mmol/L)	5.79(5.30,6.56)	4.34(3.81,4.78)	-11.506	<0.001
HDL(mmol/L)	2.16(1.86,2.37)	1.94(1.71,2.17)	-4.207	<0.001
LDL(mmol/L)	3.46(2.82,4.13)	2.59(2.13,3.01)	-8.189	<0.001
Apolipoprotein A1(g/L)	2.03(1.75,2.37)	1.45(1.22,1.74)	-8.714	<0.001
Apolipoprotein B(g/L)	1.04(0.87,1.27)	0.78(0.67,0.91)	-8.239	<0.001
Lipoprotein(mg/L)	334.20(245.00,368.55)	72.20(38.65,117.95)	-12.398	<0.001
IVF(n (%))			3.879	0.049
Yes	109(87.2)	118(94.4)		
No	16(12.8)	7(5.6)		
GPR120(mmol/L)	4.19(2.25,8.00)	0.98(0.66,1.72)	-10.773	<0.001

3.2 Constructing a prediction model based on LASSO and logistic regression analyses in the training dataset

Herein, 5 potential predictors with non-zero coefficients were chosen from 26 features for developing the LASSO regression model, including FPG, pregnancy BMI, TC, lipoprotein, and GPR120 levels, which could be used as the GDM risk factors (Figure 2). A binomial deviance curve against log (λ) was plotted, where λ indicates the tuning hyperparameter. Furthermore, the solid vertical lines denoted the binomial deviance ± standard error (SE). Also, the 1-SE criteria were employed for drawing the dotted vertical lines at optimal values. The LASSO model used an optimal λ value with the 10-fold cross-validation with 1-SE criterion (Figure 2B). The final risk prediction model included FPG, TC, lipoprotein, and GPR120 levels using multivariate logistic regression (Table 2). An algorithm that reflected the contribution of these 4 factors to GDM probability (GDMP) was derived from the training cohort data using a logistic regression model: GDMP = 2.504*FPG + 1.528*TC +0.019*Lipoprotein + 0.544*GPR120 - 30.625. Figure 3 shows the predictive model and its application as a nomogram. For instance, the nomogram model was used for anticipating the probability of a woman with GDM, who showed an FPG level of 4.49 mmol/L, TC levels of 6.11 mmol/L, lipoprotein levels of 356.2 mg/L, and GPR120 levels of 1.68 mmol/L, which was seen to be 95% (Figure 3B). In this study, the GPR120 expression level during the first trimester was regarded as an independent risk factor for GDM. Thereafter, the performance of GPR120 as a predictive biomarker for GDM was assessed after developing Model 2 containing only GPR120. As presented in Figures 4A, B, Model 2 showed an AUC value of 0.88 (95% confidence interval [CI]: 0.829–0.931) for the training set, while it showed a value of 0.936 (95% CI: 0.873-0.998) for the validation set. Model 2 showed an AIC of 192.73 in the training set. Multivariable logistic regression indicated that the FPG level was significantly and positively related to the higher GDM risk (odds ratio [OR]= 12.236, 95% CI= 2.094–71.494, p = 0.005). FPG is a traditional risk factor for GDM. Therefore, Model 3, which included only FPG levels, was established. The AUC of Model 3 (Figure 4A) was 0.935 (95% CI: 0.895–0.976, p < 0.001) for the training set, while it was 0.875 (95% CI: 0.782–0.968) for the validation set. Model 3 showed an AIC of 100.42 for the training set.

3.3 Validating the predictive model

The discriminatory abilities of the above three predictive models were determined using the ROC curve. The ROCs of the nomogram were plotted with the data derived from the training and validation datasets. The nomogram showed AUCs of 0.996 (95% CI: 0.989–0.999) and 0.992 (95% CI: 0.9793–0.999) for the training and validation sets, respectively, and the specificity and sensitivity values were 0.977 and 0.989, respectively. The specificity and sensitivity of Model 2(Model 3)for predicting GDM in early pregnancy was 0.954 and 0.774(specificity 0.855 and sensitivity 0.935), respectively. The nomograms showed significantly higher AUCs compared to those displayed by Models 2 and 3 for the training and validation sets.

The nomogram showed an AIC of 37.961. The results implied that the nomogram displayed lower AIC values in comparison to those displayed by the remaining two models displaying the favorable discrimination capability of the nomogram for estimating the likelihood of developing GDM. This predictive model was calibrated by means of the Hosmer–Lemeshow test and calibration plot. The nomogram's calibration curves exhibited a higher accuracy between the predicted and observed values. The Hosmer–Lemeshow test exhibited a higher consistency between the predicted and actual probabilities (training set, p = 0.788; validation set, p = 0.289) (Figures 4C, D). The decision curves for the nomogram in the validation and training sets displayed a relatively good model performance for clinical applications (Figures 4E, F). Furthermore, graphical DCA results showed that the nomogram offered a greater net advantage compared to other

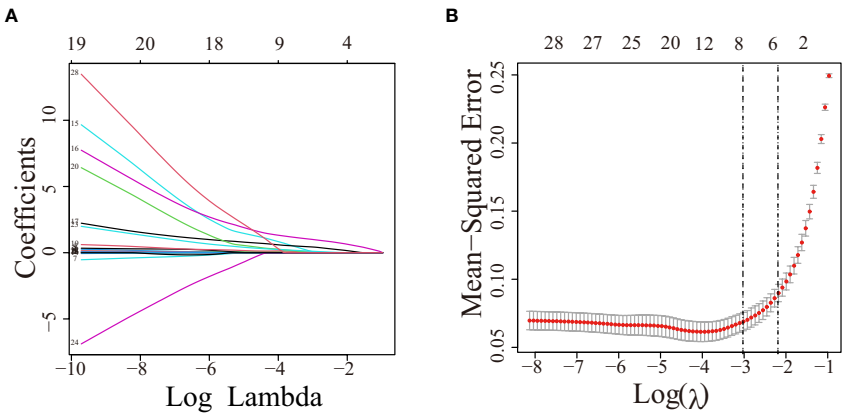

FIGURE 2
The variable filtering process during LASSO regression. **(A)** Twenty-six variables with non-zero coefficients were chosen by determining the optimal λ. **(B)** After validating the optimal (λ) parameter using the LASSO model, a partial likelihood deviance (binomial deviance) curve was plotted against log (λ), and dotted vertical lines were drawn based on the 1-standard error criteria. LASSO, Least absolute shrinkage and selection operator.

TABLE 2 Multivariable logistic regression to predict GDM based on Lasso regression.

Variables	Coefficient	*P* value	Adjusted OR(95%CI)
BMI2	0.191	0.370	1.211(0.797,1.841)
FPG	2.504	0.005	12.236(2.094,71.494)
TC	1.528	0.004	4.609(1.630,13.032)
Lipoprotein	0.019	0.001	1.019(1.008,1.031)
GPR120	0.544	0.001	1.722(1.235,2.402)

models over the pertinent threshold range in the entire cohort (Figures 4E, F).

4 Discussion

In this cohort study, a novel predictive nomogram was constructed that included GPR120 levels and clinical risk factors (such as FPG, TC, and lipoprotein levels). The results indicated that the inclusion of these factors significantly enhanced the nomogram's ability to detect the onset and progression of GDM in pregnant women in their first trimester. Furthermore, it was noted that the women with GDM showed significantly higher GPR120 expression levels within their first trimester compared to healthy pregnant women. Furthermore, this nomogram displayed a higher level of discrimination and exhibited an AUC of 0.996. Thus, clinicians can use this prediction model to identify the patients showing a high risk of GDM, thus developing effective and targeted treatment strategies.

GDM is a common, comprehensive, obstetric, and gynecological disease syndrome that is related to abnormal lipid and glucose metabolism during pregnancy. Although GDM presents a significant threat to maternal and fetal safety during pregnancy (31), very less information regarding its pathogenesis is available. Our data showed that some women diagnosed with GDM exhibited abnormal glucose and blood lipid metabolism during the first trimester (Table 1). Wang et al. found that lipid metabolism disorders noted in the early months of pregnancy were associated with the risk of GDM. Immanuel and Simmons reported that many women with GDM (15–70%) present signs of hyperglycemia before 24 weeks of gestation (5, 32), which was similar to the results presented in this study. Currently, early clinical treatment generally focuses on regulating the patients' diet and exercise (33, 34) and implementing blood glucose management plans in the first trimester, which are important for both fetal and maternal health (35). However, the GDM diagnosis is generally carried out in the 24^{th}–28^{th} weeks of pregnancy, which presents a limited time for intervention. Thus, an early GDM prediction model needed to be developed for improving the prevention, treatment, and prognosis of GDM and decreasing the economic burden (36).

This prospective cohort study recruited 250 patients for constructing a nomogram based on multiple variables that were screened by means of the LASSO regression analysis. The traditional biochemical indicators of GDM exhibit strong collinearity. LASSO regression, which is better than univariate analysis, helps in addressing the issue of multicollinearity among the variables. Figure 2 illustrates the LASSO penalty selection process. A majority of the earlier studies used statistical techniques that combined univariate analysis and multivariate logistic regression methods for analyzing the data (36, 37). The findings in this report indicated that in comparison to the multivariate logistic regression analysis, a combination of LASSO regression and multivariate logistic regression analyses yields a better AUC. Herein, multivariate logistic regression analyses implied that the TC, FPG, lipoprotein, and GPR120 levels could be used as independent predictive factors for GDM. Earlier studies showed that the FPG and lipoprotein levels were independent risk factors for GDM, which were further validated by the findings noted

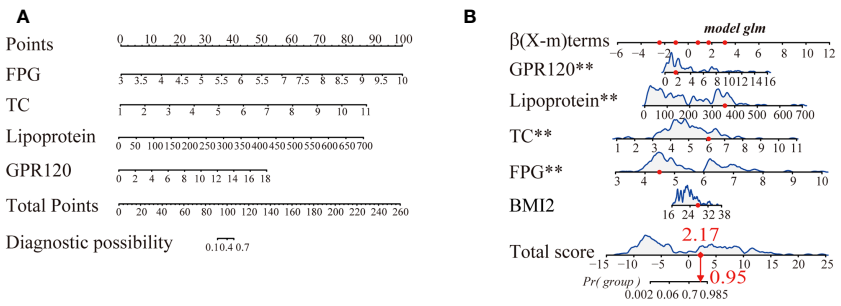

FIGURE 3
(A) A nomogram for predicting gestational diabetes mellitus (GDM). For this purpose, predictor points were determined on the uppermost point scale corresponding to every variable used for the pregnant participants and then added. The numerical value that was projected to the bottom scale highlights the probability of GDM. **(B)** Dynamic nomogram served used as an example. Herein, Participant 1 has been listed as the example (expressed in red). The sum (2.17) of these points is located on the Total score axis, and a line is drawn downward to the probability of developing GDM (95%). **p < 0.01.

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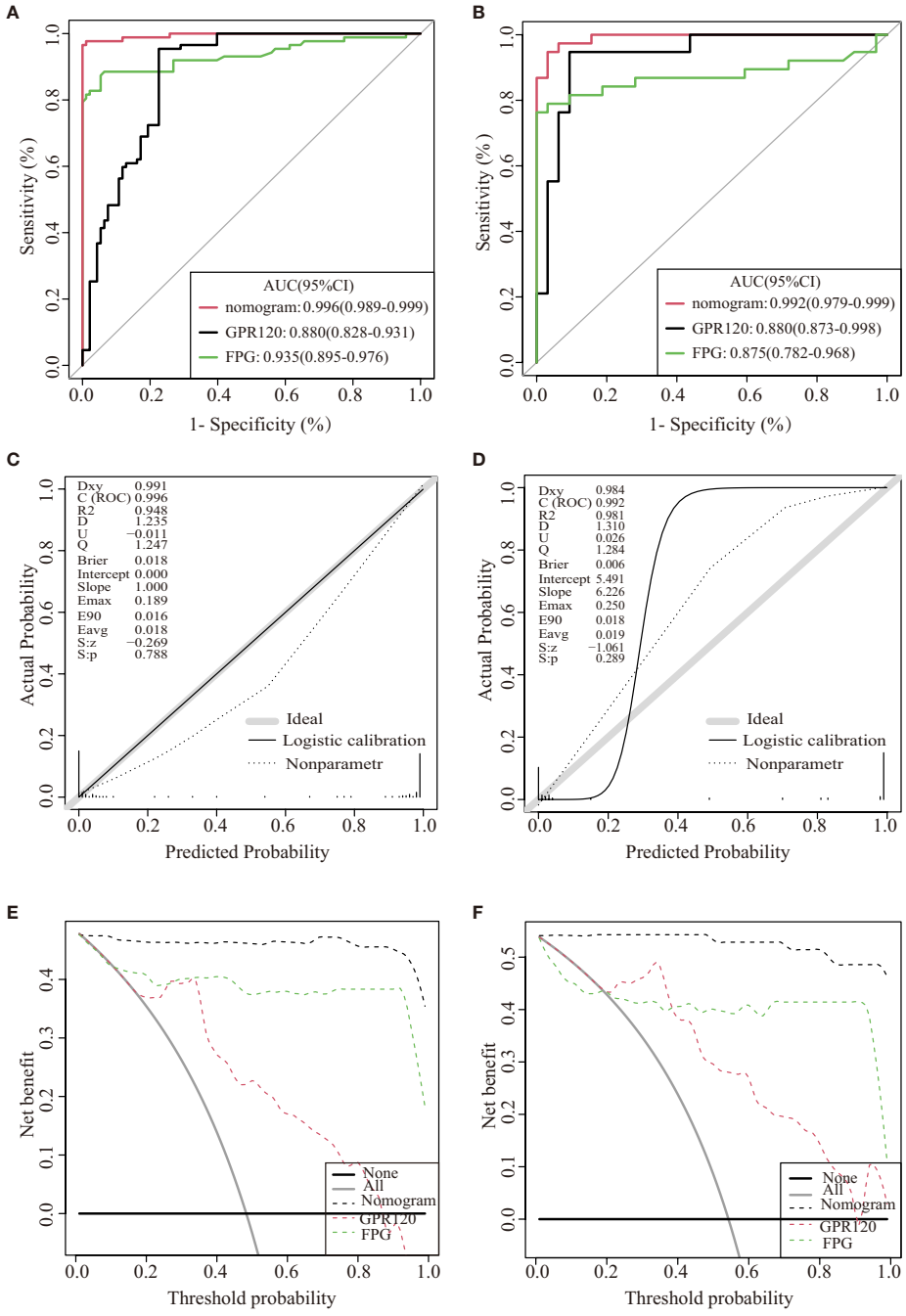

FIGURE 4
Internal validation of the three models for GDM. ROC curves of the three models for the training dataset **(A)** and validation dataset **(B)**. The calibration curve was derived from the nomogram to predict GDM in the training set **(C)** and validation dataset **(D)**. DCA values were used to predict the performance of the three models in the training **(E)** and validation datasets **(F)**.

in this study (10, 11, 38). However, several studies in the past have conducted univariate logistic regression analysis for identifying GDM-related risk factors (39, 40). This may be due to an indirect correlation between exposure and outcome among the research variables included in the model, which makes TC insignificant in the multivariate analysis. This contradictory event demonstrates the disparity between the statistical methodologies as well as the prospective advantages of the multivariate analysis. The findings of the univariate regression analyses indicated the significance of a single factor based on the presumption that this factor operates independently without taking into consideration its interaction with other relevant factors. However, due to the strong interactions between various GDM-related factors, the findings of the univariate analysis could not present a subjective conclusion. A multivariate analysis assists in overcoming these limitations.

GPR120 is involved in the lipid and glucose metabolism processes, where medium-to-long-chain fatty acids serve as ligands (41). Since GDM shows a similar pathology as T2DM,

GDM can be regarded as an early T2DM stage (42). GPR120 protects against obesity and T2DM (25–27), however, its actual role in GDM is unclear. However, several hypotheses have been proposed. Fasting plasma glucose (FPG) is diagnosis maker for diabetes. Meanwhile, the main role of GPR120 is to elicit free fatty acids regulation on metabolism homeostasis and GPR120 agonism correlates with prevention of the occurrence and development of metabolic disorders such as obesity and diabetes. Thus, the disorder of GPR120 expression may cause the level of FPG raised. In this study, we demonstrated that GPR120 levels increased the risk of developing GDM. This phenomenon is linked to the upregulated GPR120 expression levels, which protect individuals from various lipid disorders. Therefore, it was speculated that the GPR120 agonists could exhibit a therapeutic value among GDM individuals. However, the mechanism used by GPR120 to regulate lipid metabolism is not defined and needs to be further investigated.

We constructed a nomogram for GDM, which, for the first time, demonstrated that GPR120 expression levels during the first trimester could be utilized for predicting the development of GDM. This nomogram showed a considerable degree of discrimination (AUC = 0.996) and calibration ($p = 0.788$). Tong et al. reported that FPG could serve as an independent risk factor for GDM during the initial trimester and could be employed as a screening tool for determining risky GDM-related pregnancies and predicting adverse pregnancy outcomes. The findings noted in this study suggested that the developed nomogram showed a better predictive ability compared to the two other models in all cohorts. Therefore, GPR120 was selected to enhance the model's ability to identify the onset of GDM during the first trimester. Different first-trimester-related GDM nomograms were proposed in the past. However, a majority of GDM risk prediction models that have been established earlier are based on the primary characteristics of pregnant women, like pre-pregnancy BMI or age, and do not include GPR120 levels. Most studies on this topic are retrospective, which restricts the clinical significance of all the results. The previously established nomograms have limited diagnostic accuracy (11, 43, 44), and the AUC of these models is less than 0.8 (36, 45), which is lower than that of our model. Furthermore, the results of the DCA curve showed that the constructed nomogram displayed a positive effect, which validated the better clinical value of this model compared to other models.

Despite the advantages presented in this study, it shows a few limitations. This single-center study had a limited sample size, where the population showed a restricted ethnicity. Furthermore, the mechanism used by GPR120 for GDM regulation is not known. Thus, in the future, multicenter studies with large sample sizes should be conducted for verifying the results noted in this study. Furthermore, the specific mechanism responsible for the interaction between GPR120 and GDM requires further investigation.

5 Conclusions

To conclude, patients with GDM showed high GPR120 transcriptional levels during their early trimester. The novel nomogram that was constructed in this study included the GPR 120 levels within the first 3 months of pregnancy, and it displayed good predictive and discrimination values.

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 studies involving humans were approved by Institutional Ethics Committee of the Wuxi Maternity and Child Health Care Hospital. 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.

Author contributions

QH and ML are responsible for the collection of data and writing of the original manuscript. RY and are ZW responsible for the concept development, revision, review of the manuscript. RY is responsible for funding acquisition. 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. Coustan DR. Gestational diabetes mellitus. *Clin Chem* (2013) 59:1310–21. doi: 10.1373/clinchem.2013.203331

2. Farrar D, Simmonds M, Bryant M, Sheldon TA, Tuffnell D, Golder S, et al. Hyperglycaemia and risk of adverse perinatal outcomes: systematic review and meta-analysis. *BMJ* (2016) 13:354–65. doi: 10.1136/bmj.i4694

3. Kc K, Shakya S, Zhang H. Gestational diabetes mellitus and macrosomia: a literature review. *Ann Nutr Metab* (2015) 2:14–20. doi: 10.1159/000371628

4. Szmuilowicz ED, Josefson JL, Metzger BE. Gestational diabetes mellitus. *Endocrinol Metab Clin North Am* (2019) 48:479–93. doi: 10.1016/j.ecl.2019.05.001

5. Juan J, Yang H. Prevalence, prevention, and lifestyle intervention of gestational diabetes mellitus in China. *Int J Environ Res Public Health* (2020) 17:9517–28. doi: 10.3390/ijerph17249517

6. Wang Y, Huang Y, Wu P, Ye Y, Sun F, Yang X, et al. Plasma lipidomics in early pregnancy and risk of gestational diabetes mellitus: a prospective nested case-control study in Chinese women. *Am J Clin Nutr* (2021) 114:1763–73. doi: 10.1093/ajcn/nqab242

7. You H, Hu J, Liu Y, Luo B, Lei A. Risk of type 2 diabetes mellitus after gestational diabetes mellitus: A systematic review & meta-analysis. *Indian J Med Res* (2021) 154:62–77. doi: 10.4103/ijmr.IJMR_852_18

8. Zhu Y, Zhang C. Prevalence of gestational diabetes and risk of progression to type 2 diabetes: a global perspective. *Curr Diabetes Rep* (2016) 16:7–19. doi: 10.1007/s11892-015-0699-x

9. Sweeting A, Wong J, Murphy HR, Ross GP. A clinical update on gestational diabetes mellitus. *Endocr Rev* (2022) 43:763–93. doi: 10.1210/endrev/bnac003

10. Tong JN, Wu LL, Chen YX, Guan XN, Tian FY, Zhang HF, et al. Fasting plasma glucose in the first trimester is related to gestational diabetes mellitus and adverse pregnancy outcomes. *Endocrine* (2022) 75:70–81. doi: 10.1007/s12020-021-02831-w

11. An R, Ma S, Zhang N, Lin H, Xiang T, Chen M, et al. AST-to-ALT ratio in the first trimester and the risk of gestational diabetes mellitus. *Front Endocrinol (Lausanne).* (2022) 13:1017448. doi: 10.3389/fendo.2022.1017448

12. Zhang M, Yang H. Perspectives from metabolomics in the early diagnosis and prognosis of gestational diabetes mellitus. *Front Endocrinol (Lausanne).* (2022) 13:967191. doi: 10.3389/fendo.2022.967191

13. Li G, Xing Y, Wang G, Wu Q, Ni W, Jiao N, et al. Does recurrent gestational diabetes mellitus increase the risk of preterm birth? A population-based cohort study. *Diabetes Res Clin Pract* (2023) 199:110628–37. doi: 10.1016/j.diabres.2023.110628

14. Wei X, Song H, Yin L, Rizzo MG, Sidhu R, Covey DF, et al. Fatty acid synthesis configures the plasma membrane for inflammation in diabetes. *Nature* (2016) 539:294–8. doi: 10.1038/nature20117

15. Xu B, Allard C, Alvarez-Mercado AI, Fuselier T, Kim JH, Coons LA, et al. Estrogens promote misfolded proinsulin degradation to protect insulin production and delay diabetes. *Cell Rep* (2018) 24:181–96. doi: 10.1016/j.celrep.2018.06.019

16. Kang M, Zhang H, Zhang J, Huang K, Zhao J, Hu J, et al. A novel nomogram for predicting gestational diabetes mellitus during early pregnancy. *Front Endocrinol (Lausanne).* (2021) 12:779210. doi: 10.3389/fendo.2021.779210

17. Milovanović Z, Filimonović D, Soldatović I, Karadžov Orlić N. Can thyroid screening in the first trimester improve the prediction of gestational diabetes mellitus? *J Clin Med* (2022) 11:3916–27. doi: 10.3390/jcm11133916

18. Zhu B, Han Y, Deng F, Huang K, Yan S, Hao J, et al. The role of triiodothyronine (T3) and T3/free thyroxine (fT4) in glucose metabolism during pregnancy: the Ma'anshan birth cohort study. *Endocr Connect.* (2021) 10:685–93. doi: 10.1530/EC-21-0088

19. Benhalima K, Van Crombrugge P, Moyson C, Verhaeghe J, Vandeginste S, Verlaenen H, et al. Estimating the risk of gestational diabetes mellitus based on the 2013 WHO criteria: a prediction model based on clinical and biochemical variables in early pregnancy. *Acta Diabetol* (2020) 57:661–71. doi: 10.1007/s00592-019-01469-5

20. Kotzaeridi G, Blätter J, Eppel D, Rosicky I, Mittlböck M, Yerlikaya-Schatten G, et al. Performance of early risk assessment tools to predict the later development of gestational diabetes. *Eur J Clin Invest.* (2021) 51:e13630. doi: 10.1111/eci.13630

21. Jiang B, Zhang J, Sun X, Yang C, Cheng G, Xu M, et al. Circulating exosomal hsa_circRNA_0039480 is highly expressed in gestational diabetes mellitus and may be served as a biomarker for early diagnosis of GDM. *J Transl Med* (2022) 20:5–18. doi: 10.1186/s12967-021-03195-5

22. Furse S, Koulman A, Ozanne SE, Poston L, White SL, Meek CL. Altered lipid metabolism in obese women with gestational diabetes and associations with offspring adiposity. *J Clin Endocrinol Metab* (2022) 107:e2825–2832. doi: 10.1210/clinem/dgac206

23. Zhang X, Macielag MJ. GPR120 agonists for the treatment of diabetes: a patent review (2014 present). *Expert Opin Ther Pat.* (2020) 30:729–42. doi: 10.1080/13543776.2020.1811852

24. He Q, Zhu S, Lin M, Yang Q, Wei L, Zhang J, et al. Increased GPR120 level is associated with gestational diabetes mellitus. *Biochem Biophys Res Commun* (2019) 512 (2):196–201. doi: 10.1016/j.bbrc.2019.03.034

25. Ichimura A, Hirasawa A, Poulain-Godefroy O, Bonnefond A, Hara T, Yengo L, et al. Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. *Nature* (2012) 483:350–4. doi: 10.1038/nature10798

26. Oh DY, Walenta E, Akiyama TE, Lagakos WS, Lackey D, Pessentheiner AR, et al. . doi: 10.1038/nm.3614

27. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* (2010) 142:687–98. doi: 10.1016/j.cell.2010.07.041

28. Ghislain J, Poitout V. Targeting lipid GPCRs to treat type 2 diabetes mellitus - progress and challenges. *Nat Rev Endocrinol* (2021) 17:162–75. doi: 10.1038/s41574-020-00459-w

29. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care* (2010) 33:676–82. doi: 10.2337/dc09-1848

30. Friedman J, Hastie T, Tibshirani R. Regularization paths for generalized linear models via coordinate descent. *J Stat Software* (2010) 33:1–22. doi: 10.18637/jss.v033.i01

31. Chiefari E, Arcidiacono B, Foti D, Brunetti A. Gestational diabetes mellitus: an updated overview. *J Endocrinol Invest.* (2017) 40:899–909. doi: 10.1007/s40618-016-0607-5

32. Immanuel J, Simmons D. Screening and treatment for early-onset gestational diabetes mellitus: a systematic review and meta-analysis. *Curr Diabetes Rep* (2017) 17:115–24. doi: 10.1007/s11892-017-0943-7

33. Rasmussen L, Poulsen CW, Kampmann U, Smedegaard SB, Ovesen PG, Fuglsang J. Diet and healthy lifestyle in the management of gestational diabetes mellitus. *Nutrients* (2020) 12:3050–61. doi: 10.3390/nu12103050

34. American Diabetes Association. 14. Management of diabetes in pregnancy: standards of medical care in diabetes-2020. *Diabetes Care* (2020) 43:S183–92. doi: 10.2337/dc20-S014

35. Viana LV, Gross JL, Azevedo MJ. Dietary intervention in patients with gestational diabetes mellitus: a systematic review and meta-analysis of randomized clinical trials on maternal and newborn outcomes. *Diabetes Care* (2014) 37:3345–55. doi: 10.2337/dc14-1530

36. Guo F, Yang S, Zhang Y, Yang X, Zhang C, Fan J. Nomogram for prediction of gestational diabetes mellitus in urban, Chinese, pregnant women. *BMC Pregnancy Childbirth.* (2020) 20:43–54. doi: 10.1186/s12884-019-2703-y

37. Liu C, Wang Y, Zheng W, Wang J, Zhang Y, Song W, et al. Putrescine as a novel biomarker of maternal serum in first trimester for the prediction of gestational diabetes mellitus: A nested case-control study. *Front Endocrinol (Lausanne).* (2021) 12:759893. doi: 10.3389/fendo.2021.759893

38. Lamri A, Limbachia J, Schulze KM, Desai D, Kelly B, de Souza RJ, et al. The genetic risk of gestational diabetes in South Asian women. *Elife* (2022) 11:e81498–07. doi: 10.7554/eLife.81498

39. Zheng Y, Hou W, Xiao J, Huang H, Quan W, Chen Y. Application value of predictive model based on maternal coagulation function and glycolipid metabolism indicators in early diagnosis of gestational diabetes mellitus. *Front Public Health* (2022) 10:850191. doi: 10.3389/fpubh.2022.850191

40. Zheng Y, Hou W, Xiao J, Huang H, Quan W, Chen Y. Application value of predictive model based on maternal coagulation function and glycolipid metabolism indicators in early diagnosis of gestational diabetes mellitus. *Front Public Health* (2022) 10:850191. doi: 10.3389/fpubh.2022.850191

41. Sankoda A, Harada N, Kato T, Ikeguchi E, Iwasaki K, Yamane S, et al. Free fatty acid receptors, G protein-coupled receptor 120 and G protein-coupled receptor 40, are essential for oil-induced gastric inhibitory polypeptide secretion. *J Diabetes Investig* (2019) 10:1430–7. doi: 10.1111/jdi.13059

42. Samuel VT, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. *J Clin Invest.* (2016) 126:12–22. doi: 10.1172/JCI77812

43. Zhang D, Zhang S, Li G, Lai Y, Hao YT, Chen WQ, et al. A clinical model and nomogram for early prediction of gestational diabetes based on common maternal demographics and routine clinical parameters. *J Obstet Gynaecol Res* (2022) 48:2738–47. doi: 10.1111/jog.15380

44. Zhang X, Zhao X, Huo L, Yuan N, Sun J, Du J, et al. Risk prediction model of gestational diabetes mellitus based on nomogram in a Chinese population cohort study. *Sci Rep* (2020) 10:21223–34. doi: 10.1038/s41598-020-78164-x

45. Adam S, Rheeder P. Selective screening strategies for gestational diabetes: A prospective cohort observational study. *J Diabetes Res* (2017) 2017:2849346–58. doi: 10.1155/2017/2849346

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Case Report: Abruptio placentae and epileptic seizure after occurrence of perinatal hyperglycaemia in woman with gestational diabetes mellitus and hypertriglyceridemia-induced acute pancreatitis

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Hypertriglyceridemia-induced acute pancreatitis seldom occurs in the second trimester of pregnancy with gestational diabetes mellitus. For these patients, the existing knowledge on concomitant hyperglycemia is not sufficient. We report a case of abruptio placentae and epileptic seizure following perinatal hyperglycaemia in woman with gestational diabetes mellitus and hypertriglyceridemia-induced acute pancreatitis. The occurrence of abruptio placentae and epileptic seizure may be associated with concomitant hyperglycemia, and the epileptic seizure was terminated after she underwent treatment with insulin. We should pay more attention to the adverse effects of perinatal hyperglycemia and continue to give appropriate insulin treatment even if patients have passed the acute phase of hypertriglyceridemia-induced acute pancreatitis.

KEYWORDS

abruptio placentae, epileptic seizure, hyperglycaemia, hypertriglyceridemia-induced, gestational diabetes mellitus

1 Introduction

Pregnant women with gestational diabetes mellitus(GDM) usually return to normal blood glucose after delivery due to reduced insulin resistance (1, 2). As a result, the effects and management of perinatal hyperglycemia, especially in women with acute pancreatic disease, have been poorly studied. In addition, hypertriglyceridemia-induced acute pancreatitis (HTG AP) rarely occurs in the second trimester of pregnancy (3–5). Here, we describe a rare case of

abruptio placenta and epileptic seizure following perinatal hyperglycaemia in woman with GDM and HTG AP in the second trimester, discuss possible causes, and compare treatment options for concomitant hyperglycaemia in the perinatal period.

2 Case description

In October 2022, a 29-year-old multipara with 27 + 2 weeks of amenorrhea was admitted to the emergency department of Jiangxi Provincial People's Hospital with acute abdominal pain. There was no previous history of gastrointestinal ulcer or pancreatitis. During two previous pregnancies, the patient developed gestational diabetes mellitus. In this pregnancy, she underwent a 75g oral glucose tolerance test at 24 weeks of gestation and found a fasting blood glucose (FBG) level of 8.0mmol/l (>7mmol/l) and a 1-hour postprandial blood glucose level of 10.5mmol/l (>10mmol/l). She was diagnosed with gestational diabetes mellitus according to the latest guideline (6). However, she did not regularly monitor her glucose levels. She has recently been taking in a bit more lipid than usual.

One day before admission, the patient suddenly developed persistent epigastric pain with nausea and vomiting. Next, she began experiencing pain in her right lower abdomen and vaginal bleeding, and was rushed to the hospital. Upon admission, the patient was in a coma, physical examination: heart rate 126 pulses per minute, blood pressure 123/51mmHg (supported by norepinephrine 0.5ug/kg/min), epileptic seizures, uncooperative nervous system examination. The rest of the physical examination was unremarkable. Her triglyceride was 31.92mmol/L (reference range 0.45 to 1.7mmol/L), amylase 401.2U/L (reference range 35 to 135U/L), white blood cell 20.87*10^9/L (reference range 4 to 10*10^9/L), procalcitonin 4.68ng/ml (reference range 0 to 0.05ng/mL), random blood glucose 15.0mmol/L. Blood gas analysis showed pH7.35, lactic acid 0.79mmol/L (reference range 0.5 to 1.7mmol/L), and urine ketone bodies were negative. Computed tomography (CT) of the head (Figure 1) was normal, and CT of the abdomen (Figure 2) showed that: pancreatic morphology was abnormal and combined with extensive peripheral exudation. Because ultrasound (Figure 3) showed mixed echoes posterior to the placenta, abruptio placentae was considered. An emergency Caesarean section was performed on the lower uterine segment to terminate the pregnancy. Unfortunately, the newborn died. After the operation, she started developing epileptic seizures again and transferred to critical care medicine department.

Combined with the typical symptoms of persistent upper abdominal pain, significant increases in blood amylase and triglycerides, imaging findings from abdominal CT, and the exclusion of common causes of acute pancreatitis such as gallstones and alcohol history, we considered acute pancreatitis induced by hypertriglyceridemia as the primary disease. Our treatment measures include active fluid resuscitation, fasting, plasma exchange, nasogastric tube decompression, inhibition of gastric acid secretion and pancreatic enzyme secretion, broad-spectrum antibiotics to prevent infection, nutritional support, analgesia and sedation, invasive ventilator-assisted respiration, traditional Chinese medicine rhubarb to induce diarrhoea, and

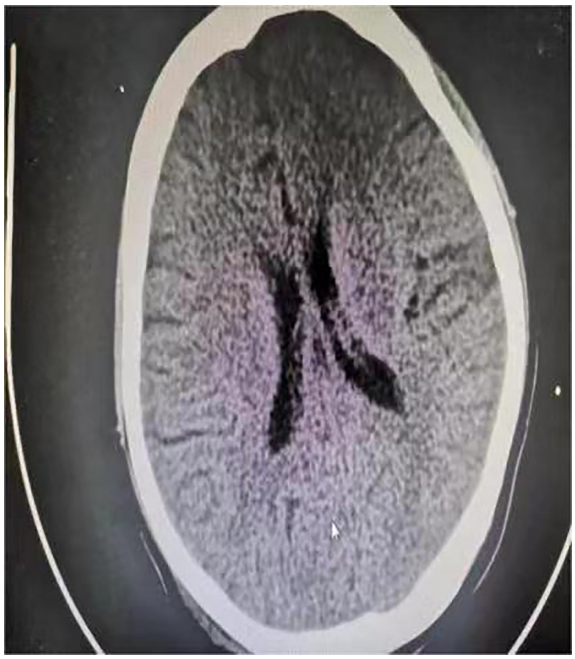

FIGURE 1
Computed tomography of the patient's head after cesarean section.

additional supportive therapies. Given her history of GDM, insulin was continuously infused by micropump and the rate was adjusted based on hourly capillary glucose checks.

After aggressive treatment, patient gradually regained consciousness from the second day, seizures disappeared, blood pressure stabilized, and laboratory tests showed significant reductions in blood glucose, blood lipids, and inflammatory markers. On the fourth to fifth day in the hospital, her condition improved further. On the evening of the fifth day in the hospital, we considered her to have passed the acute phase due to significant improvement in vital signs, inflammatory markers, and imaging findings. She was transferred to the gastroenterology department for continued treatment. However, the patient refused to continue with insulin and was switched to oral metformin 500mg three times daily and acarbose 50mg three times daily to control blood glucose according to the latest guidelines (6).

On the sixth day in the hospital, the patient had a postprandial seizure. She had no history of epilepsy. Temperature, blood pressure and neurological examination were not abnormal.Blood gas analysis showed a pH of 7.37, osmotic pressure of 304mOsm/kgH2O, negative urinary ketone bodies, and normal blood calcium levels. The only positive result was that blood glucose levels exceeded 20 mmol/l during each seizure (Table 1), which terminated approximately 5 to 10 minutes after the subcutaneous insulin injection.

On the eighth day in the hospital, we evaluated the patient's glucose metabolism again, referring to the random blood glucose values of the previous two days, glycosylated hemoglobin(HbA1c) 9.9% (normal range 4-6%), fructosamine 2.38 (normal range 1.10-2.14). We asked the endocrinology department to help manage glucose. Patients' glucose monitoring was changed to every 2 hours,

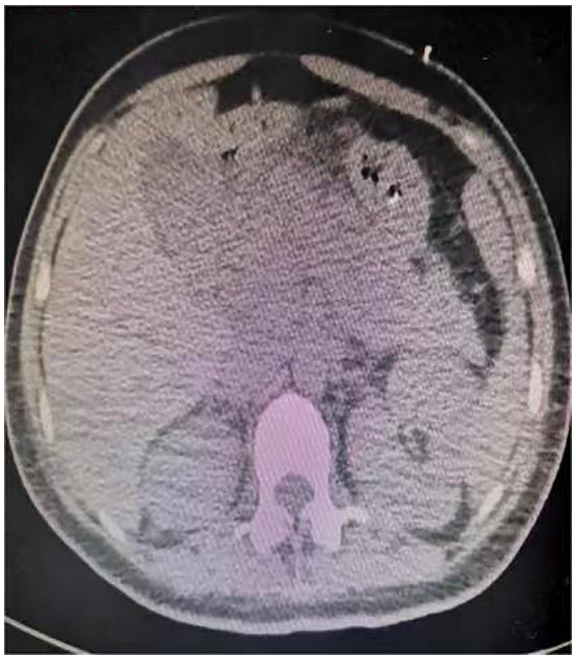

FIGURE 2
Computed tomography of the patient's abdomen after cesarean section.

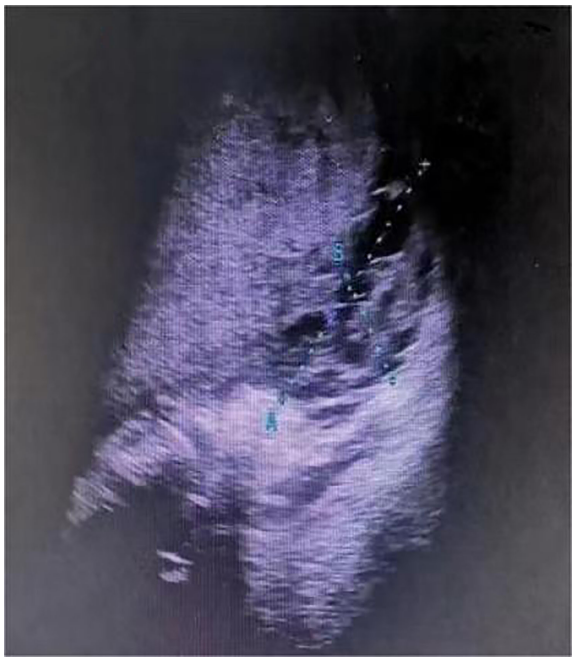

FIGURE 3
Obstetric ultrasound of the patient before cesarean section.

oral antidiabetic medications were discontinued, and Insulin Degludec/Insulin Aspart 18u was administered subcutaneously 5 minutes before breakfast and dinner. The patient's epileptic seizure did not return and her glucose levels steadily decreased (Table 1). On the twelfth day in the hospital, her fructosamine was 2.08. She can be discharged and continued to use Insulin Degludec/Insulin Aspart for a month.

Three months after discharge, we followed up with the patient again in the outpatient department. Without using any drugs, the fasting blood glucose, glycosylated hemoglobin, and blood lipids of the patient were normal without any obvious sequelae.

3 Discussion

To the best of our knowledge, this is the first reported case of abruptio placentae and epileptic seizure emerged after the occurrence of perinatal hyperglycaemia in woman with GDM and HTG AP in the second trimester. Some reasons may explain this phenomenon. On the one hand, acute pancreatitis usually occurs in the third trimester (52%), postpartum (30%), and rarely in the second trimester (3–5). Because gestational lipids typically peak in the third trimester of pregnancy, which is determined by estrogen-induced triglyceride synthesis and very low-density lipoprotein (7). In particular, HTG AP accounts for only 5% of cases (8) (Table 2). On the other hand, the morbidity of abruptio placentae and seizure decreased with the improvement of prenatal screening and medical care. Although the incidence is decreasing, both are still serious adverse events and can be seriously harmful during pregnancy. According to the literature, abruptio placentae and epileptic seizure are not considered to be common complications of HTG AP. As a result, the current knowledge of clinicians is likely to be insufficient in the event of a bursty abruptio placentae and epileptic seizure in patients with GDM and HTG AP.

Abruptio placentae is a pregnancy complication that can endanger the life and health of the mother and fetus. Previous literature studies have suggested that the common causes of abruptio placentae include pregnancy-induced hypertension syndrome, severe stress, trauma, improper obstetric care, smoking, etc (9, 10). HTG AP can be considered as severe stress. However, cases of abruptio placentae after HTG AP alone have been extremely rare in previous studies, suggesting that other mechanisms may be involved. Theoretically, hyperglycemia during pregnancy can lead to placental vascular endothelial dysfunction (11–14), hypercoagulable state of the blood system (15, 16), fetal distress (17), etc. It may facilitate the occurrence of abruptio placentae, but the specific mechanism needs to be investigated further. Consistent with the above studies, our patient had HTG AP which occurred with GDM. Next, abruptio placentae did occur after occurrence of perinatal hyperglycemia. Therefore, for pregnant women with HTG AP and GDM, if they have lower abdominal pain, vaginal bleeding, and other suspected manifestations, clinicians should increase the awareness of abruptio placentae, and early diagnosis is important because in severe abruptio placentae, the fetal mortality rate is nearly 100%, and the maternal mortality rate can be up to 5% (18, 19).

Previous studies have suggested that seizure during pregnancy is more common in epilepsy, eclampsia and stroke (20), and the fact that the patient's previous medical history, blood pressure and cranial CT were normal at the time of the attack essentially ruled out the possibility of the above conditions. In addition, the patient had normal body temperature and serum calcium, which also

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TABLE 1 Partial capillary glucose checks on the patient's 6th day to 12th day.

	Sixth day	Seventh day	Eighth day	Ninth day	Tenth day	Eleventh day	Twelfth day
03:00	14.4mmol/L no seizure	19.4mmol/L slight seizure	14.5mmol/L no seizure	12.3mmol/L no seizure	8.5mmol/L no seizure	6.3mmol/L no seizure	4.8mmol/L no seizure
09:00	18.8mmol/L no seizure	21.1mmol/L seizure	17.1mmol/L no seizure	12.0mmol/L no seizure	5.7mmol/L no seizure	4.8mmol/L no seizure	Refuse to measure
15:00	25.1mmol/L seizure	24.6mmol/L seizure	17.1mmol/L no seizure	14.2mmol/L no seizure	8.3mmol/L no seizure	Refuse to measure	discharge
21:00	20.1mmol/L seizure	18.5mmol/L no seizure	18.1mmol/L no seizure	9.0mmol/L no seizure	Refuse to measure	10.5mmol/L no seizure	discharge

excluded the possibility of hyperpyretic convulsion and hypocalcemic convulsion. The cause of epileptic seizure in this patient was unknown. In previous reports, uncontrolled hyperglycemia can also cause seizure (21–23), which may be related to its brain damage (23–27). Most cases have been described in patients with non-ketotic hyperglycemia (NKH), which is a common complication of type 2 diabetes (28, 29). Fewer cases have been described in patients with GDM. Taken together with our case, epileptic seizure may occur only in specific states of stress. In agreement with previous findings (30), this patient's seizure ceased after the hyperglycemia was corrected. Therefore, for those patients, if an unexplained epileptic seizure occurs, rapid recognition of a hyperglycemic state is vital because the hyperglycemia-induced seizure is commonly refractory to anti-epileptic drugs, and some treatments (phenytoin) may even aggravate them.

Based on the above discussion, these two rare complications in this patient do not seem to rule out the effect of hyperglycemia. However, previous studies on the treatment of pregnant women with HTG AP have focused on lipid reduction, as it has been established in numerous studies that lipid levels are positively correlated with the severity of the disease and adverse fetal outcomes (31, 32), and that early lipid reduction can reduce complications and mortality (33). As a result, numerous studies (34, 35) have focused on the design of different lipid-lowering regimens and the comparison of their efficacy that these regimens did achieve excellent results in reducing mortality and critical illness rate. Thus, the importance of glycemic control in reducing complications is overshadowed. Given the association of prolonged glucose load with increased risk of diabetes-related complications and mortality (36, 37), effective early glycemic control is confirmed critical to achieve sustained and long-term reductions in diabetes-related complications and thereby to reduce mortality and cost of diabetes care related to Type 1 diabetes or Type 2 diabetes (38–40). Yet very little is known about perinatal hyperglycemia. Due to a lack of understanding of its rare complications and deleterious effects, glycemic management was initially neglected after she passed the acute stage. Then the patient's blood glucose went out of control and seizures returned. As a result, hyperglycemia may not be easy to control after the onset of HTG AP and it is critical to give stricter management of glucose for puerperal women with a history of GDM. Insulin therapy in the acute phase is well defined. However, there is no uniform standard for the selection of hypoglycemic agents for puerperal women who have passed the acute phase of HTG AP.

TABLE 2 The etiology of Pancreatitis in pregnancy.

Proportion of the etiology
Gallstones (65–68%)
Alcohol abuse (5–10%)
Familial hypertriglyceridemia-induced pancreatitis (5%)
diopathic (15%)
Drugs-induced AP (thiazide diuretics) (cases)
Pancreatitis associated with pregnancy-induced hypertension (cases)
Acute fatty liver of pregnancy associated with AP (cases)
Hyperparathyrodism (cases)
Gene mutations (cases)
Cationic trypsinogen (PRSS1)
CFTR
PSTI
PPARG

AP, acute pancreatitis; CFTR, cystic fifibrosis transmembrane conductance regulator; PPARG, peroxisome proliferator-activated receptor gamma; PSTI, pancreatic secretory trypsin inhibitor.

Because most postpartum women have lactation needs, the drug selection is generally the same as for pregnant women. As a result, only a limited number of oral drugs are currently available for clinical use. Metformin, the most studied oral hypoglycemic drug, is labeled as a Class B drug, meaning there is no strong evidence of a contraindication in pregnant women (41).In terms of the actual efficacy of glycemic control, a systematic analysis involving a total of 4533 GDM patients (42)confirmed that compared to insulin, metformin still had a significantly stronger 2h-postprandial blood glucose control (22 studies, 2301 patients, MD, −1.11; 95% CI −1.50 to −0.72; $p < 0.00001$), lower HbA1c (15 studies, 1370 patients, MD, −1.04; 95% CI −1.47 to −0.61; $P<0.00001$), lower gestational FBG(32 studies 2996 patients, MD, −0.89; 95% CI−1.19 to−0.58; $P<0.00001$). This is consistent with several previous meta-analyses showing that metformin is no less effective or even better than insulin in controlling the primary outcome of GDM (43–46). In addition, there is additional evidence of the advantages of metformin such as ease of administration, ease of patient

education, better adherence, and lower cost (47–49). Thus, patients may prefer metformin to insulin in clinical practice (50).

Based on these advantages, metformin has been recommended in the latest Chinese guidelines for the treatment of GDM when patients refuse to use insulin, cannot safely inject insulin, or cannot afford the cost of insulin (6). Our patient was in a similar situation and had passed the acute period. Following the guidelines, we tried metformin to lower blood glucose, but there was no significant reduction in glucose. Given the damage caused by pancreatitis, the slow onset time of oral medication, and the short duration of use, this result should be interpreted with caution and cannot be entirely denied for the effect of metformin. In addition, considering that the long-term effects of metformin on neonates through milk secretion have not been completely elucidated, its safety cannot be absolutely guaranteed. For puerperal women with a history of GDM, the use of metformin to control glucose may not be appropriate even if they have passed the acute phase of HTG AP, and it is still necessary to consider the benefits and risks with caution before using metformin.

Insulin is another agent that can be used to lower blood glucose levels in pregnant women. Considering the long-term safety and non-teratogenicity of insulin, the American Diabetes Association (ADA) and the American College of Obstetricians and Gynecologists (ACOG) had recommended insulin as the primary medical treatment for GDM if lifestyle interventions do not meet glycemic treatment goal (51, 52). For women with the acute disease in the perinatal period, the principle of controlling maternal hyperglycemia with insulin has long been recognized, while there remains no nationwide or international consensus about the choice of infusion method and the type of insulin, and most national endocrine and obstetric governing bodies have not published specific guidelines.

In the intrapartum period, the American College of Obstetricians and Gynecologists (ACOG) recommended a continuous insulin infusion to maintain blood glucose levels at rv100 mg/dL using a protocol adapted from Coustan (1, 53). The protocol did not adequately take into account differences in insulin resistance levels among pregnant women. However, various institutions still choose continuous glucose and insulin infusion to manage intrapartum glucose, despite poor evidence for this decision (54). Another protocol, from Northwestern Memorial Hospital's Prentice Women's Hospital, involved administration of insulin and dextrose titration by an endocrinologist based on every 2 hours capillary blood glucose. Its medical decisions relied heavily on the clinical experience of numerous specialized endocrinologists, which is obviously cumbersome and inefficient. In 2011, Northwestern University began developing a new protocol for managing glucose, creating standardized algorithms in which registered nurses titrated insulin at different rates based on hourly capillary glucose checks. They also designed a series of tables (Table 3) to instruct providers on insulin administration, depending on the patient's total daily dose of insulin combined with the patient's cumulative basal and bolus insulin requirements and insulin resistance (55). This protocol was simple to implement and improved the consistency of glucose management. Moreover, it was once tailored to the individual needs of different patients. Our patients who received this regimen in the intensive care unit had excellent glycemic control and no seizures. However, this protocol requires frequent glucose measurements by specialist nurses and its relative complexity and intensiveness when glucose levels may change rapidly, which is difficult to administer in general wards. We need further research to clarify the optimal glucose infusion protocol for patients in general wards.

In the postpartum period, the Guideline of Committee on Practice Bulletins—Obstetrics states that women with gestational diabetes discontinue insulin at postpartum stage (56), which is consistent with clinical practice. Therefore, there is relatively little data on the use of insulin in the treatment of postpartum hyperglycemia, especially in patients with combined pancreatitis. In our patient, after the acute phase, she preferred subcutaneous injections of insulin analogue twice daily to continuous subcutaneous insulin infusion. However, the results showed acceptable effects of glucose control. Thus, intermittent injection appears to be an alternative in postpartum hyperglycemia.

Another controversial issue is the type of insulin used. In the current consensus, short-acting and intermediate-acting human insulin are the preferred insulin regimens for GDM (57). However, it is unclear whether this applies to postpartum, and the specific insulin has not been confirmed. Numerous studies of GDM have used Novolin 30R as an object. However, a meta-analysis by Li et al. (42) confirmed that Novolin 30R's efficacy was even inferior to that of metformin. Additionally, like other premixed insulin, it has the inability to adjust the long- and short-acting components separately or adequately treat post-lunch and early-morning hyperglycemia (58). Finding appropriate insulin is a key issue in current postpartum glucose management. We used Insulin Degludec/Insulin Aspart(IDegAsp) in our case. IDegAsp is the first fixed-ratio co-formulation of insulin degludec, which provides long-lasting basal insulin coverage, and insulin aspart, which targets postprandial glucose (59). It has the advantages of rapid onset, longer half-life, flat and stable glucose lowering profile, less 24-H variability, and lower risk of hypoglycemia (60). As a result, it has fewer injections and is more acceptable to patients. Many high-quality meta-analyses have confirmed its positive glucose lowering effects in type 2 diabetes. However, little is known about its use in postpartum hyperglycemia. Our case provides a valuable reference for its application to postpartum hyperglycemia. However, the long-acting component"degludec insulin" is not approved and is a category C agent in pregnancy yet. Given the potential risks, this recommendation may only be appropriate for those who do not need to breastfeed postpartum.

It must be admitted that there are some limitations in this study. First, with only one case reported in this study, there is relatively limited evidence-based evidence to support its conclusions, which limits its generalizability. Second, there were confounding factors in the study, such as irregular prenatal check-ups, unclear maternal pregnancy status and fetal intrauterine development, lack of pre-onset glucose monitoring, and no confirmation of seizure by electroencephalogram. All of these factors may affect the interpretation of the results. Finally, there are no published randomized controlled trials of IDegAsp in pregnant women, the pregnancy safety of IDegAsp is not sufficiently established, which may inherently limit its clinical applicability in pregnant women.

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TABLE 3 The new protocol for managing glucose from Northwestern University.

Table 1: Total Daily Dose of Insulin≤60 Units/24 hours							
Hourly	Initial Dose of Insulin		Continuous infusion	CBG UNCHANGED or INCREASING		CBG DECREASING	
CBG Mg/dL	Bolus Units IV push	Basal Units/hour	D_{10} W ml/hr	Bolus Units IV push	Basal Units/hour	Bolus Units IV push	Basal Units/hour
<70	0	0	50	0	0	0	↓0.5
70-100	0	0	50	0	no△	0	↓0.3
101-130	1	0.5	50	1	↑0.5	0	no△
131-160	2	0.5	50	2	↑0.5	0	↑0.5
161-190	3	0.5	0	3	↑0.7	1	↑0.5
191-220	4	0.5	0	4	↑0.7	2	↑0.8
>220	5	0.5	0	5	↑0.8	3	↑0.8
Table 2 Total Daily Dose of Insulin 61-120 Units/24 hours							
Hourly	Initial Dose of Insulin		Continuous infusion	CBG UNCHANGED or INCREASING		CBG DECREASING	
CBG Mg/dL	Bolus Units IV push	Basal Units/hour	D_{10} W ml/hr	Bolus Units IV push	Basal Units/hour	Bolus Units IV push	Basal Units/hour
<70	0	0	50	0	0	0	↓0.4
70-100	0	0	50	0	no△	0	↓0.4
101-130	2	1.0	50	2	↑0.6	0	no△
131-160	3	1.0	50	3	↑0.6	0	↑0.6
161-190	4	1.0	0	3	↑0.8	2	↑0.6
191-220	5	1.0	0	5	↑0.8	3	↑0.8
>220	6	1.0	0	6	↑1.0	4	↑0.8

If CBG is < 70, give 100mL of D10 W over 10 minutes followed by the 50mL/hr continuous infusion.
↑ It represents an increase in the insulin dose.
no△ It represents insulin dosage does not need to change.

Thus, the conclusions still require further careful interpretation and clinical identification.

4 Conclusion

The harms of perinatal hyperglycemia are still not fully understood and can be exacerbated by co-morbidities such as HTG AP and GDM. However, as serious and rare complications can be triggered, effective glucose management is extremely critical. For perinatal women, timely adjustment of continuous insulin infusion according to blood glucose monitoring seems to be the optimal plan, but for women who have survived the acute phase of the disease or be in general wards, our case supports that intermittent subcutaneous injection of a fixed-ratio co-formulation of insulin analogues (such as IDegAsp)may be a suitable alternative. More research is needed to clarify the management of perinatal hyperglycemia in both acute and chronic conditions.

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

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. Written informed consent was obtained from the participant/patient(s) for the publication of this case report.

Author contributions

YH and JC conceived and designed the study. ZH and CW collected the data. YH and JC drafted the manuscript. All authors read, edited, and approved the final manuscript. All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. JC is responsible for the overall content of the manuscript, and serves as the guarantor.

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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. ACOG Committee on Practice Bulletins. ACOG Practice Bulletin. Clinical management guidelines for obstetrician-gynecologists. Number 60, March 2005. Pregestational diabetes mellitus. *Obstet Gynecol* (2005) 105(03):675–85. doi: 10.1097/00006250-200503000-00049

2. Gabbe SG, Carpenter LB, Garrison EA. New strategies for glucose control in patients with type 1 and type 2 diabetes mellitus in pregnancy. *Clin Obstet Gynecol* (2007) 50(04):1014–24. doi: 10.1097/GRF.0b013e31815a6435

3. Pitchumoni CS, Yegneswaran B. Acute pancreatitis in pregnancy. *World J Gastroenterol* (2009) 15(45):5641–6. doi: 10.3748/wjg.15.5641

4. Ducarme G, Maire F, Chatel P, Luton D, Hammel P. Acute pancreatitis during pregnancy: a review. *J Perinatol* (2014) 34(2):87–94. doi: 10.1038/jp.2013.161

5. Ramin KD, Ramin SM, Richey SD, Cunningham FG. Acute pancreatitis in pregnancy. *Am J Obstet Gynecol* (1995) 173(1):187–91. doi: 10.1016/0002-9378(95)90188-4

6. Obstetrics Subgroup, Chinese Society of Obstetrics and Gynecology, Chinese Medical Association, Chinese Society of Perinatal Medicine, Chinese Medical Association and Committee of Pregnancy with Diabetes Mellitus, et al. Guideline of diagnosis and treatment of hyperglycemia in pregnancy (2022) [Part two]. *Zhonghua Fu Chan Ke Za Zhi* (2022) 57(2):81–90. doi: 10.3760/cma.j.cn112141-20210917-00529

7. Lippi G, Albiero A, Montagnana M, Salvagno GL, Scevarolli S, Franchi M, et al. Lipid and lipoprotein profile in physiological pregnancy. *Clin Lab* (2007) 53(3-4):173–7.

8. Papadakis EP, Sarigianni M, Mikhailidis DP, Mamopoulos A, Karagiannis V. Acute pancreatitis in pregnancy: an overview. *Eur J Obstet Gynecol Reprod Biol* (2011) 159(2):261–6. doi: 10.1016/j.ejogrb.2011.07.037

9. Oyelese Y, Ananth CV. Placental abruption. *Obstet Gynecol* (2006) 108(4):1005–16. doi: 10.1097/01.AOG.0000239439.04364.9a

10. Naeye RL. Abruptio placentae and placenta previa: frequency, perinatal mortality, and cigarette smoking. *J Obstet Gynecol* (1980) 55(6):701–4.

11. Cornejo M, Fuentes G, Valero P, Vega S, Grismaldo A, Toledo F, et al. Gestational diabesity and foetoplacental vascular dysfunction. *Acta Physiol (Oxf)* (2021) 232(4):e13671. doi: 10.1111/apha.13671

12. Subiabre M, Silva L, Villalobos-Labra R, Toledo F, Paublo M, López MA, et al. Maternal insulin therapy does not restore foetoplacental endothelial dysfunction in gestational diabetes mellitus. *Biochim Biophys Acta Mol Basis Dis* (2017) 1863 (11):2987–98. doi: 10.1016/j.bbadis.2017.07.022

13. Silva L, Subiabre M, Araos J, Sáez T, Salsoso R, Pardo F, et al. Insulin/adenosine axis linked signalling. *Mol Aspects Med* (2017) 55:45–61. doi: 10.1016/j.mam.2016.11.002

14. Cabalín C, Villalobos-Labra R, Toledo F, Sobrevia L. Involvement of A2B adenosine receptors as anti-inflammatory in gestational diabesity. *Mol Aspects Med* (2019) 66:31–9. doi: 10.1016/j.mam.2019.01.001

15. Ozbasli E, Takmaz O, Karabuk E, Gungor M. Comparison of factor XII levels in gestational diabetes, fetal macrosomia, and healthy pregnancies. *BMC Pregnancy Childbirth* (2020) 20(1):752. doi: 10.1186/s12884-020-03455-0

16. Liu Y, Sun X, Tao J, Song B, Wu W, Li Y, et al. Gestational diabetes mellitus is associated with antenatal hypercoagulability and hyperfibrinolysis: a case control study of Chinese women. *J Matern Fetal Neonatal Med* (2022) 35(15):2995–8. doi: 10.1080/14767058.2020.1818202

17. Bianchi C, Taricco E, Cardellicchio M, Mandò C, Massari M, Savasi V, et al. The role of obesity and gestational diabetes on placental size and fetal oxygenation. *Placenta* (2021) 103:59–63. doi: 10.1016/j.placenta.2020.10.013

18. Practice bulletin no ACOG. 134: fetal growth restriction. *Obstet Gynecol* (2013) 121(5):1122–33. doi: 10.1097/01.AOG.0000429658.85846.f9

19. Gotsch F, Cruciani L, Ghezzi F, Ogge G, Yeo L, Romero JR. Doppler interrogation of the fetal circulation. In: Fleischer AC, Toy EC, Lee W, Manning FA, Romero R, editors. *Sonography in obstetrics & gynecology: principles and practice, 7th edition*. McGraw–Hill Medical (2011). p. 257–308.

20. Hart LA, Sibai BM. Seizures in pregnancy: epilepsy, eclampsia, and stroke. *Semin Perinatol* (2013) 37(4):207–24. doi: 10.1053/j.semperi.2013.04.001

21. Baltyde D, De Toffol B, Nacher M, Sabbah N. Epileptic seizures during Non-Ketotic Hyperglycemia (NKH) in French Guiana: A retrospective study. *Front Endocrinol (Lausanne)* (2022) 13:946642. doi: 10.3389/fendo.2022.946642

22. Alakkas A, Chen P, Chen M, Longardner K, Piccioni DE. Hyperglycaemia-related occipital lobe seizures. *JRSM Open* (2020) 11(6):2054270420920999. doi: 10.1177/2054270420920999

23. Jain A, Sankhe S. Hyperglycemia-induced seizures and blindness. *Indian J Radiol Imag* (2020) 30(2):245–7. doi: 10.4103/ijri.IJRI_41_20

24. Moghadami M, Moghimi A, Jalal R, Behnam-Rasouli M, Mahdavi-Shahri N. Effects of infantile repeated hyperglycemia on neuronal density of hippocampus and pentylentetrazol induced convulsions in male wistar rats. *Iran J Basic Med Sci* (2012) 15(4):951–7.

25. Liang Y, Lei Z, Zhang H, Xu Z, Cui Q, Xu ZC. Toll-like receptor 4 is associated with seizures following ischemia with hyperglycemia. *Brain Res* (2014) 1590:75–84. doi: 10.1016/j.brainres.2014.09.020

26. De Martino SRM, Toni F, Spinardi L, Cirillo L. Magnetic resonance imaging findings in patients with non-ketotic hyperglycaemia and focal seizures. *Neuroradiol J* (2020) 33(5):416–23. doi: 10.1177/1971400920929554

27. Hiremath SB, Gautam AA, George PJ, Thomas A, Thomas R, Benjamin G. Hyperglycemia-induced seizures - Understanding the clinico- radiological association. *Indian J Radiol Imag* (2019) 29(4):343–9. doi: 10.4103/ijri.IJRI_344_19

28. Chen CC, Chai JW, Wu CH, Chen WS, Hung HC, Lee SK. Neuroimaging in seizure patients associated with nonketotic hyperglycemia. *Neuroradiol J* (2011) 24 (2):215–20. doi: 10.1177/197140091102400208

29. Lee EJ, Kim KK, Lee EK, Lee JE. Characteristic MRI findings in hyperglycaemia-induced seizures: diagnostic value of contrast-enhanced fluid-attenuated inversion recovery imaging. *Clin Radiol* (2016) 71(12):1240–7. doi: 10.1016/j.crad.2016.05.006

30. Urbach H, Berger B, Solymosi L, Zimmermann J. "Negative T2 shine through" in patients with hyperglycemia and seizures: a frequently overlooked MRI pattern. *Neuroradiology* (2020) 62(7):895–9. doi: 10.1007/s00234-020-02408-w

31. Zhang T, Wang G, Cao Z, Huang W, Xiao H, Wei H, et al. Acute pancreatitis in pregnancy: a 10-year, multi-center, retrospective study in Beijing. *BMC Pregnancy Childbirth* (2022) 22(1):414. doi: 10.1186/s12884-022-04742-8

32. Luo L, Zen H, Xu H, Zhu Y, Liu P, Xia L, et al. Clinical characteristics of acute pancreatitis in pregnancy: experience based on 121 cases. *Arch Gynecol Obstet* (2018) 297(2):333–9. doi: 10.1007/s00404-017-4558-7

33. Zádori N, Gede N, Antal J, Szentesi A, Alizadeh H, Vincze Á, et al. EarLy Elimination of Fatty Acids iN hypertriglyceridemia-induced acuTe pancreatitis (ELEFANT trial): Protocol of an open-label, multicenter, adaptive randomized clinical trial. *Pancreatology* (2020) 20(3):369–76. doi: 10.1016/j.pan.2019.12.018

34. Webb CB, Leveno M, Quinn AM, Burner J. Effect of TPE vs medical management on patient outcomes in the setting of hypertriglyceridemia-induced

acute pancreatitis with severely elevated triglycerides. *J Clin Apher* (2021) 36(5):719–26. doi: 10.1002/jca.21922

35. Jin M, Peng JM, Zhu HD, Zhang HM, Lu B, Li Y, et al. Continuous intravenous infusion of insulin and heparin vs plasma exchange in hypertriglyceridemia-induced acute pancreatitis. *J Dig Dis* (2018) 19(12):766–72. doi: 10.1111/1751-2980.12659

36. International Diabetes Federation. *IDF Diabetes Atlas. 9th Ed*. Brussels, Belgium: International Diabetes Federation (2019).

37. World Health Organization 2016. *Global report on diabetes*. Geneva,Switzerland: WHO Press (2016). Available at: http://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf.

38. Baxter M, Hudson R, Mahon J, Bartlett C, Samyshkin Y, Alexiou D, et al. Estimating the impact of better management of glycaemic control in adults with Type 1 and Type 2 diabetes on the number of clinical complications and the associated fifinancial benefifit. *Diabetes Med* (2016) 33:1575–81. doi: 10.1111/dme.13062

39. Lanhers C, Walther G, Chapier R, Lesourd B, Naughton G, Pereira B, et al. Long-term cost reduction of routine medications following a residential programme combining physical activity and nutrition in the treatment of type 2 diabetes: a prospective cohort study. *BMJ Open* (2017) 7:e013763. doi: 10.1136/bmjopen-2016-013763

40. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* (2008) 359:1577– 89. doi: 10.1056/NEJMoa0806470

41. Rai L, Meenakshi D, Kamath A. Metformin–a convenient alternative to insulin for Indian women with diabetes in pregnancy. *Indian J Med Sci* (2009) 63(11):491–7. doi: 10.4103/0019-5359.58878

42. Li F, Liu L, Hu Y, Marx CM, Liu W. Efficacy and safety of metformin compared to insulin in gestational diabetes: a systemic review and meta-analysis of Chinese randomized controlled trials. *Int J Clin Pharm* (2022) 44:1102–13. doi: 10.1007/s11096-022-01438-z

43. Balsells M, García-Patterson A, Solà I, Roqué M, Gich I, Corcoy R, et al. Glibenclamide, metformin, and insulin for the treatment of gestational diabetes: a systematic review and meta-analysis. *BMJ* (2015) 350:h102. doi: 10.1136/bmj.h102

44. Bo S, Juan N, Bin L, Jiang G, Lin X, Li H, et al. Short-term neonatal outcomes in women with gestational diabetes treated using metformin versus insulin: a systematic review and meta-analysis of randomized controlled trials. *Acta Diabetol* (2023) 60:595–608. doi: 10.1007/s00592-022-02016-5

45. Wang X, Liu W, Chen H, Chen Q. Comparison of insulin, metformin, and glyburide on perinatal complications of gestational diabetes mellitus: a systematic review and meta-analysis. *Gynecol Obstet Invest* (2021) 86(3):218–30. doi: 10.1159/000515893

46. Bao LX, Shi WT, Han YX. Metformin versus insulin for gestational diabetes: a systematic review and meta-analysis. *J Matern Fetal Neonatal Med* (2021) 34(16):2741–53. doi: 10.1080/14767058.2019.1670804

47. Ryu RJ, Hays KE, Hebert MF. Gestational diabetes mellitus management with oral hypoglycemic agents. *Semin Perinatol* (2014) 38(8):508–15. doi: 10.1053/j.semperi.2014.08.012

48. Finneran MM, Landon MB. Oral agents for the treatment of gestational diabetes. *Curr Diabetes Rep* (2018) 18(11):119. doi: 10.1007/s11892-018-1093-2

49. Gelaw BK, Mohammed A, Tegegne GT, Defersha AD, Fromsa M, Tadesse E, et al. Nonadherence and contributing factors among ambulatory patients with antidiabetic medications in Adama Referral Hospital. *J Diabetes Res* (2014) 204:617041. doi: 10.1155/2014/617041

50. Rowan JA, Hague WM, Gao W, Battin MR, Moore MPMiG Trial Investigators, et al. Metformin versus insulin for the treatment of gestational diabetes. *N Engl J Med* (2008) 358(19):2003–15. doi: 10.1056/NEJMoa0707193

51. Committee on Practice B-O. ACOG practice bulletin no. 190: gestational diabetes mellitus. *Obstet Gynecol* (2018) 131:e49–64. doi: 10.1097/AOG.0000000000002501

52. Simmons D, McElduff A, McIntyre HD, Elrishi M. Gestational diabetes mellitus: NICE for the U.S.? A comparison of the American Diabetes Association and the American College of Obstetricians and Gynecologists guidelines with the U.K. National Institute for health and clinical excellence guidelines. *Diabetes Care* (2010) 33(1):34–7. doi: 10.2337/dc09-1376

53. Coustan D. Delivery: timing, mode, and management. In: Reece EACD, Gabbe SG, editors. *Diabetes Mellitus in Women: Adolescence through Pregnancy and Menopause. 3*. Philadelphia, PA: Lippincott Williams and Wilkins (2004).

54. Rosenberg VA, Eglinton GS, Rauch ER, Skupski DW. Intrapartum maternal glycemic control in women with insulin requiring diabetes: a randomized clinical trial of rotating fluids versus insulin drip. *Am J Obstet Gynecol* (2006) 195(04):1095–9. doi: 10.1016/j.ajog.2006.05.051

55. Dude A, Niznik CM, Szmuilowicz ED, Peaceman AM, Yee LM. Management of diabetes in the intrapartum and postpartum patient. *Am J Perinatol* (2018) 35(11):1119–26. doi: 10.1055/s-0038-1629903

56. Committee on Practice Bulletins—Obstetrics. Practice Bulletin No 180: gestational diabetes mellitus. *Obstet Gynecol* (2017) 130(01):e17–37. doi: 10.1097/AOG.0000000000002159

57. Gangopadhyay KK, Mukherjee JJ, Sahay RK. Consensus on use of insulins in gestational diabetes. *J Assoc Physicians India* (2017) 65(3 Suppl):16–22.

58. Rizvi AA, Ligthelm RJ. The Use of premixed insulin analogues in the treatment of patients with type 2 diabetes mellitus: Advantages and limitations. *Insulin* (2007) 2:68–79. doi: 10.1016/S1557-0843(07)80018-2

59. Demir T, Turan S, Unluhizarci K, Topaloglu O, Tukek T, Gogas Yavuz D. Use of insulin degludec/insulin aspart in the management of diabetes mellitus: expert panel recommendations on appropriate practice patterns. *Front Endocrinol (Lausanne)* (2021) 12:616514. doi: 10.3389/fendo.2021.616514

60. Glastras SJ, Cohen N, Dover T, Kilov G, MacIsaac RJ, McGill M, et al. The clinical role of insulin degludec/insulin aspart in type 2 diabetes: an empirical perspective from experience in Australia. *J Clin Med* (2020) 9(4):1091. doi: 10.3390/jcm9041091

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Free triiodothyronine (FT3)-to-free thyroxine (FT4) ratio identified as a risk factor for gestational diabetes in euthyroid pregnant women: insights from a Chinese population cohort study

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Background: To explore the association between thyroid hormones and gestational diabetes mellitus in euthyroid pregnant women, with the aim of preventing the occurrence of gestational diabetes mellitus.

Methods: In this prospective study, a total of 1222 euthyroid pregnant women in their first trimester were recruited at Peking University International Hospital between December 2017 and March 2019. These participants underwent an oral glucose tolerance test during the 24-28 weeks of gestation.

Results: During early pregnancy, the gestational diabetes mellitus group displayed lower levels of free thyroxine when compared to the non-gestational diabetes mellitus group. Additionally, the ratio of free triiodothyronine to free thyroxine in the gestational diabetes mellitus group during early pregnancy was significantly higher ($p<0.05$). The ratio of free triiodothyronine to free thyroxine during early pregnancy showed a positive correlation with blood glucose levels at 0, 60, and 120 min both before and after glucose loading (all $p<0.05$). During early pregnancy, there was a negative relationship between free thyroxine levels and fasting blood glucose. The free triiodothyronine levels were positively correlated to blood glucose levels at 120 min following glucose loading (all $p<0.05$).

Conclusion: The ratio of free triiodothyronine-to-free thyroxine is an independent risk factor for gestational diabetes mellitus and has the potential to be a predictor for gestational diabetes mellitus in euthyroid pregnant women.

KEYWORDS

thyroid hormone, gestational diabetes mellitus, oral glucose tolerance test, glycosylated hemoglobin, pregnancy

1 Introduction

As the economic level has risen, there has been a notable increase in the risk of various endocrine disorders during pregnancy (1), including gestational diabetes mellitus (GDM), thyroid disease (TD), hyperlipidemia, and so on. GDM is a frequent complication of pregnancy that can have negative impacts on the well-being of both mothers and their children (2). Thus, early detection and treatment of GDM are advisable (3) and an exploration of risk factors associated with the development of GDM would provide valuable clinical insights and benefits.

The intricate physiological transformations that occur during pregnancy also influence the metabolic alterations in thyroid function. Recent studies, both domestic and international, have explored how abnormalities in thyroid hormone (TH) levels are associated with the occurrence of GDM through different mechanisms (4, 5). Nonetheless, there is still a dearth of evidence-based research regarding the association between GDM and thyroid hormone (TH) during the first trimester of pregnancy, and the underlying mechanisms of this association remain unclear.

A recent study found that initiating treatment for gestational diabetes before the 20th week of pregnancy resulted in a slightly reduced occurrence of a combination of negative neonatal outcomes compared to no early treatment (6). In clinical practice, for women identified as being at a high risk for GDM, early implementation of lifestyle interventions is essential to minimize the incidence of GDM.The objective of this study is to study the association between TH in early pregnancy and the development of GDM and to identify predictive factors for the occurrence of GDM.

2 Materials and methods

2.1 Ethics statement

The study was approved by the Ethics Bioethics Committee of Peking University International Hospital. All protocols followed the ethical guidelines of the institution and national committee and complied with the 1964 Declaration of Helsinki and subsequent amendments. All participants provided written informed consent.

Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine; GDM, gestational diabetes mellitus; OGTT, oral glucose tolerance test; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; HbA1c, glycosylated hemoglobin; FBG, fasting blood glucose; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; SCr, serum creatinine; TT4, total thryroxine; TT3, total triiodothyronine; TSH, thyroid-stimulating hormone; HPLC, high-performance liquid chromatography; ROC, receiver operating characteristic; AUC, area under the curve; TH, thyroid hormones; T3, triiodothyronine; T4, thyroxine; SCH, subclinical hypothyroidism; HOMA-IR, homeostasis model assessment for insulin resistance; GLUT2, glucose transporter 2; GLUT4, glucose transporter 4; T2DM, type 2 diabetes mellitus.

2.2 General information

The age, parity, family history, and personal history of GDM, family history of TD, and the gestational week of pregnant women were recorded at the time of enrollment, data on blood pressure (both systolic blood pressure and diastolic blood pressure), as well as measurements of height and weight, were obtained, and body mass index was calculated and recorded. BMI was calculated using the formula BMI (kg/m^2) = weight(kg)/body height^2(m^2). The pregnant women participating in this study had their fasting blood glucose levels and TH including antibodies measured before becoming pregnant.

Sample size calculation: The sample size calculation formula for survey research is used to determine the sample size: $n=U\alpha^2 * \pi(1-\pi)/\delta^2$. The π is the overall rate of GDM and π=0.20, δ is for an error of 2%, and U α= 1.96. Therefore, 1,537 subjects will be included in this study.

Inclusion criteria for the study were as follows: (1) Age of 18 years or older. (2) Willing to undergo an oral glucose tolerance test (OGTT) between the 24th and 28th weeks of gestation. (3) Planned to receive prenatal check-ups and deliver their baby at the hospital. (4) Consented to participate in the relevant questionnaire survey and agreed to the collection of blood samples after being informed about the content of the survey.

The exclusion criteria for the study were as follows: (1) Pre-existing diagnoses of cardiovascular, cerebrovascular, hematological, liver, renal, or respiratory diseases, or pre-pregnancy diabetes mellitus, or pre-thyroid diseases including positive antibodies. (2) Carrying multiple pregnancies. (3) Lack of essential baseline data. Finally, 1,222 subjects with complete data were recruited in this study (Figure 1).

2.2 Biochemical index detection

All the subjects had fasting 5 ml of venous blood collected in the morning during the 7-12 weeks of their gestation period. The detection indexes included glycosylated hemoglobin (HbA1c), fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), uric acid (UA), and serum creatinine (SCr). Additionally, the TH levels were measured, including total thyroxine (TT4), total triiodothyronine (TT3), free thyroxine (FT4), free triiodothyronine (FT3), thyroid-stimulating hormone (TSH), thyroid peroxidase antibodies (TPOAb), and thyroglobulin antibodies (TgAb), and FT3/FT4 ratio was calculated. The biochemical indices were analyzed in the laboratory of Peking University International Hospital Center. HbA1c levels were determined using high-performance liquid chromatography (HPLC) and a Dongcao G8 analyzer.

2.3 Diagnosis of GDM

The pregnant women were screened for GDM through a 75g OGTT at 24–28 weeks of gestation. To perform this test, the

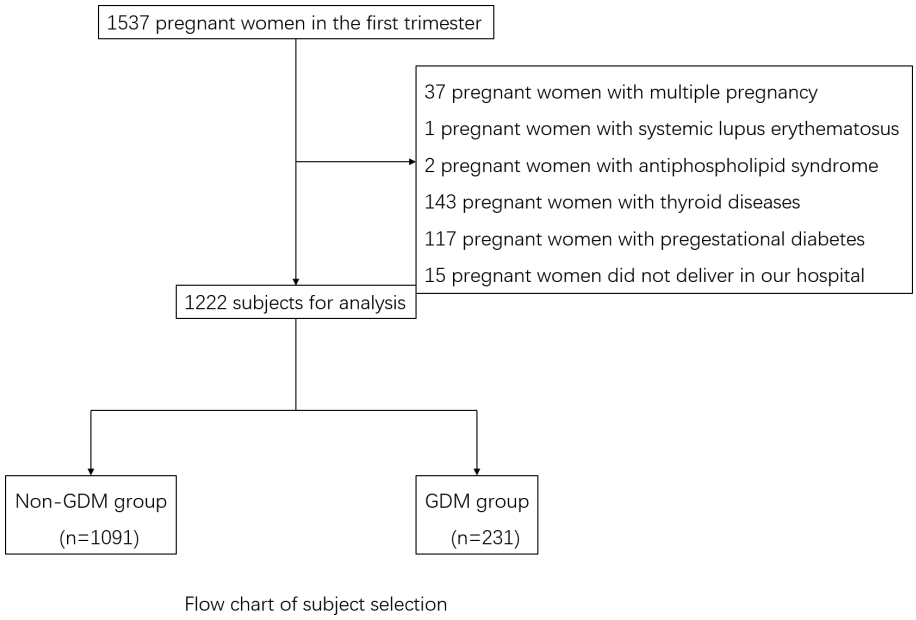

FIGURE 1
Flow chart of subject selection.

pregnant women were admitted to the hospital in the morning after fasting for 8–12 h. They were provided with 75g of glucose powder, which was dissolved in 250ml–300ml of warm boiled water, and they had to consume it quickly within 5 min. Blood glucose levels were measured at three specific time points during the oral glucose tolerance test: before taking the glucose solution (GLU0min), at 1 hour after taking the glucose solution (GLU60min), and at 2 h after taking the glucose solution (GLU120min).

The diagnostic criteria for GDM in this study were based on the IADPSG (International Association of the Diabetes and Pregnancy Study Groups) guidelines (7). According to these criteria, the blood glucose values at different time points during the OGTT should be as follows: GLU0min should be lower than 5.1 mmol/L, GLU60min should be lower than 10.0 mmol/L, and GLU120min should be lower than 8.5 mmol/L. If any of the blood glucose values reach or exceed these specified criteria, a diagnosis of GDM is made.

The weight of the pregnant women at 24–28 weeks of gestation was documented, and their weight gain during this period was calculated and recorded.

2.4 Statistical analysis

All data were analyzed using SPSS 22.0. Data were tested for normality. Normally distributed data were expressed as means ± standard deviation (x ± s) and compared using t-tests, while non-normally distributed data were expressed as medians (P25, P75) and compared using rank sum tests. The counting data were expressed as rates, and comparisons between the two groups were made using $\chi 2$ tests. Spearman correlations were used to assess associations between variables, while univariate and multivariate analyses were conducted using unconditional logistic regression models. These models were used to calculate the odds ratio (OR) and its corresponding 95% confidence interval (95%CI). Receiver operating characteristic (ROC) curves were plotted, and the areas under the curve (AUC) were calculated. All statistical tests were two-sided, and $p<0.05$ was considered statistically significant.

3 Results

3.1 Comparison of general conditions and biochemical indexes between the two groups in the first trimester of pregnancy and OGTT results

Out of the 1,222 patients, 231 were diagnosed with GDM during the second trimester, resulting in an incidence rate of 18.90%. All the patients tested negative for TPOAb and TGAb. The levels of HbA1c ranged from 4.0% to 6.4% in the non-GDM group and 4.5% to 9.5% in the GDM group. In comparison to the non-GDM group, there was a notable increase in the proportion of individuals with a personal history and family history of GDM in the GDM group ($\chi 2 = 10.21$ and $\chi 2 = 9.87$, all $p<0.05$). When compared to the non-GDM group, there was a significant increase in the proportion of multipara in the GDM group ($\chi 2 = 9.94$, $p<0.05$). Women with GDM tended to have higher BMI than those without and also showed higher levels of both HbA1c and FBG in the first trimester of pregnancy (all $p<0.05$). The levels of TG TC, LDL-C, and UA were also higher in the GDM group (all $p<0.05$). Women with GDM also showed lower levels of FT4 and a significantly higher FT3/FT4 ratio than those without GDM (all $p<0.05$). The levels of

TT4, TT3, FT3, and TSH did not differ significantly between the two groups during early pregnancy (all p>0.05) (Table 1).

3.2 Association between TH and blood glucose before and after glucose loading

Positive correlations were found between BMI, TG, TC, LDL-C, UA, and blood glucose levels before and after glucose loading (all p<0.05). There were positive correlations between weight gain and blood glucose levels before and after glucose loading (all p<0.05). There were positive correlations between FT3/FT4 ratio in the first trimester of pregnancy and blood glucose levels before and after glucose loading (all p<0.05). The FT4 levels in the first trimester were negatively correlated with GLU0min and FT3 levels were positively correlated with GLU120min (all p<0.05). There were no significant correlations between TT3, TT4, TSH, and glucose levels before and after glucose loading (all p>0.05) (Tables 2, 3).

3.3 Logistic regression analyses of TH and GDM

Multivariate logistic regression was conducted using GDM as the dependent variable and variables that were shown to be significant in the univariate analysis as independent variables.

TABLE 1 Comparison of general conditions and biochemical indexes between the two groups in the first pregnancy and OGTT results.

Index	non-GDM group	GDM group	t(X2)	p
	(n=991)	(n=231)		
Age (years)	30.94 ± 3.64	30.77 ± 3.86	0.63	0.52
BMI (kg/m^2)	21.65 ± 3.01	23.31 ± 3.16	-7.62	<0.05
Personal history of GDM	10 (1.01%)	43(18.61%)	10.21	<0.05
Family history of GDM	5 (0.50%)	21(9.10%)	9.87	<0.05
Family history of TD	37(3.73%)	11(4.76%)	3.21	0.12
Parity				
0	588(59.33%)	111(48.05%)	9.94	<0.05
≥1	403(40.67%)	120(51.95%)		
SBP (mmHg)	110.06 ± 10.64	109.71 ± 10.26	0.45	0.65
DBP (mmHg)	66.48 ± 8.91	64.77 ± 9.08	2.62	<0.05
TC (mmol/L)	3.93 ± 0.69	4.06 ± 0.67	-2.69	<0.05
TG (mmol/L)	0.95 ± 0.58	1.13 ± 0.47	-4.46	<0.05
LDL-C(mmol/L)	2.03 ± 0.55	2.12 ± 0.54	-2.28	<0.05
HDL-C(mmol/L)	1.41 ± 0.28	1.42 ± 0.29	-0.51	0.61
UA (umol/L)	211.92 ± 46.54	227.80 ± 47.95	-4.67	<0.05
sCr (umol/L)	49.67 ± 7.11	48.98 ± 6.78	1.33	0.18
HbA1c (%)	5.08 ± 0.26	5.29 ± 0.30	-5.62	<0.05
FBG (mmol/L)	4.87 ± 0.40	5.04 ± 0.41	-6.19	<0.05
gestational weight gain(kg)	9.34 ± 1.23	12.09 ± 2.32	-5.43	<0.05
FT4 (pmol/l)	16.84 ± 1.92	16.40 ± 1.95	3.13	<0.05
FT3 (pmol/l)	4.62 ± 0.50	4.67 ± 0.50	-1.40	0.16
TT4 (nmol/l)	121.01 ± 22.24	118.88 ± 20.70	1.31	0.19
TT3 (nmol/l)	1.76 ± 1.19	1.83 ± 1.09	-0.93	0.35
TSH (uIU/ml)	2.03 ± 0.35	2.06 ± 0.36	-1.16	0.25
FT3/FT4	0.27 ± 0.04	0.29 ± 0.04	-3.92	<0.05

BMI is for body mass index, SBP is systolic blood pressure, DBP is for diastolic blood pressure, FBG is for fasting blood glucose, HbA1c is for glycosylated hemoglobin, sCr is for serum creatinine, UA is for uric acid, TC is for total cholesterol, TG is for triglycerides, LDL-C is for low-density lipoprotein cholesterol, HDL-C is for high-density lipoprotein cholesterol, TT4 is for total thyroxine,TT3 is for total triiodothyronine, FT4 is for free thyroxine, FT3 is for free triiodothyronine, TSH is for thyroid-stimulating hormone, GLU0min is for fasting blood glucose before OGTT, GLU60min is for blood glucose 60 min after OGTT, GLU120min is for blood glucose 120 min after OGTT.

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TABLE 2 Correlation Analysis between biochemical indexes and blood glucose before and after glucose loading.

Index	GLU0min		GLU60min		GLU120min	
	r	p	r	p	r	p
BMI (kg/m^2)	0.25	<0.05	0.18	<0.05	0.26	<0.05
SBP (mmHg)	-0.06	0.06	-0.07	<0.05	-0.03	0.38
DBP (mmHg)	-0.07	<0.05	-0.10	<0.05	-0.07	<0.05
TC (mmol/L)	0.08	<0.05	0.12	<0.05	0.13	<0.05
TG (mmol/L)	0.12	<0.05	0.15	<0.05	0.15	<0.05
LDL-C(mmol/L)	0.11	<0.05	0.12	<0.05	0.12	<0.05
HDL-C(mmol/L)	-0.10	<0.05	-0.01	0.84	-0.04	0.14
UA (umol/L)	0.16	<0.05	0.14	<0.05	0.16	<0.05
sCr(umol/L)	-0.02	0.54	-0.01	0.70	-0.03	0.30
gestational weight gain(kg)	0.09	<0.05	0.04	0.09	0.11	<0.05

BMI is for body mass index, SBP is systolic blood pressure, DBP is for diastolic blood pressure, FBG is for fasting blood glucose, HbA1c is for glycosylated hemoglobin, sCr is for serum creatinine, UA is for uric acid, TC is for total cholesterol, TG is for triglycerides, LDL-C is for low-density lipoprotein cholesterol, HDL-C is for high-density lipoprotein cholesterol.

After adjustment for age, BMI, parity, blood lipid, blood pressure, UA, and sCr, the FT3/FT4 ratio was an independent risk factor for GDM (Table 4).

3.4 Single variable predicting model of GDM

The model for predicting the risk of GDM using individual variables including FT3, FT4 and FT3/FT4 showed that the AUCs were ranked FT3/FT4 (0.59) > FT4 (0.57) > FT3 (0.51). The cut-off points of FT4, FT3, and FT3/FT4 were 15.55 pmol/L, 119.7 pmol/L, and 0.27, respectively (Table 5).

In the multivariate predictive model, GDM was used as the dependent variable, and age, BMI, parity, blood lipid, blood pressure, UA, and FT3/FT4 were used as independent variables. The regression equation is - 11.61649 + 6.46352 * FT3/FT4 + 0.00026 * Age + 0.12568 * BMI + 0.10806 * TC + 0.07897 * TG- 0.006666 * SBP + 0.44134 * Parity + 1.07293 * HbA1c. The model had an AUC of 0.708 (95% CI 0.66, 0.76), a specificity of 73.83%, a sensitivity of 58.39%, and an accuracy of 70.87% (Figure 2).

4 Discussion

GDM can result in significant perinatal complications, including macrosomia, shoulder dystocia, cesarean section, and neonatal hypoglycemia, Additionally, GDM can have long-term effects on the mother's risk of developing type 2 diabetes mellitus (T2DM) and can contribute to obesity in the child. During pregnancy, the thyroid gland enlarges to meet the increased hormonal demands of pregnancy, growing by approximately 40%. This expansion is accompanied by changes in the secretion of FT3 and FT4 levels and an overall increase in metabolic activity (8).

TH plays a crucial role in regulating balanced glucose metabolism. Its involvement in insulin signal transduction and the maintenance of glucose homeostasis is considered a potential factor in human pathophysiology. Both a deficiency or an excess of TH can disrupt the normal regulation of glucose in the body. T3 is the main bioactive hormone responsible for glucose-related metabolic activities. Elevated TSH levels can cause harm to the pancreatic islets and possibly hinder the function of beta cells, resulting in insulin resistance and elevated blood glucose levels (8). Studies in rats with subclinical hypothyroidism (SCH) have shown a

TABLE 3 Correlation Analysis between thyroid hormone and blood glucose before and after glucose loading.

Index	GLU0min		GLU60min		GLU120min	
	r	p	r	p	r	p
FT4 (pmol/l)	-0.06	<0.05	-0.05	0.06	-0.04	0.20
FT3 (pmol/l)	0.03	0.34	0.05	0.09	0.06	<0.05
TT4 (nmol/l)	-0.01	0.67	-0.03	0.31	-0.03	0.29
TT3 (nmol/l)	0.02	0.46	0.03	0.39	0.02	0.28
TSH (uIU/ml)	0.06	0.06	0.06	0.06	0.05	0.05
FT3/FT4	0.07	<0.05	0.08	<0.05	0.09	<0.05

TT4 is for total thyroxine, TT3 is for total triiodothyronine, FT4 is for free thyroxine, FT3 is for free triiodothyronine, TSH is for thyroid-stimulating hormone, GLU0min is for fasting blood glucose before OGTT, GLU60min is for blood glucose 60 min after OGTT, GLU120min is for blood glucose 120 min after OGTT.

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TABLE 4 Logistic regression between thyroid function in first trimester and GDM.

Index	Crude OR	95%CI	p	Adjust OR	95%CI	p
FT4(pmol/L)						
Low:12.01-16.70	1			1		
Medium:16.71-18.86	0.78	0.55-1.09	0.59	0.84	0.58,1.20	0.33
High:18.87-22.00	0.67	0.47-0.95	<0.05	0.75	0.52-1.09	0.13
FT3(pmol/L)						
Low:3.20-4.62	1			1		
Medium:4.62-4.81	1.27	0.86-1.85	0.23	1.22	0.82-1.82	0.32
High:4.81-6.39	1.41	0.98-2.03	0.06	1.32	0.90-1.94	0.15
FT3/FT4						
Low:0.17-0.28	1			1		
Medium:0.28-0.32	1.58	1.08-2.30	<0.05	1.45	1.01-2.01	<0.05
High:0.32-0.41	1.95	1.35-2.80	<0.05	1.67	1.14-2.46	<0.05

FT4 is for free thyroxine, FT3 is for free triiodothyronine.

reduction in glucose production in the liver, along with a decreased rate of glucose utilization in skeletal muscles and adipose tissues (9, 10).

Our results demonstrated a significant association between the FT3/FT4 ratio and blood glucose levels in the second trimester of pregnancy. It was found that the FT3/FT4 ratio was the only parameter that showed a significant positive correlation with blood glucose levels after glucose loading during the second trimester. T4 is usually considered as a pre-hormone. As the precursor to the biologically active form, T3, the FT3/FT4 ratio is utilized to assess deiodinase activity. In a cross-sectional study, it was observed that elevated deiodinase activities in women with normal TH levels were associated with higher BMI and increased deiodinase activity was significantly correlated with higher blood glucose levels. It is theorized that the increased deiodinase activity triggered by BMI may elevate the risk of GDM by amplifying the effects of T3 (11). A recent study has reported similar findings, indicating that a higher FT3-to-FT4 ratio during the later stages of pregnancy was linked to an increased risk of GDM, adverse pregnancy outcomes, and an adverse metabolic profile in the early postpartum period (12). Nonetheless, it is worth noting that the study had a relatively small sample size, and it was unable to demonstrate the predictive value of FT3/FT4 for the occurrence of GDM. Another study, however, did establish associations between FT3/FT4 ratio in the first trimester and GLU0min and concluded that FT3/FT4 was an independent risk factor for the development of GDM. This finding aligns with the outcomes of our study (13, 14). However, the outcomes of these studies also indicated significant associations between FT3 and TSH levels and GDM. It is important to note that the subjects in these studies consisted of pregnant women with SCH or T4 levels, which might introduce confounding variables and affect the analysis of the relationship between TH and GDM in euthyroid women. Another study with large number subjects has shown that lower concentration of serum FT4 or higher FT3/FT4 ratio in early pregnancy was associated with an increased risk of GDM (OR = 1.43; 95% CI 1.06, 1.93, p= 0.01) after adjusting for potential confounders. However, the study failed to find the cut-off of the FT3/FT4 ratio for predicting GDM (15). Our study focused on euthyroid women during the first trimester of pregnancy, intentionally excluding women with TD or those taking medication that could potentially confound the analysis of the relationship between TH and GDM. In our research, we employed ROC analysis to evaluate the predictive value of FT3, FT4, and the FT3/FT4 ratio for the occurrence of GDM. The AUCs were ranked from smallest to largest as follows: FT3/FT4 (0.59) > FT4 (0.57) > FT3 (0.51). Additionally, it was determined that the cut-off points for FT4, FT3, and FT3/FT4 in predicting GDM were 15.55 pmol/L, 119.7 pmol/L, and 0.27, respectively.

The interaction between TH and blood glucose may be influenced by several factors. (1) TH has the capacity to regulate the expression of glucose transporter 2 (GLUT2) (11). Intrahepatic gluconeogenesis is a process that can lead to the swift transport of

TABLE 5 Univariate predictive model of GDM.

Index	AUC (95%CI)	Specificity	Sensitivity	Cut-off
FT4	0.57 (0.53, 0.61)	0.74	0.35	15.55
FT3	0.51 (0.47, 0.56)	0.54	0.54	4.65
FT3/FT4	0.59 (0.55, 0.63)	0.45	0.68	0.27

TT4 is for total thyroxine, FT4 is for free thyroxine, FT3 is for free triiodothyronine.

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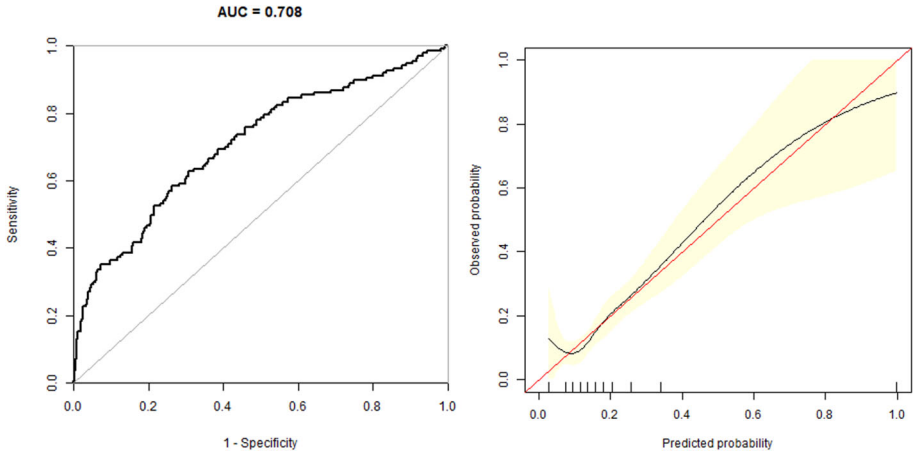

FIGURE 2
The overall predictive accuracy of multivariate predictive model for the risk of GDM. The AUC is 0.708 (95% CI 0.66, 0.76), a specificity of 73.83%, a sensitivity of 58.39% and an accuracy of 70.87%.

glucose across the cytoplasmic membrane of the liver. This glucose efflux, which occurs at the hepatic cytoplasmic membrane, is facilitated by a protein called GLUT2. The pathway involving gluconeogenesis, kinesin, and glucose transporter interactions may have secondary effects on hepatocytes and could result in reduced sensitivity to insulin in the liver (16). Some studies have shown that T3 can induce the expression of glucose transporter 4 (GLUT4) and GLUT4 is known to potentially enhance insulin sensitivity (17), therefore, T3 might have the capacity to induce insulin sensitivity. (2) It has been confirmed that abnormal TH levels may have adverse effects on mitochondrial function (18). T3 has the ability to directly bind to specific T3 binding sites in mitochondria. Additionally, it can exert its influence on the cell nucleus, indirectly impacting the transcription of genes associated with the regulation of cellular metabolism and mitochondrial function (19). Mitochondria play a crucial role in glucose metabolism within pancreatic cells. Any defects in mitochondrial function can make individuals more susceptible to cellular dysfunction, which, in turn, may increase the risk of developing T2DM.

Our study observed slightly higher TT3 and FT3 levels in patients with GDM than those without GDM, and the FT3 level was positively associated with the GLU120min level after glucose loading ($r=0.06$, $p<0.05$). However, after adjusting for BMI, blood pressure, and other variables in logistic regression, the association between FT3 and GDM risk was not sustained.

In a retrospective analysis involving a total of 27,513 pregnant women, which included 3,697 cases in the GDM group, the relationship between various TH levels and GDM was examined. The findings revealed that pregnant women with GDM had lower FT4 levels in comparison to those without GDM ($p<0.01$). A lower FT4 level during the first trimester of pregnancy was found to be linked with the development of GDM ($p<0.01$) (20). Many research studies have verified the association between reduced FT4 levels during the second and third trimesters of pregnancy and an elevated risk of GDM. Nevertheless, the exact cause-and-effect relationship between these two factors remains unclear. A meta-analysis showed that isolated maternal hypothyroxinaemia (IMH) was associated with increased GDM, preterm premature rupture of membranes, preterm birth, fetal distress, and macrosomia outcomes in IMH compared to euthyroid women, and the relative risks were 1.42, 1.50, 1.33, 1.75 and 1.62, respectively. IMH was not associated with placenta previa, gestational hypertension, pre-eclampsia, intrauterine growth restriction, and off-spring outcomes like birth weight, low birth weight infants, fetal macrosomia, neonatal intensive care, neonatal death, or fetal head circumference (21). However, certain studies have indicated that, even after accounting for confounding variables, there is no statistically significant correlation between FT4 and GDM (6, 22). The majority of previous research has primarily focused on pregnant women with SCH or low T4 levels, but there has been a scarcity of research findings concerning pregnant women with normal TH. In our study, we identified a negative correlation between FT4 and blood glucose levels following OGTT in pregnant euthyroid women.

In addition, it has been reported that TSH binding to adipocyte receptors stimulates the secretion of IL-6 from the cells, leading to proliferation, differentiation, and leptin production in both preadipocytes and adipocytes (23). These findings indicate that adipocytes may play a role in connecting insulin resistance with TSH. A recent study identified a positive association between TSH levels and the homeostasis model assessment of insulin resistance (HOMA-IR) in both individuals with diabetes and those without diabetes. This observation suggests an independent and direct correlation between insulin resistance and TSH levels (24). A population-based Chinese study also showed an independent correlation between TSH during the first trimester of pregnancy and GDM. This association was particularly significant among women with higher BMI values prior to pregnancy (25). In our own study, we did not find this association, which could be attributed to the fact that the study participants had normal TH levels, and their TSH levels fell within the normal range. This did not allow us to determine whether the risk of GDM increases when TSH levels exceed the normal range.

Some studies have shown that thyroid disease and GDM could share some common risk factors, such as age, BMI, vitamin D deficiency, selenium level and so on (26). Additionally, our study identified parity, BMI, blood pressure, blood lipid, UA, and HbA1c levels as independent risk factors for GDM, confirming the findings of earlier studies (27, 28). In order to avoid the biases, the multivariate logistic regression was conducted using GDM as the dependent variable after adjustment for age, BMI, parity, blood lipid, blood pressure, UA, and sCr. In our investigation, we established a predictive model with GDM as the dependent variable and age, parity, blood pressure, BMI, blood lipid, HbA1c, UA, and FT4/FT3 as independent variables. The AUC of the prediction model was 0.708 (95% CI 0.66,0.76), the specificity was 73.83%, the sensitivity was 58.39%, and the accuracy was 70.87%, which indicates that the model possesses a certain degree of predictive value for the early diagnosis of GDM.

The study has several limitations. Firstly, the subjects were enrolled from a single hospital and the sample size was limited. In future research, it would be beneficial to involve multiple centers and a larger sample of subjects to enhance the generalizability of the findings. Secondly, our study solely incorporated TH data from the first trimester of pregnancy, and we did not perform a dynamic assessment of TH throughout the entire pregnancy. In future research, it would be valuable to include TH measurements from various stages of pregnancy to provide a more comprehensive understanding of the relationship between TH and GDM. Furthermore, in future studies, we plan to conduct a more extensive investigation into the correlation between TH and insulin sensitivity as well as insulin resistance. This will help clarify the specific relationship between TH and glucose metabolism. Finally, the correlation analysis between TH and blood glucose revealed statistically significant but not particularly high correlation values (r values). To better elucidate the causal relationship between TH and blood glucose, we further conducted a logistic regression analysis. The results of our logistic regression analysis have indicated a causal relationship between TH and diabetes. We believe that while the r values may not be very high, their statistical significance still suggests a trend in the correlation between these two variables. In future research, as the sample size continues to grow, it may become more likely to observe a stronger and more significant linear relationship between the variables.

5 Conclusions

This study delved into the association between the FT3/FT4 ratio and GDM in pregnant women with normal thyroid function. It is worth noting that the study featured a substantial sample size, effectively excluding the influence of abnormal TH on the research outcomes. Furthermore, we constructed a prediction model for GDM based on the FT3/FT4 ratio, and an ROC curve was generated to evaluate its performance. The study also identified a specific cut-off value for predicting GDM. The FT3/FT4 ratio is an independent risk factor for GDM in the first trimester of pregnancy. When compared with the individual components, FT4 and FT3, the FT3/FT4 ratio exhibits greater predictive value for the development of GDM.

Data availability statement

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

Ethics statement

The studies involving humans were approved by bioethics committee of Peking University International Hospital. 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.

Author contributions

XZ: Conceptualization, Formal Analysis, Methodology, Software, Writing – original draft. JS: Conceptualization, Formal Analysis, Methodology, Software, Writing – review & editing. NY: Data curation, Writing – review & editing. XMZ: Funding acquisition, Project administration, Supervision, Writing – review & editing.

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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. Jenum AK, Mørkrid K, Sletner L, Vangen S, Torper JL, Nakstad B, et al. Impact of ethnicity on gestational diabetes identified with the WHO and the modified International Association of Diabetes and Pregnancy Study Groups criteria: a population-based cohort study. *Eur J Endocrinol* (2012) 166:317–24. doi: 10.1530/eje-11-0866

2. Thompson D, Berger H, Feig D, Gagnon R, Kader T, Keely E, et al. Diabetes and pregnancy. *Can J Diabetes* (2013) 37(Suppl 1):S168–83. doi: 10.1016/j.jcjd.2013.01.044

3. Viana LV, Gross JL, Azevedo MJ. Dietary intervention in patients with gestational diabetes mellitus: a systematic review and meta-analysis of randomized clinical trials on maternal and newborn outcomes. *Diabetes Care* (2014) 37:3345–55. doi: 10.2337/dc14-1530

4. Sert UY, Buyuk GN, Engin Ustun Y, Ozgu Erdinc AS. Is there any relationship between thyroid function abnormalities, thyroid antibodies and development of gestational diabetes mellitus (GDM) in pregnant women? *Medeni Med J* (2020) 35:195–201. doi: 10.5222/mmj.2020.29964

5. Demiral Sezer S, Topaloglu O. Effects of thyroid hormones in women with gestational diabetes. *Gynecol Endocrinol* (2022) 38:588–91. doi: 10.1080/09513590.2022.2076832

6. Simmons D, Immanuel J, Hague WM, Teede H, Nolan1 CJ, Peek MJ, et al. Treatment of gestational diabetes mellitus diagnosed early in pregnancy. *N Engl J Med* (2023) 388(23):2132–44. doi: 10.1056/NEJMoa2214956

7. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care* (2010) 33:676–82. doi: 10.2337/dc09-1848

8. Potenza M, Via MA, Yanagisawa RT. Excess thyroid hormone and carbohydrate metabolism. *Endocr Pract* (2009) 15:254–62. doi: 10.4158/ep.15.3.254

9. Cettour-Rose P, Theander-Carrillo C, Asensio C, Klein M, Visser TJ, Burger AG, et al. Hypothyroidism in rats decreases peripheral glucose utilisation, a defect partially corrected by central leptin infusion. *Diabetologia* (2005) 48:624–33. doi: 10.1007/s00125-005-1696-4

10. Fu X, Xia H, Mao H, Zhao S, Wang Z. The effect of glycaemic control in type 2 diabetic patients with subclinical hypothyroidism. *J Endocrinol Invest* (2016) 39:1453–8. doi: 10.1007/s40618-016-0519-4

11. Lyu J, Imachi H, Yoshimoto T, Fukunaga K, Sato S, Ibata T, et al. Thyroid stimulating hormone stimulates the expression of glucose transporter 2 via its receptor in pancreatic β cell line, INS-1 cells. *Sci Rep* (2018) 8:1986. doi: 10.1038/s41598-018-20449-3

12. Raets L, Minschart C, Van den Bruel A, Van den Bogaert E, Van Crombrugge P, Moyson C, et al. Higher thyroid fT3-to-fT4 ratio is associated with gestational diabetes mellitus and adverse pregnancy outcomes. *J Clin Med* (2022) 11:5016. doi: 10.3390/jcm11175016

13. Liu ZM, Li G, Wu Y, Zhang D, Zhang S, Hao YT, et al. Increased central and peripheral thyroid resistance indices during the first half of gestation were associated with lowered risk of gestational diabetes-analyses based on Huizhou birth cohort in South China. *Front Endocrinol (Lausanne)* (2022) 13:806256. doi: 10.3389/fendo.2022.806256

14. Duntas LH, Orgiazzi J, Brabant G. The interface between thyroid and diabetes mellitus. *Clin Endocrinol (Oxf)* (2011) 75:1–9. doi: 10.1111/j.1365-2265.2011.04029.x

15. Wang Y, Sun F, Wu P, Huang Y, Ye Y, Yang X, et al. A prospective study of early-pregnancy thyroid markers, lipid species, and risk of gestational diabetes mellitus. *J Clin Endocrinol Metab* (2022) 107(2):e804–14. doi: 10.1210/clinem/dgab637

16. Lanni A, Moreno M, Goglia F. Mitochondrial actions of thyroid hormone. *Compr Physiol* (2016) 6:1591–607. doi: 10.1002/cphy.c150019

17. Feldthusen AD, Larsen J, Pedersen PL, Toft Kristensen T, Kvetny J. Pregnancy-induced alterations in mitochondrial function in euthyroid pregnant women and pregnant women with subclinical hypothyroidism; relation to adverse outcome. *J Clin Transl Endocrinol* (2014) 1:e13–e7. doi: 10.1016/j.jcte.2013.12.003

18. Haddow JE, Metzger BE, Lambert-Messerlian G, Eklund E, Coustan D, Catalano P, et al. Peripheral deiodinase activity, and plasma glucose: relationships between white women in the HAPO study. *J Clin Endocrinol Metab* (2019) 104:2593–600. doi: 10.1210/jc.2018-02328

19. Rawal S, Tsai MY, Hinkle SN, Zhu Y, Bao W, Lin Y, et al. A longitudinal study of thyroid markers across pregnancy and the risk of gestational diabetes. *J Clin Endocrinol Metab* (2018) 103:2447–56. doi: 10.1210/jc.2017-02442

20. Yang S, Shi FT, Leung PC, Huang HF, Fan J. Low thyroid hormone in early pregnancy is associated with an increased risk of gestational diabetes mellitus. *J Clin Endocrinol Metab* (2016) 101:4237–43. doi: 10.1210/jc.2016-1506

21. Zhuo L, Wang Z, Yang Y, et al. Obstetric and offspring outcomes in isolated maternal hypothyroxinaemia: a systematic review and meta-analysis. *J Endocrinol Invest* (2023) 46(6):1087–101. doi: 10.1007/s40618-022-01967-4

22. Gu Y, Su X, Li Y, Tang Y, Bao Y, Ying H. Do free thyroxine levels influence the relationship between maternal serum ferritin and gestational diabetes mellitus in early pregnancy? *Diabetes Res Clin Pract* (2019) 151:114–9. doi: 10.1016/j.diabres.2019.03.033

23. Antunes TT, Gagnon A, Chen B, Pacini F, Smith TJ, Sorisky A. Interleukin-6 release from human abdominal adipose cells is regulated by thyroid-stimulating hormone: effect of adipocyte differentiation and anatomic depot. *Am J Physiol Endocrinol Metab* (2006) 290:E1140–4. doi: 10.1152/ajpendo.00516.2005

24. Zhu P, Liu X, Mao X. Thyroid-stimulating hormone levels are positively associated with insulin resistance. *Med Sci Monit* (2018) 24:342–7. doi: 10.12659/msm.905774

25. Leng J, Li W, Wang L, Zhang S, Liu H, Li W, et al. Higher thyroid-stimulating hormone levels in the first trimester are associated with gestational diabetes in a Chinese population. *Diabetes Med* (2019) 36:1679–85. doi: 10.1111/dme.14106

26. Pinto S, Croce L, Carlier L, Cosson E, Rotondi M, et al. Thyroid dysfunction during gestation and gestational diabetes mellitus: a complex relationship. *J Endocrinol Invest* (2023) 46(9):1737–59. doi: 10.1007/s40618-023-02079-3

27. Wang J, Li Z, Lin L. Maternal lipid profiles in women with and without gestational diabetes mellitus. *Med (Baltimore)* (2019) 98:e15320. doi: 10.1097/md.0000000000015320

28. Ryckman KK, Spracklen CN, Smith CJ, Robinson JG, Saftlas AF. Maternal lipid levels during pregnancy and gestational diabetes: a systematic review and meta-analysis. *Bjog* (2015) 122:643–51. doi: 10.1111/1471-0528.13261

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Employing fasting plasma glucose to safely limit the use of oral glucose tolerance tests in pregnancy: a pooled analysis of four Norwegian studies

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Background/objective: There is no international consensus about the optimal approach to screening and diagnosis of gestational diabetes mellitus (GDM). Fasting plasma glucose (FPG) has been proposed as an alternative universal screening test to simplify the diagnosis of GDM. We investigate the ability of the FPG to predict a 2-hour glucose value below the cut-off for GDM, thereby "ruling out" the necessity of a full OGTT and assess the proportion of GDM-related complications associated with the identified FPG level.

Materials and methods: This study included secondary data from four Norwegian pregnancy cohorts (2002-2013), encompassing 2960 women universally screened with late mid-pregnancy 75g OGTT measuring FPG and 2-hour glucose. For a range of FPG thresholds, we calculated sensitivity to predict elevated 2-hour glucose, number of OGTTs needed and percentage of GDM cases missed, applying modified World Health Organization (WHO) 2013 criteria (^{2013}WHO) and 2017 Norwegian criteria (^{2017}Norwegian). We analyzed pregnancy outcomes for women above and below our selected threshold.

Results: The prevalence of GDM was 16.6% (^{2013}WHO) and 10.1% (^{2017}Norwegian). A FPG threshold of 4.7 mmol/L had a sensitivity of 76% (^{2013}WHO) and 80% (^{2017}Norwegian) for detecting elevated 2-hour glucose, with few missed GDM cases (2.0% of those ruled out and 7.5% of all GDM cases for ^{2013}WHO, and 1.1% of those ruled out and 7% of all GDM cases for ^{2017}Norwegian). When excluding women with FPG <4.7mmol/l and those with GDM based on FPG, only 24% (^{2013}WHO) and 29% (^{2017}Norwegian) would require

OGTT. Women with FPG <4.7mmol/l, including missed GDM cases, had low risk of large-for-gestational-age newborns, cesarean section and operative vaginal delivery.

Conclusion: A FPG threshold of 4.7mmol/l as a first step when screening for GDM could potentially eliminate the need for OGTT in 70-77% of pregnancies. Women with FPG below this threshold appear to carry low risk of GDM-associated adverse pregnancy outcomes.

KEYWORDS

gestational diabetes, screening, pregnancy outcomes, fasting plasma glucose, OGTT 3

Introduction

Gestational diabetes mellitus (GDM) is one of the most common disorders of pregnancy, responsible for several adverse outcomes in both mother and child during gestation and in the longer term (1). Despite extensive research over the past decades, there is still no consensus about the optimal approach to screening strategies and diagnostic criteria for GDM, reflected by substantial variations in clinical recommendations throughout the world (2, 3).

Although different diagnostic criteria for the identification of GDM are used, the oral glucose tolerance test (OGTT) is endorsed by all diabetes and health organizations as the "gold standard" diagnostic test for GDM. The use of OGTT in a clinical setting, however, poses several challenges. The test is poorly reproducible (4), time-consuming, and not user-friendly (5, 6), leading to a significant burden on the healthcare system in terms of infrastructure and cost. While the International Federation of Gynecology and Obstetrics strongly recommends universal testing (7) several European countries, including Norway, practice risk-factor based selective screening with the intention to identify the most severe cases of GDM and, concurrently, limit the number of OGTT's. However, this selection process is also demanding for healthcare providers, requiring screening of about 70% of the pregnant population (8).

Fasting plasma glucose (FPG) has been proposed as an alternative universal screening test for GDM (9), as it is easy to administer, less time-consuming for patients and healthcare providers, and inexpensive (10). During the Covid-19 pandemic, in order to minimize transmission of the virus and reduce use of medical resources, several health authorities and professional bodies suggested limiting the OGTT to women with FPG above a certain threshold value, "ruling out" those with lower FPG values where the GDM risk was considered low (11). If still recommended today, these new strategies should, however, be balanced by the need to ensure the best possible pregnancy outcomes for women and their infants. To date, a number of studies have proposed FPG cut-offs to accurately rule in and rule out GDM, with wide variation amongst different geographical regions in the world (12–16), but few have evaluated pregnancy complications potentially detected or missed (13, 17).

In light of this context, we aimed to explore the use of FPG to identify women at low risk for GDM and GDM-related adverse outcomes, limiting the need for an oral glucose tolerance test (OGTT). Our primary aim was to investigate the ability of the FPG to predict a 2-hour glucose value below the cut-off for GDM, thereby "ruling out" the necessity of a full OGTT, based on two different diagnostic GDM criteria in a Norwegian pregnant population. The secondary aim was to assess the proportion of GDM-associated complications for the identified FPG level in order to evaluate whether pregnancies ruled out can be safely excluded from further post-load glucose testing.

Material and methods

Study population and setting

We used secondary data from four Norwegian population-based birth cohort studies with a special focus on GDM. The criteria for the selection of studies have been previously described in detail (8). Participant characteristics for all studies are summarized in Table S1. The merged dataset consisted of two cohort studies (18, 19) and two randomized controlled trials (RCT) (20, 21) collecting data between 2002 and 2013. The interventions in the two trials consisted of either an exercise program (20) or a combination of a physical activity component and dietary counselling (21), but these interventions demonstrated no effect on the incidence of GDM or the outcomes of LGA and caesarean section. We excluded women with multiple pregnancies, those lacking glucose values, and infants with missing birthweight. We also excluded fetal deaths, as all except one had missing OGTT and/or outcome data (Flow chart, Figure S1). The Norwegian Regional Ethics committees (REC) had approved each study, and the current study was approved by the REC South East. All participants provided written informed consent.

Data collection

All women were offered a 75 g OGTT measuring fasting and 2-hour (2-h) glucose levels. In the STORK Groruddalen study (22) venous blood samples were collected in tubes containing

ethylenediaminetetraacetic acid according to standardized protocols, and glucose was analyzed on site in fresh, whole EDTA blood, using HemoCue 201+ glucose analyser (Angelholm, Sweden) calibrated for plasma. In two studies (19, 23), glucose levels were measured in serum by the routine methods used at the participating hospital laboratories, and blood samples were stored at -80°C. The Fit for Delivery study measured glucose in plasma using a Cobas 6000 c501 chemistry analyzer (Roche Diagnostics) (24). Inter-essay coefficients for each study are reported in Table S1 (CV 2.0-3.6%), and further details about the laboratory measurements can be found in the original studies.

During data collection, the diagnosis of GDM was made according to the 1999 World Health Organization (WHO) criteria (^{1999}WHO) (FPG ≥7.0 mmol/l or 2-h glucose ≥7.8 mmol/l). We retrospectively applied modified 2013 WHO (^{2013}WHO) diagnostic cut-offs (FPG ≥5.1 mmol/l or 2-h glucose ≥8.5 mmol/L, as 1-hour glucose was not measured in the respective studies) and the 2017 Norwegian (^{2017}Norwegian) cut-offs (FPG ≥ 5.3 mmol/l or 2-h glucose ≥ 9.0 mmol/L) to the same OGTT. Women with GDM by 1999-WHO criteria were informed and referred to their general practitioner or specialist care according to protocol. Women received standard GDM treatment in accordance with either global guidelines in place at the time (25) or local guidelines specific to each hospital (treatment targets provided in Table S1). However, we lack specific information about the treatment provided to each woman, including whether the clinicians adhered to the guidelines. Only 12 women have been documented as receiving pharmacological treatment.

All participants provided questionnaire data, self-reported (19–21) or through interviews (18), with information on maternal age, parity, smoking status and their highest level of education. Ethnic origin was defined by the pregnant woman's mother's country of birth and further merged into three groups in the current study: European (predominantly Scandinavian), Middle Eastern/African, and Asian (primarily South and East Asian ethnicity). Height was measured directly on site while weight prior to pregnancy was self-reported. Pre-pregnancy body mass index (BMI) was defined as weight (kg) divided by height $(m)^2$ and categorized as normal weight (≤24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obesity (≥30 kg/m^2).

Pregnancy and delivery outcome data

Outcome data collected at the time of birth were birthweight (grams), gestational age at birth, delivery method (normal vaginal delivery, cesarean section (planned or emergency), operative vaginal delivery (vacuum extraction or forceps)), preeclampsia or severe hypertensive disorder, and preterm delivery (<37 weeks). As in clinical practice in Norway, LGA (sex and gestational age-specific birthweight >90th percentile) was calculated using Norwegian national references (26), while macrosomia for the present study was defined as birthweight >4000 g.

Statistical analyses

The area (AUC) under the receiver operating characteristic (ROC) curve was used to analyze the discriminative power of FPG to predict an elevated 2-h glucose value, using the modified ^{2013}WHO criteria and the ^{2017}Norwegian criteria. Elevated 2-h glucose was used instead of the diagnosis of GDM since the latter also includes those diagnosed based on FPG. Using standard definitions, we assessed diagnostic accuracy measures such as sensitivity, specificity, and negative predictive value (NPV) of a range of threshold values of FPG (varying from 4.4 to 5.0 mmol/l). The number of OGTTs needed was analyzed after excluding women who had GDM based on FPG alone (FPG≥5.3 mmol/l or ≥5.1 mmol/l, depending on the diagnostic criteria). In addition, we calculated the proportion of missed GDM cases (women with GDM according to the 2-hour glucose but "ruled out" and excluded from the OGTT because of the specified FPG threshold). In the process of selecting the "optimal" FPG threshold, options that demonstrated good/acceptable sensitivity were considered (27). The thresholds were then reviewed individually according to diagnostic needs and clinical usefulness, with particular emphasis on the number of required OGTTs and missed GDM cases.

Characteristics of the women were categorized by FPG-status, and the groups were compared using X^2 statistic for categorical data and ANOVA for continuous variables. Data are presented as frequencies and percentages for categorical variables and mean and standard deviations (SD) for continuous variables.

To examine the risk of pregnancy complications among missed GDM cases (GDM according to the 2-hour glucose, but potentially excluded from the OGTT based on low FPG), we stratified women further into two groups: FPG below or FPG at/above the proposed threshold (4.7 mmol/l). For both strata, multivariable logistic regression models were performed for the pregnancy outcomes LGA, cesarean section and operative vaginal delivery, with elevated 2-h glucose (categorized as less than or at/above 9.0 mmol/l) as the main exposure. We adjusted for pre-specified confounders such as maternal age, pre-pregnancy BMI, ethnicity, parity, cohort, smoking and gestational age at birth. The effect estimates for 2-h glucose (less than or at/above 9.0 mmol/l) is presented as odds ratios (OR) with 95% confidence intervals (CI). The level of significance was set as 0.05. Statistical analyses were performed using statistical package IBM SPSS (version 23.0. Armonk, NY: IBM Corp).

Results

Of the 2970 women included in the present study, 16.6% fulfilled the modified ^{2013}WHO criteria for GDM, while 10.1% met the ^{2017}Norwegian criteria. More than 80% of all GDM cases were identified by elevated FPG, both by the modified ^{2013}WHO (≥5.1 mmol/L) and the ^{2017}Norwegian (≥5.3 mmol/L) criteria, while 16.0% and 17.6% were identified by elevated 2-h glucose alone

[^{2013}WHO (≥8.5 mmol/L) and ^{2017}Norwegian criteria (≥9.0 mmol/L)] (Table S2).

The ability of FPG to 'rule-out' the need for a full OGTT

The ROC curves along with the AUC quantifying the performance of the FPG to predict an elevated 2-h glucose (diagnostic for GDM) were assessed graphically (Figure 1). The AUC was 0.81 (95% CI 0.76-0.85) using the ^{2017}Norwegian criteria and slightly lower (0.78, 95% CI 0.75-0.82) when the modified ^{2013}WHO criteria were used to define GDM. A separate ROC analysis for women with non-European background gave an AUC of 0.70 (95% CI 0.6-0.8) using the ^{2017}Norwegian criteria (Figure S2).

Table 1A lists a range of threshold values for FPG with the associated sensitivity, specificity, and negative predictive value (NPV) using the Norwegian criteria. As the cut-off value rises, the sensitivity of the screening test decreases and the specificity increases. Conversely, a lower FPG threshold has high sensitivity and identifies most women with GDM but an excessive number of women without GDM will need to undergo the OGTT due to the corresponding poor specificity. Based on test properties (27) and careful clinical judgment the threshold value 4.7 mmol/L for FPG was selected, as this threshold had an acceptable sensitivity (78.9%) to predict elevated 2-hour glucose and appeared to offer the best trade-off to limit the number of missed GDM cases while avoiding unnecessary OGTTs. In total, 1855 women (62.5%) had FPG below this threshold and could potentially be 'ruled-out' as non-GDM. Of these women, 20 (1.1% of those ruled out and 6.6% of all GDM cases) had an elevated 2-hour glucose value, and would hence be "misclassified" as non-GDM, with the NPV being 98.9%. Of the remaining 1111 women with FPG above the 4.7mmol/l threshold, 248 (8.4% of the entire cohort) had FPG ≥5.3 mmol/l, i.e. GDM according to the ^{2017}Norwegian diagnostic criteria (Table 2). Thus, only the remaining 864 (29.1% of the entire cohort) would have to undergo the complete OGTT.

Similar results were found for the modified ^{2013}WHO criteria (Table 1B), although sensitivity to predict elevated 2-hour glucose was slightly reduced (75.8%), and the number of missed GDMs cases was slightly higher (2.0% of those ruled out and 7.5% of all GDM cases) for the same FPG threshold. The proportion of women requiring further evaluation to define their GDM status on the basis of FPG 4.7-5.0 mmol/L (number of OGTT needed) was, on the other hand, slightly lower using these criteria (23.5%).

Thus, if the FPG was offered to all women and a FPG threshold of 4.7 mmol/l was used to decide whether the OGTT was needed or not, 70.9% of women in the cohort would not require further testing when using the ^{2017}Norwegian diagnostic thresholds, and 76.5% when using the modified ^{2013}WHO criteria.

Comparison of women below or above the selected FPG threshold

Table 2 presents maternal characteristics and the proportion of pregnancy outcomes found among women classified as low FPG (<4.7 mmol/l), indeterminate FPG (4.7-5.2/5.0 mmol/l) and elevated FPG (FPG ≥5.3/5.1 mmol/l) according to the ^{2017}Norwegian criteria and ^{2013}WHO criteria, respectively. Women in the low FPG group had the lowest pre-pregnancy BMI. They also had the highest proportion of primiparas and the lowest proportion of women with a non-European ethnicity. Furthermore, the lowest proportion of LGA, macrosomia (>4000g) and total cesarean section was observed in women with FPG <4.7 mmol/L and the highest proportion in the elevated FPG groups. None of the women with FPG <4.7 mmol/l and GDM by ^{1999}WHO criteria received insulin or other antidiabetic medication.

Table 3 reports the proportion of pregnancy complications amongst women with GDM based on an elevated 2-hour glucose value (≥9.0 mmol/L), after dividing the sample into those below or

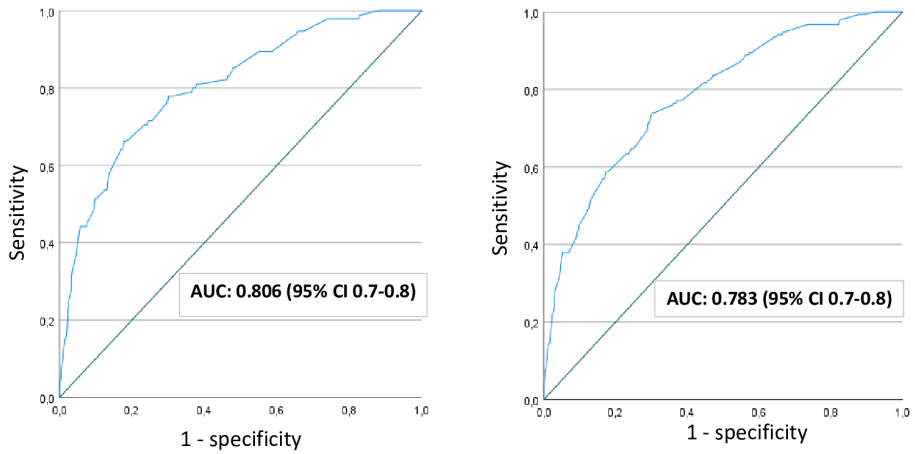

FIGURE 1
ROC curve to assess the performance of fasting plasma glucose to predict elevated 2-hour glucose, applying 2017-Norwegian criteria (left) and applying modified 2013-WHO criteria (right). AUC, area under the curve; CI, confidence interval; WHO, World Health Organization.

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TABLE 1A Overview of the sensitivity of different thresholds of FPG to the need for an OGTT to screen for GDM (Norwegian 2017 criteria).

Threshold FPG (mmol/l)	No. of women below threshold, n (%)	No. of OGTT needed*, n (%)	No. of GDM cases missed^, n (%)°	Sensitivity for 2-h glucose, % n/N	Sensitivity for GDM, % n/N	Specificity, %	NPV (%)
4,4	1051 (34,2)	1703 (57.4)	6 (1.9)	93,7 (89/95)	98,0 (295/301)	35.2	99,4
4,5	1298 (43,8)	1420 (47.9)	10 (3.3)	89.5 (85/95)	96,7 (291/301)	44.9	99.2
4,6	1586 (53,5)	1132 (38.1)	17 (5.6)	82.1 (78/95)	94,4 (284/301)	54.7	98.9
4,7	1855 (62.5)	863 (29.0)	20 (6.6)	78,9 (75/95)	93,3 (281/301)	63.9	98,9
4,8	2052 (69,2)	666 (22.4)	23 (7.6)	75,8 (72/95)	92,3 (278/301)	70.7	98,9
4,9	2222 (74,9)	526 (17.7)	28 (9.3)	70.5 (67/95)	90,0 (273/301)	76.7	98,7
5.0	2414 (81,4)	304 (10.2)	34 (11.3)	64.2 (61/95)	88,7 (267/301)	82.9	98,6

*Excluding women diagnosed with FPG ≥5.3 mmol/L (248 women).
^Women with FPG below stated threshold but 2-hour glucose above diagnostic criteria (>9.0 mmol/l), i.e. false negative cases.
FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; GDM, gestational diabetes mellitus; NPV, negative predictive value; no, number.
n: total number of cases; %: percentage of the total study cohort; °: percentage of total GDM cases.

TABLE 1B Overview of the sensitivity of different thresholds of FPG to the need for an OGTT to screen for GDM 2013 WHO criteria.

Threshold FPG (mmol/l)	No. of women below threshold, n (%)	No. of OGTT needed*, n (%)	No. of GDM cases missed^, n (%)°	Sensitivity for 2-h glucose, % n/N	Sensitivity for GDM, % n/N	Specificity, %	NPV (%)
4,4	1051 (34,2)	1536 (51.7)	10 (2.0)	93.5 (143/153)	98,0 (484/494)	35.8	99.0
4,5	1298 (43,8)	1254 (42.2)	20 (4.0)	86.8 (133/153)	95,9 (474/494)	45.4	98.5
4,6	1586 (53,5)	965 (32.5)	28 (5.7)	81.7 (125/153)	94,3 (466/494)	55.4	98.2
4,7	1855 (62.5)	697 (23.4)	37 (7.5)	75.8 (116/153)	92,5 (457/494)	64.6	98.0
4,8	2052 (69,2)	499 (16.8)	47 (9.5)	69.3 (106/153)	90,5 (447/494)	71.3	97.7
4,9	2222 (74,9)	329 (11.0)	56 (11.3)	63.4 (97/153)	88,7 (438/494)	77.0	97.5
5.0	2414 (81,4)	137 (4.6)	66 (13.4)	56.9 (87/153)	86,6 (428/494)	83.5	97.3

*Excluding women diagnosed with FPG ≥5.1 mmol/L (415 women).
^Women with FPG below listed threshold but 2-hour glucose above diagnostic criteria (>8.5 mmol/l), i.e. false negative cases.
FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; GDM, gestational diabetes mellitus; NPV, negative predictive value; no, number.
n: total number of cases; %: percentage of the total study cohort; °: percentage of total GDM cases.

at/above the FPG threshold of 4.7 mmol/l. For women with FPG ≥4.7 mmol/l [including those meeting current GDM criteria (FPG ≥5.3 mmol/L)], 2-h glucose ≥9.0 mmol/l was associated with higher risk for LGA (OR 2.61; 95%CI 1.37-4.95) but not for cesarean section and operative vaginal delivery. For women with FPG <4.7 mmol/l, who would not be offered an OGTT according to the proposed strategy, 2-h glucose ≥9.0 mmol/l was not associated with an increased risk for any of these outcomes.

Discussion

Main findings

Using data from a Norwegian sample offered universal mid-pregnancy GDM screening, we found that a FPG threshold of 4.7 mmol/L demonstrated an acceptable sensitivity of 76-80% to predict an elevated 2-hour glucose value (using modified ^{2013}WHO and ^{2017}Norwegian criteria respectively); 63% of participants had FPG below this threshold and could be "ruled-out" from further testing regardless of criteria used. Importantly, this group appears to carry a low risk of a range of pregnancy complications commonly associated with GDM. Furthermore, because FPG is included in the diagnostic criteria, we could identify ("rule-in") over 80% of GDM cases using FPG alone in our sample. This implies that if a rule-in/rule-out approach was used, OGTT would be needed in only 24-29% of our population, i.e. only those with FPG in the range 4.7-5.0/5.2 mmol/L.

Interpretation

FPG thresholds previously suggested as the preferred cut-off to avoid unnecessary OGTT's include 5.0 mmol/l in Mexican adolescents (14), 4.8 mmol/l in Swedish women (16), 4.4 mmol/l in both an Arab (12) and Chinese population (15), and 4.3 mmol/l in studies from South Asia (28) and Belgium (13). The diagnostic performance of FPG as a screening test is dependent on the

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TABLE 2 Comparison of characteristics and pregnancy outcomes between women with fasting plasma glucose below and at/above 4.7 and ≥5.3/5.1 mmol/l (2017 Norwegian cut offs and 2013 WHO cut offs).

Characteristics	Total		Norwegian 2017 Criteria			2013 WHO criteria		
		<4.7 mmol/L	4.7-5.2 mmol/L	≥5.3 mmol/L	*p*	4.7-5.0 mmol/L	≥5.1 mmol/L	*p*
n	2967	1855 (62.5)	864 (29.1)	248 (8.4)		697 (23.5)	415 (14.0)	
Maternal age (years)	30.0 (4.4)	29.9 (4.2)	30.5 (4.5)	30.7 (5.0)	0.001	30.4 (4.4)	30.6 (5.0)	0.001
Pre-pregnancy BMI (kg/m²)	23.7± 3.9	22.9 (3.3)	24.5 (4.1)	26.5 (5.7)	0.000	24.3 (4.0)	25.9 (5.3)	0.000
normalweight	2127 (71.6)	1457 (78.5)	552 (63.9)	116 (46.8)		455 (65.3)	213 (51.3)	
overweight	610 (20.5)	315 (17.0)	225 (26.0)	69 (27.8)		175 (25.1)	119 (28.7)	
obesity	233 (7.8)	83 (4.5)	87 (10.1)	63 (25.4)		67 (9.6)	83 (20.0)	
Ethnicity					0.000			0.000
European	2570 (86.6)	1705 (91.9)	708 (81.9)	157 (63.3)		584 (83.8)	281 (67.7)	
Middle East/African	174 (5.9)	68 (3.7)	68 (7.9)	38 (15.3)		48 (6.9)	58 (14.0)	
Asian	223 (7.5)	82 (4.4)	88 (10.2)	53 (21.4)		65 (9.3)	76 (18.3)	
Primipara, n (%)	1814 (61.1)	1174 (63.3)	517 (59.8)	123 (49.6)	0.000	424 (60.8)	216 (52.0)	0.000
Education, n (%)					0.000			0.000
Primary or less	145 (4.9)	61 (3.3)	51 (5.9)	34 (13.7)		33 (4.7)	52 (12.5)	
High school education	637 (21.4)	329 (17.7)	231 (26.7)	80 (32.3)		184 (26.4)	127 (30.6)	
Higher education	2180 (73.4)	1465 (79.0)	582 (67.4)	134 (54.0)		480 (68.9)	236 (56.9)	
Current smoker, n (%)	80 (2.8)	41 (2.3)	30 (3.7)	9 (4.0)	0.064	24 (3.7)	15 (3.9)	0.063
Fasting glucose at OGTT (mmol/L)	4.6± 0.5	4.3 (0.2)	4.9 (0.1)	5.7 (0.4)	0.000	4.8 (0.4)	5.4 (0.4)	0.000
2-hour glucose at OGTT (mmol/L)	6.1± 1.3	5.7 (1.1)	6.4 (1.3)	7.2 (1.6)	0.000	6.3 (1.2)	7.0 (1.5)	0.000
Gestational age at OGTT (weeks)	30.8 ± 2.5	31.4 (2.6)	30.1 (2.1)	29.5 (2.0)	0.000	30.2 (2.1)	29.6 (2.0)	0.000
GDM treatment								
Insulin/metformin	12 (0.4)	0	4	8		4	8	
Delivery								
Gestational age at delivery (weeks)	39.8 (1.6)	38.9 (1.5)	39.7 (1.6)	39.5 (1.6)	0.009	39.7 (1.6)	39.6 (1.6)	0.023
Birthweight, gram	3520 (522)	3485.7 (501)	3567.9 (529)	3607.1 (560)	0.000	3556.8 (532)	3610.1 (542)	0.000
LGA, n (%)	230 (7.7)	117 (6.3)	73 (8.4)	40 (16.1)	0.000	60 (8.6)	53 (12.8)	0.000
Macrosomia >4000g, n (%)	507 (17.1)	274 (14.8)	168 (19.4)	65 (26.2)	0.000	133 (19.1)	100 (24.1)	0.000
Total cesarean section, n (%)	446 (15.0)	244 (13.2)	143 (16.6)	60 (24.2)	0.000	113 (16.2)	90 (21.7)	0.000
Emergency cesarean section, n (%)	298 (10.1)	171 (9.2)	85 (9.8)	42 (16.9)	0.000	68 (9.8)	59 (14.2)	0.000

(Continued)

TABLE 2 Continued

Characteristics	Total	<4.7 mmol/L	Norwegian 2017 Criteria			2013 WHO criteria		
			4.7-5.2 mmol/L	≥5.3 mmol/L	*p*	4.7-5.0 mmol/L	≥5.1 mmol/L	*p*
Preterm birth	108 (3.6)	67 (3.6)	28 (3.2)	13 (5.2)	0.331	22 (3.2)	19 (4.6)	0.470
Preeclampsia	98 (3.6)	62 (3.7)	27 (3.3)	9 (3.8)	0.861	22 (3.4)	14 (3.6)	0.908
Operative vaginal delivery, n (%)	386 (13.0)	242 (13.1)	109 (12.6)	34 (13.7)	0.893	91 (13.1)	52 (12.5)	0.958

P values refer to comparison between the three groups using ANOVA.
FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; BMI, body mass index; GDM, gestational diabetes; WHO, World health organization; LGA, large for gestational age.

population tested, GDM prevalence and GDM criteria used (29, 30). In addition, the determination of ideal test sensitivity and specificity requires judicious assessment of harms related to missed diagnosis as well as burdens associated with large-scale testing. A low FPG threshold will have high sensitivity and identify most women with GDM but an excessive number of women without GDM will need to undergo the OGTT due to corresponding poor specificity, putting pressure on health services and medicalizing low-risk pregnancies. Similar to our findings, a recent Australian study concluded that FPG ≥4.7 mmol/l had the best sensitivity and specificity for abnormal OGTT results (31), and this preliminary test was employed in Australia during the Covid-19 pandemic.

Although more than 70% of OGTT's could be avoided by the proposed strategy in our study, applying either ^{2013}WHO or ^{2017}Norwegian criteria, 7-8% of GDMs identified with universal OGTT would be missed. Others have reported higher rates of "missed GDM" for the same threshold. Van Gemert et al. compared the use of a preliminary FPG ≥4.7 mmol/l to universal OGTT, reporting that 29% of women who would otherwise be diagnosed with GDM by ^{2013}WHO criteria could be missed (11). The contrasting finding in this study may at least to some degree be explained by additional measurements of 1-hour glucose values which we lacked. Nevertheless, recognizing and diagnosing GDM is essential, as management of GDM has been associated with reduced maternal, fetal and newborn complications (32–34). Furthermore, the identification of GDM provides a valuable opportunity to assess the women's future risk of diabetes and implement preventive measures, a possibility that would remain beyond reach without proper identification.

In our cohort, 82-84% of all GDM cases were identified based on FPG, making the idea of entirely abandoning an assessment of post-load glycemia rather appealing. A single FPG test offered to all pregnant women is a simple and low-cost option to diagnose GDM. However, the proportion of women diagnosed by FPG in our study was much higher than reported by others, including the multinational HAPO study, where 55% were diagnosed with GDM by FPG, using ^{2013}WHO criteria (35). Given the wide variability in the percentage of women diagnosed exclusively by FPG, probably explained by factors such as ethnicity and varying rates of obesity (35, 36) continued use of OGTT seems indicated.

Few previous studies have addressed whether pregnancies with FPG below a proposed threshold are in fact associated with low rates of GDM-associated complications. McIntyre et al. examined the outcomes associated with the Australian Covid-19 model of limiting GDM testing to those with FPG ≥4.7 mmol/l, using a subset of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study (37). Broadly consistent with our findings, participants with FPG <4.7 mmol/L had lower rates of pregnancy complications than those above this threshold. A recent Belgian study assessed pregnancy outcomes for a FPG threshold of 4.3 mmol/l, finding a better metabolic profile and low incidence of adverse outcomes below this cut-off (13), but the clinical relevance may be limited as

TABLE 3 Pregnancy outcomes stratified according to fasting plasma glucose (2017 Norwegian criteria).

Delivery outcomes	Total	<4.7 mmol/L			≥4.7 mmol/L		
2-hour glucose values		<9.0	>9.0	aOR* (95% CI)	<9.0	>9.0	aOR* (95% CI)
n	2967	1835	20		831	33	
Birthweight, gram	3520 (522)	3488	3243		3564	3663	
LGA, n (%)	230 (7.7)	116 (6.3)	1 (5.0)	1.01 (0.12-7.91)	67 (8.1)	6 (18.2)	**2.612 (1.37-4.95)**
Macrosomia >4000g, n (%)	507 (17.1)	237 (14.9)	1 (5.0)		161 (19.4)	7 (21.2)	
Total cesarean section, n (%)	446 (15.0)	240 (13.1)	4 (20.0)	1.20 (0.38-3.84)	137 (16.5)	6 (18.2)	1.040 (0.58-1.85)
Preterm birth	108 (3.6)	65 (3.5)	2 (10.0)		27 (3.2)	1 (3.0)	
Preeclampsia	89 (3.6)	61 (3.7)	1 (5.6)		27 (3.5)	0	
Operative vaginal delivery, n (%)	386 (13.0)	167 (9.1)	2 (10.0)	1.018 (0.37-2.75)	84 (10.1)	3 (9.1)	1.087 (0.65-1.80)

Significant values in bold.
*Adjusted for age, prepreg BMI, parity, ethnicity, cohort, smoking (LGA only) and gestational age at birth (ceserean section and operative delivery).

this threshold excludes few women from testing. Our findings indicate that women with FPG <4.7 mmol/l had a better metabolic profile, with less overweight/obesity, compared to women with higher FPG. In addition, pregnancies with FPG <4.7 mmol/l had low rates of LGA, macrosomia and total cesarean section, indicating that these women can safely continue routine care. However, long-term health risks in these women and their children, particularly related to type 2 diabetes, are unknown.

We have previously reported that selection criteria for BMI and age currently used in Norway would result in recommending OGTT to about 70% of women with European ethnicity in our sample (8). The results from the current study lend support to the universal use of FPG as an alternative to risk-profiling for selectively offering the OGTT, with the potential to limit the use of OGTT to less than 30% of all pregnancies and achieve similar sensitivity of about 80%. Additionally, it may avoid potentially stigmatizing selection based on age, BMI and ethnicity.

However, the proposed screening strategy requires certain logistics to be in place in order to make the implementation successful. Ideally, the fasting venous sample would have to be analyzed without delay by a measure with acceptable validity and reliability and at the same facility. This should be followed by an immediate decision as to whether a full OGTT is required, thereby avoiding prolonged waiting time and enabling women to complete the test on the same day as the fasting blood test. Moreover, our study is centered on GDM diagnosis made late in pregnancy. In light of a recent RCT indicating potential benefits of early screening (38), the matter of early versus late screening also warrants further consideration and exploration.

Strengths and weaknesses

Our study has several strengths. We merged previously collected maternal and offspring data from four cohorts, allowing more powerful and flexible analyses. Moreover, there is no pre-selection bias as an OGTT was offered to all included women. Importantly, most previous studies that have explored the performance of FPG provide limited or no information on adverse pregnancy outcomes.

The main limitation of our study is that glucose results were not blinded and women with GDM diagnosed by ^{1999}WHO criteria were routinely treated. This implies that conclusions drawn about likely clinical outcomes for women classified as "missed GDMs" may be inaccurate, as patients had a known diagnosis and received care, although none of these women received pharmacological treatment. Nonetheless, our results are comparable to those of McIntyre et al., which used a population blinded to OGTT results in their retrospective analysis of FPG and pregnancy outcomes (17). Additionally, our sample had slightly lower rates of obesity than our background population (7.8% vs. 12-12.5% nationally in 2007-2013) (39). This may affect the prevalence of GDM and its associated outcomes, and the proportion of GDM identified by FPG. The pre-analytical processing and measurement of glucose is critical for accuracy in GDM diagnosis. Differences in sampling and analytical procedures across studies (one study used point-of-care whole blood glucose and two studies used serum) is another weakness of the current study, potentially affecting the uniformity of GDM diagnosis. Despite high precision for glucose measurement in all studies (small CV's), we cannot rule out that minor bias may have been introduced. Furthermore, the 1-hour glucose was not measured in any of the four cohorts. In the HAPO study population, 5.7% additional GDMs were identified by the 1-hour values when using the ^{2013}WHO criteria (40), and a higher prevalence of GDM in our study could be expected if such data were available. Finally, very few women were diagnosed based on 2-hour glucose alone (16-18% of GDM cases) which makes analysis of women in this category difficult due to power limitations. Importantly, the proposed approach may not circumvent as many OGTTs in other populations as indicated by our study and such differences may need to be considered when extrapolating our results to other settings (i.e. to more high-risk populations). If implemented, this screening procedure should be followed by careful assessment of any potential increase in unwanted pregnancy outcomes. Further studies are needed to compare current risk-factor based screening strategies with a "rule-in, rule-out" procedure with focus on birth outcomes and cost-effectiveness.

Conclusion

Our study suggests that a two-step approach to GDM screening, with an initial universal FPG and exclusion of low-risk women from further testing, could potentially limit the use of OGTT to less than 30% of all pregnancies. A FPG threshold of 4.7 mmol/l appears to identify women at low risk of both elevated 2-hour glucose and GDM-associated adverse pregnancy outcomes. Additional studies are needed to validate our findings and confirm the safety of this screening approach, including long-term health outcomes, especially in populations where a higher proportion of women are diagnosed with GDM from post-load values.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The datasets analyzed during the current study are not publicly available due to the dataset containing clinical data which cannot be shared publicly, and as the study is part of a PhD work. The data are available from the corresponding author on reasonable request. Requests to access these datasets should be directed to Line Sletner, line.sletner@medisin.uio.no.

Ethics statement

The studies involving humans were approved by Norwegian Regional Ethics Committee South East. 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.

Author contributions

AR: Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. LS: Supervision, Data curation, Writing – review & editing. AJ: Conceptualization, Investigation, Data curation, Supervision, Writing – review & editing. NØ: Supervision, Data curation, Writing – review & editing. SS: Data curation, Writing – review & editing. EQ: Data curation, Writing – review & editing. AP: Formal analysis. LRS: Supervision, Data curation, Writing – review & editing.

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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.2023.1278523/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Flowchart of included studies and excluded participants from each study.

SUPPLEMENTARY FIGURE 2
ROC curve to assess the performance of fasting plasma glucose to predict elevated 2-hour glucose in women with non-European background **(A)** applying ^{2017}Norwegian criteria **(B)** applying modified ^{2013}WHO criteria AUC, area under the curve; CI, confidence interval; WHO, World Health Organization.

References

1. Hod M, Kapur A, Sacks DA, Hadar E, Agarwal M, Di Renzo GC, et al. The International Federation of Gynecology and Obstetrics (FIGO) Initiative on gestational diabetes mellitus: A pragmatic guide for diagnosis, management, and care. *Int J Gynaecol Obstet.* (2015) 131(Suppl 3):S173–211. doi: 10.1016/S0020-7292(15)30007-2

2. Benhalima K, Mathieu C, Van Assche A, Damm P, Devlieger R, Mahmood T, et al. Survey by the European Board and College of Obstetrics and Gynaecology on screening for gestational diabetes in Europe. *Eur J Obstet Gynecol Reprod Biol* (2016) 201:197–202. doi: 10.1016/j.ejogrb.2016.04.003

3. White SL, Ayman G, Bakhai C, Hillier TA, Magee LA. Screening and diagnosis of gestational diabetes. *BMJ.* (2023) 381:e071920. doi: 10.1136/bmj-2022-071920

4. Catalano PM, Avallone DA, Drago NM, Amini SB. Reproducibility of the oral glucose tolerance test in pregnant women. *Am J Obstet Gynecol.* (1993) 169(4):874–81. doi: 10.1016/0002-9378(93)90019-F

5. Davidson MB. Counterpoint: the oral glucose tolerance test is superfluous. *Diabetes Care* (2002) 25(10):1883–5. doi: 10.2337/diacare.25.10.1883

6. Agarwal MM, Punnose J, Dhatt GS. Gestational diabetes: problems associated with the oral glucose tolerance test. *Diabetes Res Clin Pract* (2004) 63(1):73–4. doi: 10.1016/j.diabres.2003.08.005

7. Hod M, Pretty M, Mahmood T. Joint position statement on universal screening for GDM in Europe by FIGO, EBCOG and EAPM. *Eur J Obstet Gynecol Reprod Biol* (2018) 228:329–30. doi: 10.1016/j.ejogrb.2018.05.037

8. Rai AS, Sletner L, Jenum AK, Øverby NC, Stafne SN, Lekva T, et al. Identifying women with gestational diabetes based on maternal characteristics: an analysis of four Norwegian prospective studies. *BMC Pregnancy Childbirth.* (2021) 21(1):615. doi: 10.1186/s12884-021-04086-9

9. Agarwal MM, Dhatt GS, Shah SM. Gestational diabetes mellitus: simplifying the international association of diabetes and pregnancy diagnostic algorithm using fasting plasma glucose. *Diabetes Care* (2010) 33(9):2018–20. doi: 10.2337/dc10-0572

10. Poomalar GK, Rangaswamy V. A comparison of fasting plasma glucose and glucose challenge test for screening of gestational diabetes mellitus. *J Obstet Gynaecol.* (2013) 33(5):447–50. doi: 10.3109/01443615.2013.771156

11. van Gemert TE, Moses RG, Pape AV, Morris GJ. Gestational diabetes mellitus testing in the COVID-19 pandemic: The problems with simplifying the diagnostic process. *Aust N Z J Obstet Gynaecol.* (2020) 60(5):671–4. doi: 10.1111/ajo.13203

12. Babaniamansour S, Aliniagerdroudbari E, Afrakhteh M, Hosseinpanah F, Farzaneh F, Niroomand M. Can fasting plasma glucose replace oral glucose-tolerance test for diagnosis of gestational diabetes mellitus? *Diabetol Int* (2021) 12 (3):277–85. doi: 10.1007/s13340-020-00484-0

13. Beunen K, Neys A, Van Crombrugge P, Moyson C, Verhaeghe J, Vandeginste S, et al. Fasting plasma glucose level to guide the need for an OGTT to screen for gestational diabetes mellitus. *Acta Diabetol* (2022) 59(3):381–94. doi: 10.1007/s00592-021-01812-9

14. Reyes-Muñoz E, Sandoval-Osuna NL, Reyes-Mayoral C, Ortega-González C, Martínez-Cruz N, Ramírez-Torres MA, et al. Sensitivity of fasting glucose for gestational diabetes mellitus screening in Mexican adolescents based on International Association of Diabetes and Pregnancy Study Groups criteria: a diagnostic accuracy study based on retrospective data analysis. *BMJ Open* (2018) 8(4):e021617. doi: 10.1136/bmjopen-2018-021617

15. Zhu WW, Fan L, Yang HX, Kong LY, Su SP, Wang ZL, et al. Fasting plasma glucose at 24-28 weeks to screen for gestational diabetes mellitus: new evidence from China. *Diabetes Care* (2013) 36(7):2038–40. doi: 10.2337/dc12-2465

16. Saeedi M, Hanson U, Simmons D, Fadl H. Characteristics of different risk factors and fasting plasma glucose for identifying GDM when using IADPSG criteria: a cross-sectional study. *BMC Pregnancy Childbirth.* (2018) 18(1):225. doi: 10.1186/s12884-018-1875-1

17. McIntyre HD, Gibbons KS, Ma RCW, Tam WH, Sacks DA, Lowe J, et al. Testing for gestational diabetes during the COVID-19 pandemic. An evaluation of proposed protocols for the United Kingdom, Canada and Australia. *Diabetes Res Clin Pract* (2020) 167:108353. doi: 10.1016/j.diabres.2020.108353

18. Jenum AK, Sletner L, Voldner N, Vangen S, Mørkrid K, Andersen LF, et al. The STORK Groruddalen research programme: A population-based cohort study of gestational diabetes, physical activity, and obesity in pregnancy in a multiethnic population. Rationale, methods, study population, and participation rates. *Scand J Public Health* (2010) 38(5 Suppl):60–70. doi: 10.1177/1403494810378921

19. Lekva T, Bollerslev J, Godang K, Roland MC, Friis CM, Voldner N, et al. β-cell dysfunction in women with previous gestational diabetes is associated with visceral adipose tissue distribution. *Eur J Endocrinol* (2015) 173(1):63–70. doi: 10.1530/EJE-15-0153

20. Stafne SN, Salvesen K, Romundstad PR, Eggebø TM, Carlsen SM, Mørkved S. Regular exercise during pregnancy to prevent gestational diabetes: a randomized controlled trial. *Obstet Gynecol.* (2012) 119(1):29–36. doi: 10.1097/AOG.0b013e3182393f86

21. Sagedal LR, Øverby NC, Bere E, Torstveit MK, Lohne-Seiler H, Småstuen M, et al. Lifestyle intervention to limit gestational weight gain: the Norwegian Fit for Delivery randomised controlled trial. *BJOG.* (2017) 124(1):97–109. doi: 10.1111/1471-0528.13862

22. Jenum AK, Mørkrid K, Sletner L, Vangen S, Torper JL, Nakstad B, et al. Impact of ethnicity on gestational diabetes identified with the WHO and the modified International Association of Diabetes and Pregnancy Study Groups criteria: a population-based cohort study. *Eur J Endocrinol* (2012) 166(2):317–24. doi: 10.1530/EJE-11-0866

23. Odsæter IH, Åsberg A, Vanky E, Mørkved S, Stafne SN, Salvesen KÅ, et al. Hemoglobin A1c as screening for gestational diabetes mellitus in Nordic Caucasian women. *Diabetol Metab Syndr* (2016) 8(1):43. doi: 10.1186/s13098-016-0168-y

24. Sagedal LR, Vistad I, Øverby NC, Bere E, Torstveit MK, Lohne-Seiler H, et al. The effect of a prenatal lifestyle intervention on glucose metabolism: results of the Norwegian Fit for Delivery randomized controlled trial. *BMC Pregnancy Childbirth.* (2017) 17(1):167. doi: 10.1186/s12884-017-1340-6

25. *Norsk gynekologisk forening. Veilder i fødselshjelp* (2014). Available at: https://www.legeforeningen.no/foreningsledd/fagmed/norsk-gynekologisk-forening/veiledere/arkiv-utgatte-veiledere/veileder-i-fodselshjelp-2014/8-b.-svangerskapsdiabetes-ny/.

26. Skjaerven R, Gjessing HK, Bakketeig LS. Birthweight by gestational age in Norway. *Acta Obstet Gynecol Scand* (2000) 79(6):440–9. doi: 10.1034/j.1600-0412.2000.079006440.x

27. Power M, Fell G, Wright M. Principles for high-quality, high-value testing. *Evid Based Med* (2013) 18(1):5–10. doi: 10.1136/eb-2012-100645

28. Agarwal MM, Punnose J, Sukhija K, Sharma A, Choudhary NK. Gestational diabetes mellitus: using the fasting plasma glucose level to simplify the international association of diabetes and pregnancy study groups diagnostic algorithm in an adult south asian population. *Can J Diabetes* (2018) 42(5):500–4. doi: 10.1016/j.jcjd.2017.12.009

29. Agarwal MM. Gestational diabetes mellitus: Screening with fasting plasma glucose. *World J Diabetes.* (2016) 7(14):279–89. doi: 10.4239/wjd.v7.i14.279

30. Agarwal MM, Dhatt GS, Punnose J. Gestational diabetes: utility of fasting plasma glucose as a screening test depends on the diagnostic criteria. *Diabetes Med* (2006) 23 (12):1319–26. doi: 10.1111/j.1464-5491.2006.01987.x

31. d'Emden M, McLeod D, Ungerer J, Appleton C, Kanowski D. Development of a fasting blood glucose-based strategy to diagnose women with gestational diabetes mellitus at increased risk of adverse outcomes in a COVID-19 environment. *PloS One* (2020) 15(12):e0243192. doi: 10.1371/journal.pone.0243192

32. Landon MB, Spong CY, Thom E, Carpenter MW, Ramin SM, Casey B, et al. A multicenter, randomized trial of treatment for mild gestational diabetes. *N Engl J Med* (2009) 361(14):1339–48. doi: 10.1056/NEJMoa0902430

33. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *N Engl J Med* (2005) 352(24):2477–86. doi: 10.1056/NEJMoa042973

34. Committee ADAPP. 15. Management of diabetes in pregnancy: standards of medical care in diabetes-2022. *Diabetes Care* (2022) 45(Suppl 1):S232–s43. doi: 10.2337/dc22-S015

35. Sacks DA, Hadden DR, Maresh M, Deerochanawong C, Dyer AR, Metzger BE, et al. Frequency of gestational diabetes mellitus at collaborating centers based on IADPSG consensus panel–recommended criteria: the hyperglycemia and adverse pregnancy outcome (HAPO) study. *Diabetes Care* (2012) 35(3):526–8. doi: 10.2337/dc11-1641

36. Wong VW. Gestational diabetes mellitus in five ethnic groups: a comparison of their clinical characteristics. *Diabetes Med* (2012) 29(3):366–71. doi: 10.1111/j.1464-5491.2011.03439.x

37. McIntyre HD, Moses RG. The diagnosis and management of gestational diabetes mellitus in the context of the COVID-19 pandemic. *Diabetes Care* (2020) 43(7):1433–4. doi: 10.2337/dci20-0026

38. Simmons D, Immanuel J, Hague WM, Teede H, Nolan CJ, Peek MJ, et al. Treatment of gestational diabetes mellitus diagnosed early in pregnancy. *N Engl J Med* (2023) 388(23):2132–44. doi: 10.1056/NEJMoa2214956

39. *Mors kroppsmasseindeks før svangerskap* (2007-2013). Available at: http://statistikkbank.fhi.no/mfr/.

40. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care* (2010) 33 (3):676–82. doi: 10.2337/dc10-0719

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Assessment of potential risk factors associated with gestational diabetes mellitus: evidence from a Mendelian randomization study

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Background: Previous research on the association between risk factors and gestational diabetes mellitus (GDM) primarily comprises observational studies with inconclusive results. The objective of this study is to investigate the causal relationship between 108 traits and GDM by employing a two-sample Mendelian randomization (MR) analysis to identify potential risk factors of GDM.

Methods: We conducted MR analyses to explore the relationships between traits and GDM. The genome-wide association studies (GWAS) for traits were primarily based on data from the UK Biobank (UKBB), while the GWAS for GDM utilized data from FinnGen. We employed a false discovery rate (FDR) of 5% to account for multiple comparisons.

Results: The inverse-variance weighted (IVW) method indicated that the genetically predicted 24 risk factors were significantly associated with GDM, such as "Forced expiratory volume in 1-second (FEV1)" (OR=0.76; 95% CI: 0.63, 0.92), "Forced vital capacity (FVC)" (OR=0.74; 95% CI: 0.64, 0.87), "Usual walking pace" (OR=0.19; 95% CI: 0.09, 0.39), "Sex hormone-binding globulin (SHBG)" (OR=0.86; 95% CI: 0.78, 0.94). The sensitivity analyses with MR-Egger and weighted median methods indicated consistent results for most of the trats.

Conclusion: Our study has uncovered a significant causal relationship between 24 risk factors and GDM. These results offer a new theoretical foundation for preventing or mitigating the risks associated with GDM.

KEYWORDS

risk factor, gestational diabetes mellitus, Mendelian randomization, UK biobank, FinnGen

Introduction

Gestational diabetes mellitus (GDM) is the occurrence of hyperglycemia of varying severity due to impaired glucose tolerance, which is first diagnosed during pregnancy (1, 2). According to the International Diabetes Federation, it is estimated that GDM will affect one out of every six live newborns worldwide in 2019 (3). GDM significantly impacts both maternal and fetal health, as indicated by previous studies (4). Furthermore, GDM not only worsens short-term adverse outcomes during pregnancy (5–7) but also increases the long-term likelihood of developing type 2 diabetes mellitus (T2DM) among women (8, 9), which has been linked to various complications (10–16).

Observational research has identified multiple associations between various risk factors and GDM (17–19). However, these investigations are susceptible to confounding variables that may influence their findings. Additionally, the casual association proposed by these observational studies may lack statistical validity due to inconsistent study designs, conflicting findings, and substantial variability across different settings. Consequently, there is inadequate evidence available within these associations to establish a direct causal link between risk factors and GDM.

In order to address the aforementioned challenges, we employed Mendelian randomization (MR) as a method to mitigate biased estimation and reverse causation in the relationship between traits and GDM. In MR analysis, single nucleotide polymorphisms (SNPs), a type of genetic variation, are utilized as instrumental variables (IVs). Statistical techniques are utilized in this approach to evaluate the presence of a causal association between exposures and outcomes. Genetic variants serve as suitable IVs due to their random distribution during meiosis. Consequently, they exhibit reduced susceptibility to confounding influences. Hence, if these genetic variants are randomly distributed within a population, the observed causal relationships between exposures and outcomes are not likely due to potential confounders such as environmental risks, lifestyle choices, or socioeconomic status (20). Thus, MR design is employed in this study to systematically investigate the causal associations between 108 traits and GDM to identify the potential risk factors of GDM.

Methods

Study design

Utilizing datasets obtained from genome-wide association studies (GWAS), we identified significant SNPs associated with 108 traits as exposure variables. These SNPs were employed as IV, and a MR analysis was conducted to evaluate the causal relationship between the 108 traits and GDM.

Data sources

To adhere to the principles of a two-sample MR design, we sourced exposure and outcome data from distinct European populations as previously described (21–23). We extracted minimally adjusted GWAS summary statistics for our variables of interest from the largest available sample. This dataset included individuals of both sexes and European or mixed ancestry. Our selection process for summary statistics of 108 exposure variables followed a previously described procedure outlined in Supplementary Figure 1 (24). While most exposure GWAS studies utilized data from the UKBB (detailed information can be found in Supplementary Table 1), the dataset for GDM, as outcome, relied on information sourced from FinnGen, a significant biomedical research initiative based in Finland.

Selection of IVs

IVs were selected for the MR analysis based on specific criteria. The criteria included a significant genetic relationship between IVs and exposure, with a P-value < 5×10^{-8}. Independent IVs were identified by performing clumping within a 10 Mb window and considering linkage disequilibrium (LD) with an R^2 value below 0.001. Furthermore, following previous studies, only IVs with a minor allele frequency (MAF) greater than 0.01 were considered in our analysis. Palindromic SNPs were excluded from the analysis due to their intermediate allele frequencies (25). F-statistics were computed to assess the strength of the IVs; values exceeding 10 indicated reduced likelihood of weak instrument bias (refer to Supplementary Table 2) (26).

MR analysis and sensitivity analysis

The main technique employed in the MR analysis was the IVW method. Furthermore, both the weighted median technique and MR-Egger approaches were utilized. The MR-Egger intercept test was employed to evaluate the existence of horizontal pleiotropy. To address potential outliers, pleiotropy-corrected data from MR-PRESSO were incorporated. The degree of heterogeneity was examined using the Cochrane Q value. We conducted a leave-one-out sensitivity analysis to evaluate how each IV impacted causal relationships and ensure robustness of findings. The calculation of causal effects involved the use of odds ratios (ORs) along with their corresponding 95% confidence intervals (CIs). Multiple comparisons were conducted using a false discovery rate (FDR) of 5%. All MR analyses in R were conducted using the TwoSampleMR package.

Results

Out of a pool of 108 variables, SNPs were selected as IVs for potential risk factors according to predetermined inclusion and

Abbreviations: GDM, Gestational Diabetes Mellitus; MR, Mendelian randomization; IVs, instrumental variables; GWAS, genome-wide association study; UKBB, UK Biobank; IVW, inverse-variance weighted; FDR, false discovery rate; FEV1, Forced expiratory volume in 1-second; FVC, Forced vital capacity; SHBG, Sex hormone-binding globulin; HDL, High-density lipoprotein; OR, odds ratio; CI, confidence interval; T2DM, type 2 diabetes; SNPs, single nucleotide polymorphisms; LD, linkage disequilibrium; MAF, minor allele frequency; IGF-1, Insulin-like growth factor 1.

exclusion criteria. The findings were interpreted based on FDR-adjusted threshold. Using the IVW technique in MR analysis, we found significant relationships of 24 genetically predicted risk factors, such as "Apoliprotein A" (OR= 0.83; 95% CI: 0.76, 0.91), "Forced expiratory volume in 1-second (FEV1)" (OR=0.76; 95% CI: 0.63, 0.92), "Insulin-like growth factor 1 (IGF-1)" (OR=1.16; 95% CI: 1.08, 1.26) and "Usual walking pace" (OR=0.19; 95% CI: 0.09, 0.39), with GDM (Figures 1, 2, Supplementary Table 3). The F-statistics for the IVs of the 24 risk factors ranged from 28.62 to 9445.10, indicating good instrument strength (Supplementary Table 2). Except for triglycerides, we found that 23 risk factors consistently showed a significant association with GDM in the same direction when analyzed using both MR-Egger and weighted median techniques (Supplementary Table 3). The scatter plot in Figure 3 illustrated the causal relationships between all the 24 traits and GDM. The possible heterogeneity was also examined (Figure 4, Supplementary Table 4). Horizontal pleiotropy was estimated in our causality assessment based on analysis using MR-Egger technique as shown in Supplementary Table 5, and MR-PRESSO analyses indicated consistent findings after removing outlier IVs (Supplementary Table 6). The leave-one-out analysis demonstrated that no single SNP was solely responsible for the observed outcomes, as shown in Supplementary Figure 2.

Discussion

This study employed a two-sample MR analysis to investigate the causal relationship between traits and GDM. The analysis incorporated GWAS summary statistics from public databases. The findings indicated significant causal associations between 24 risk factors and the risk of GDM. These risk factors will be discussed in detail across four subsequent paragraphs based on their respective categories.

Body size measures and body composition by impedance analysis

Body size measurements and body composition evaluated using impedance analysis are important indicators for assessing obesity. Overweight or obese women have up to a four-fold increased risk of

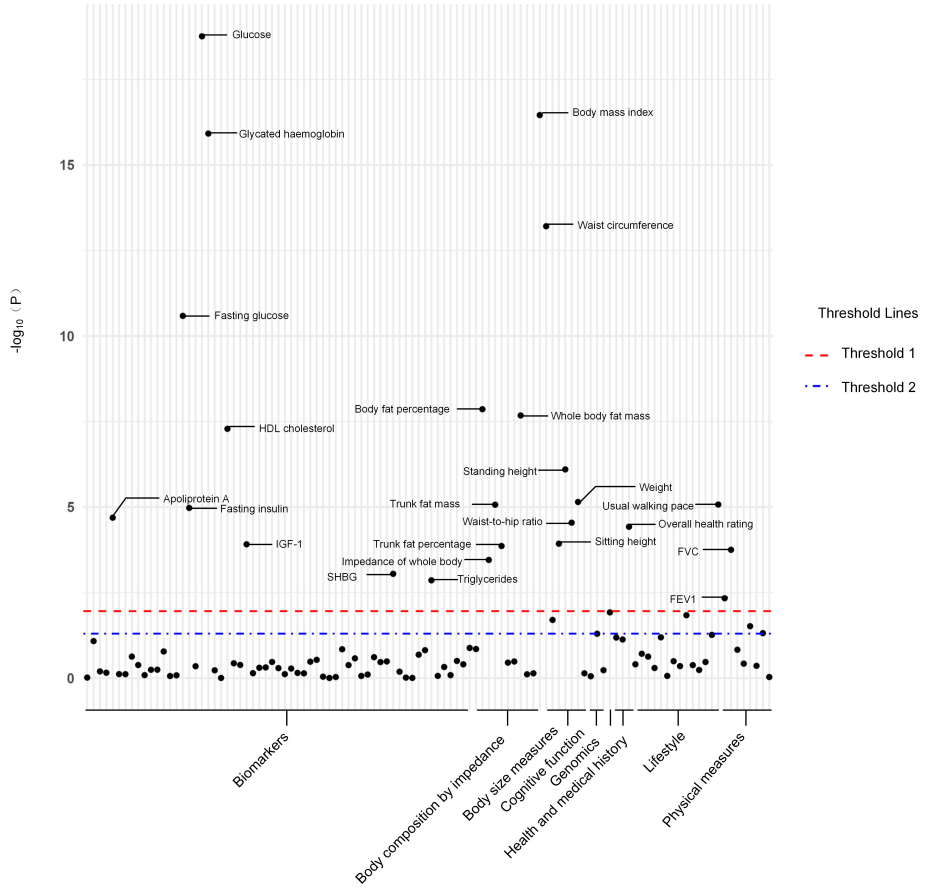

FIGURE 1
The *P*-value distribution of associations between 24 risk factors and GDM in the Mendelian randomization analysis. The red dashed line indicates the significance threshold adjusted by false discovery rate. The blue dash-dotted line indicates the suggestive significance threshold, set at $P = 0.05$. FVC, Forced vital capacity; FEV1, Forced expiratory volume in 1-second; HDL, High-density lipoprotein; IGF-1, Insulin-like growth factor 1; SHBG, Sex hormone-binding globulin.

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FIGURE 2
Associations between genetically predicted 24 risk factors and GDM examined by IVW methods. GDM, Gestational Diabetes Mellitus; IVW, inverse-variance weighted; FVC, Forced vital capacity; FEV1, Forced expiratory volume in 1-second; HDL, High-density lipoprotein; IGF-1, Insulin-like growth factor 1; SHBG, Sex hormone-binding globulin; OR, odds ratio; CI, confidence interval.

developing GDM (27). We found a significant causal relationship between GDM and various body size measurements such as body mass index (BMI), weight, waist-to-hip ratio (WHR), and waist circumference (WC) in our study. Additionally, we observed correlations among several measures of body composition determined through impedance analysis including trunk fat mass, trunk fat percentage, whole-body fat mass, and body-fat percentage. Previous studies have demonstrated a strong association between gestational weight gain and both gestational impaired glucose tolerance and GDM (28, 29). Obesity and being overweight are significant risk factors for acquiring GDM (4). A recent study revealed that obesity and visceral adiposity are correlated with an elevated risk of developing GDM. Furthermore, it highlighted that among these factors, visceral adiposity specifically poses a higher risk for GDM (17). BMI, as a measure of general obesity, has been reported to show an association with the prevalence of GDM. Specifically, there is evidence suggesting that every 1 kg/m^2 increase in pre-pregnancy BMI leads to a rise in GDM prevalence by 0.92% (30). Central obesity refers to an excessive accumulation of abdominal fat which can be assessed using markers such as WHR and WC measurements (31). Previous literature has demonstrated an association between maternal central obesity in the first trimester of pregnancy and a higher occurrence of GDM (31, 32). The presence of visceral adipose tissue can be easily explained as it directly contributes to the pathogenesis of hyperglycemia. It does so by secreting various substances such as thrombogenic agents, inflammatory compounds, and inhibitors of adiponectin. These substances negatively impact glucose metabolism, increase insulin resistance and facilitate the development of metabolic syndrome along with subsequent cardiovascular diseases (33, 34).

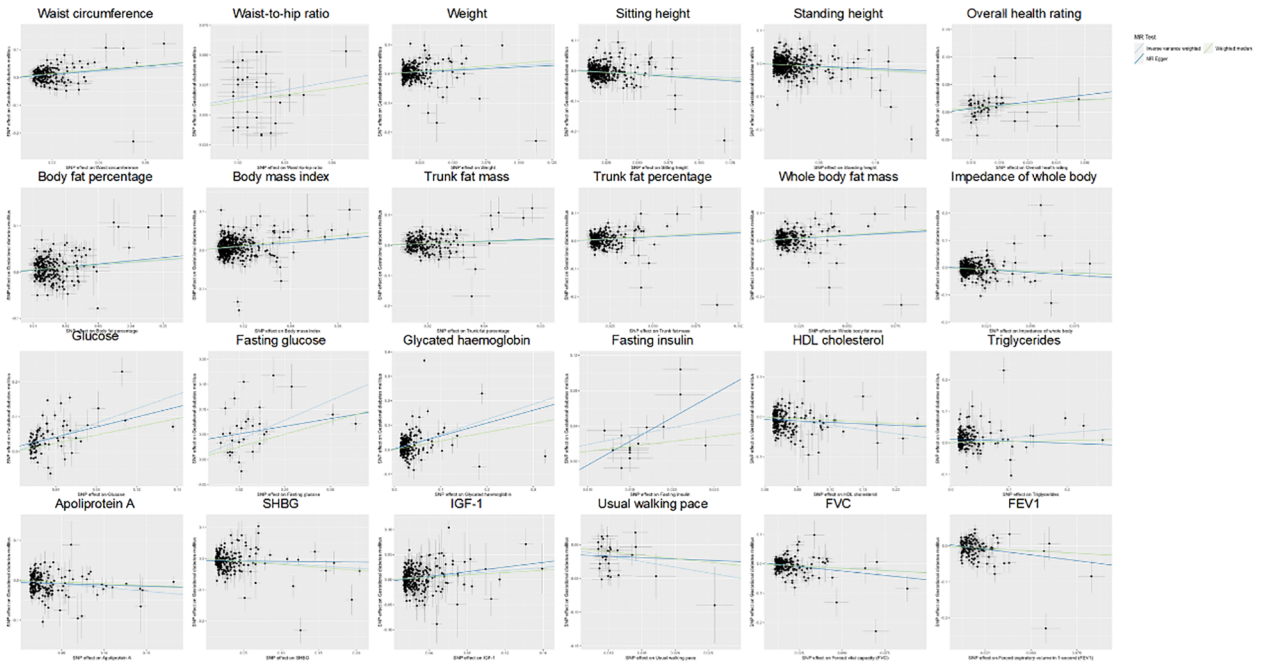

FIGURE 3
Scatter plot showing the causal effects of 24 risk factors on GDM. SNP, single nucleotide polymorphism; GDM, Gestational Diabetes Mellitus; HDL, High-density lipoprotein; SHBG, Sex hormone-binding globulin; IGF-1, Insulin-like growth factor 1; FVC, Forced vital capacity; FEV1, Forced expiratory volume in 1-second.

Biomarkers

A meta-analysis revealed a significant association between elevated fasting glucose levels and a nearly two-fold increase in the risk of developing GDM (27). Moreover, a recent study has established an association between elevated fasting glucose during the initial stages of pregnancy and the subsequent onset of GDM (35). Enquobahrie et al. reported that for every increase in triglyceride content by 20 mg/dL, there is a 10% higher likelihood of developing GDM (36). Hypertriglyceridemia increases the risk for macrosomia due to factors such as insulin resistance caused by elevated triglycerides along with reduced lipoprotein lipase function. Macrosomia results in excessive fetal growth, obesity, as well as accumulation and release of fatty acids in cord blood and fetal adipose tissue (37). Previous research demonstrates an inverse correlation between serum HDL-c concentration and the risk of GDM and macrosomia. Additionally, even a slight increase in HDL-c levels serves as a protective factor against these conditions (38). Apolipoprotein A-1 is the primary lipoprotein associated with HDL-c. In contradiction to our results, a previous cohort study reported no association between serum Apolipoprotein A-1 levels, insulin resistance, or the risk of GDM in human subjects (39). However, since this study was observational, it cannot fully eliminate potential confounding variables as contributors to this discrepancy. Sex hormone binding globulin (SHBG), derived from the liver, is expressed in the placenta and acts as a regulator of sex steroid hormones. SHBG levels in the first trimester of pregnancy have been identified as reliable biomarkers for GDM (40, 41). There exists a negative correlation between SHBG and T2DM (42). Several previous studies have not only identified HbA1c as a diagnostic tool for GDM (43–45) but have also established a relationship between an HbA1c level above 7% in early pregnancy and adverse maternal outcomes (45). Fetal IGF-1 plays a crucial role in fetal growth due to its mitogenic and metabolic properties (46). According to Schwartz et al, an increase in IGF-1 concentration within umbilical cord blood contributes to accelerated intrauterine fetal growth (47).

Physical measures

Forced vital capacity (FVC) and FEV1 are commonly used indicators of lung function (48, 49). Consistent with previous research, our study identified a significant inverse causal association between FVC, FEV1, and GDM. A prior study has reported a significant association between restrictive ventilatory dysfunction assessed through FVC and FEV1 measurements with an elevated risk of T2DM, whereas no such relationship was observed for obstructive ventilatory dysfunction evaluated using the FEV1/FVC ratio (50). Emerging evidence indicates that inflammatory markers such as C-reactive protein and interleukin-6 might contribute to the association between T2DM and decreased FEV1 and FVC (51).

Lifestyle

Our study identified a negative causal relationship between usual walking pace and GDM, consistent with prior research in this

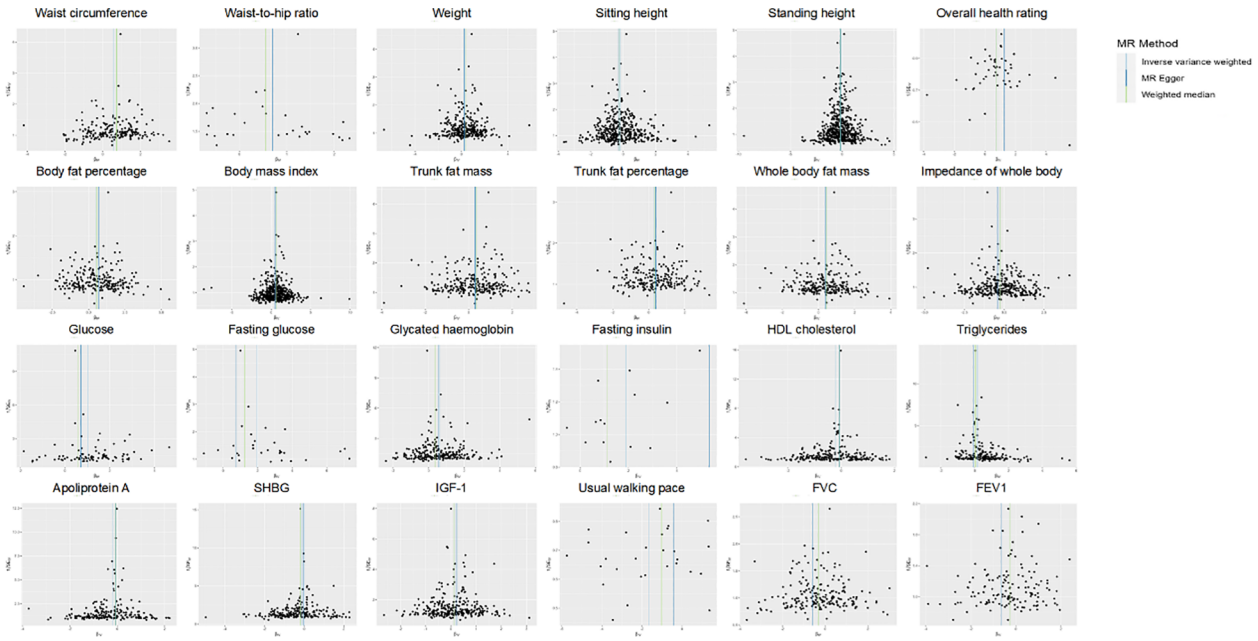

FIGURE 4
Funnel plot indicating the causal associations of 24 risk factors on GDM. SNP, single nucleotide polymorphism; GDM, Gestational Diabetes Mellitus; HDL, High-density lipoprotein; SHBG, Sex hormone-binding globulin; IGF-1, Insulin-like growth factor 1; FVC, Forced vital capacity; FEV1, Forced expiratory volume in 1-second; IV, instrumental variable; SE, standard error.

field. Previous studies have suggested a robust correlation between the usual walking pace and a decreased likelihood of developing GDM (52). Furthermore, these studies revealed that women reporting faster speeds or longer durations during regular walks exhibited reduced risks of developing GDM when compared to those with slower speeds and shorter durations (52). Exercise leads to a significant increase in muscle glucose uptake. Exercise can increase muscle glucose uptake by up to 100 times when compared to resting conditions in humans (53). Increased glucose supply to the contracting skeletal muscles is made possible by the increase in blood flow and capillary recruitment during exercise (54).

Strengths and limitations

The current study possesses three significant strengths. Firstly, previous observational studies have suggested an increased risk of GDM onset in association with long-term maternal residence in an environment characterized by a mixture of PM2.5, PM10, NO2, and PM2.5 chemical constituents (55). However, the MR method can help mitigate the influence of confounding factors on the results. This method involves selecting a SNP that is strongly associated with the exposure of interest as the IV. By utilizing this SNP, individuals can be randomly assigned to the exposure, ensuring comparability of the population in terms of both known and unknown confounders. Secondly, we conducted a comprehensive investigation of the causal relationship between 108 traits and GDM. Thirdly, we employed a variety of sensitivity analyses to verify the results of our main analyses using IVW method. Lastly, during our assessment of pleiotropy within the MR analysis framework, we utilized MR-PRESSO method to provide outlier-corrected results.

However, our study has several limitations. Firstly, the scope of our investigation was limited to people of European descent. Consequently, the generalizability is impacted by this restriction. Further studies on diverse population groups are still needed in future research. Secondly, there might be potential selection bias affecting our results as individuals who died due to competition risk related outcomes might be missed in the GWAS analysis. Thirdly, due to the utilization of GWAS summary data, we were unable to stratify the data by variables such as age and gender, potentially introducing population bias. Finally, we could not assess any potential non-linear association between risk factors and GDM. Future research utilizing extensive biobanks may offer insights into the complex relationship between traits and GDM.

Conclusion

The present study has established a substantial and causative association between multiple risk factors and GDM. The MR analysis revealed statistically significant inverse associations of usual walking pace, FEV1 and FVC with GDM risk. This finding introduces novel insights that can guide future strategies aimed at preventing or mitigating the risks associated with GDM.

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 contributions

QF: Writing – original draft. RC: Writing – original draft. SX: Writing – original draft. YD: Writing – original draft. CH: Writing – original draft. BH: Writing – original draft. TJ: Writing – original draft. BZ: Writing – original draft. MB: Writing – review & editing. SL: Writing – review & editing.

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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.2023.1276836/full#supplementary-material

References

1. Greco E, Calanducci M, Nicolaides KH, Barry EVH, Huda MSB, Iliodromiti S. Gestational diabetes mellitus and adverse maternal and perinatal outcomes in twin and singleton pregnancies: a systematic review and meta-analysis. *Am J Obstetrics Gynecology* (2023) S0002-9378(23):00545–8. doi: 10.1016/j.ajog.2023.08.011

2. Liang X, Zhang J, Wang Y, Wu Y, Liu H, Feng W, et al. Comparative study of microvascular structural changes in the gestational diabetic placenta. *Diabetes Vasc Dis Res* (2023) 20(3):14791641231173627. doi: 10.1177/14791641231173627

3. Magliano DJ, Boyko EJI.D.F.D.A.t.e.s. committee. IDF Diabetes Atlas. In: *Idf diabetes atlas*. Brussels: International Diabetes Federation© International Diabetes Federation (2021).

4. Zhou T, Du S, Sun D, Li X, Heianza Y, Hu G, et al. Prevalence and trends in gestational diabetes mellitus among women in the United States, 2006-2017: A population-based study. *Front In Endocrinol* (2022) 13:868094. doi: 10.3389/fendo.2022.868094

5. Billionnet C, Mitanchez D, Weill A, Nizard J, Alla F, Hartemann A, et al. Gestational diabetes and adverse perinatal outcomes from 716,152 births in France in 2012. *Diabetologia* (2017) 60(4):636–44. doi: 10.1007/s00125-017-4206-6

6. Farrar D, Simmonds M, Bryant M, Sheldon TA, Tuffnell D, Golder S, et al. Hyperglycaemia and risk of adverse perinatal outcomes: systematic review and meta-analysis. *BMJ (Clinical Res ed.)* (2016) 354:i4694. doi: 10.1136/bmj.i4694

7. Reece EA, Leguizamón G, Wiznitzer A. Gestational diabetes: the need for a common ground. *Lancet (London England)* (2009) 373(9677):1789–97. doi: 10.1016/S0140-6736(09)60515-8

8. Vounzoulaki E, Khunti K, Abner SC, Tan BK, Davies MJ, Gillies CL. Progression to type 2 diabetes in women with a known history of gestational diabetes: systematic review and meta-analysis. *BMJ (Clinical Res ed.)* (2020) 369:m1361. doi: 0.1136/bmj.m1361

9. Yu Y, Arah OA, Liew Z, Cnattingius S, Olsen J, Sørensen HT, et al. Maternal diabetes during pregnancy and early onset of cardiovascular disease in offspring: population based cohort study with 40 years of follow-up. *BMJ (Clinical Res ed.)* (2019) 367:l6398. doi: 10.1136/bmj.l6398

10. Su M, Hu R, Tang T, Tang W, Huang C. Review of the correlation between Chinese medicine and intestinal microbiota on the efficacy of diabetes mellitus. *Front Endocrinol (Lausanne)* (2022) 13:1085092. doi: 10.3389/fendo.2022.1085092

11. Xu Z, Zhang P, Chen Y, Jiang J, Zhou Z, Zhu H. Comparing SARC-CalF with SARC-F for screening sarcopenia in adults with type 2 diabetes mellitus. *Front Nutr* (2022) 9:803924. doi: 10.3389/fnut.2022.803924

12. Chen J, Li X, Liu H, Zhong D, Yin K, Li Y, et al. Bone marrow stromal cell-derived exosomal circular RNA improves diabetic foot ulcer wound healing by activating the nuclear factor erythroid 2-related factor 2 pathway and inhibiting ferroptosis. *Diabetes Med* (2023) 40(7):e15031. doi: 10.1111/dme.15031

13. Luo M, Cao Q, Wang D, Tan R, Shi Y, Chen J, et al. The impact of diabetes on postoperative outcomes following spine surgery: A meta-analysis of 40 cohort studies with 2.9 million participants. *Int J Surg* (2022) 104:106789. doi: 10.1016/j.ijsu.2022.106789

14. Mi W, Xia Y, Bian Y. Meta-analysis of the association between aldose reductase gene (CA)n microsatellite variants and risk of diabetic retinopathy. *Exp Ther Med* (2019) 18(6):4499–509. doi: 10.3892/etm.2019.8086

15. Yu T, Xu B, Bao M, Gao Y, Zhang Q, Zhang X, et al. Identification of potential biomarkers and pathways associated with carotid atherosclerotic plaques in type 2 diabetes mellitus: A transcriptomics study. *Front Endocrinol (Lausanne)* (2022) 13:981100. doi: 10.3389/fendo.2022.981100

16. Yang YY, Shi LX, Li JH, Yao LY, Xiang DX. Piperazine ferulate ameliorates the development of diabetic nephropathy by regulating endothelial nitric oxide synthase. *Mol Med Rep* (2019) 19(3):2245–53. doi: 10.3892/mmr.2019.9875

17. Alwash SM, McIntyre HD, Mamun A. The association of general obesity, central obesity and visceral body fat with the risk of gestational diabetes mellitus: Evidence from a systematic review and meta-analysis. *Obes Res Clin Pract* (2021) 15(5):425–30. doi: 10.1016/j.orcp.2021.07.005

18. Benhalima K, Van Crombrugge P, Moyson C, Verhaeghe J, Vandeginste S, Verlaenen H, et al. Estimating the risk of gestational diabetes mellitus based on the 2013 WHO criteria: a prediction model based on clinical and biochemical variables in early pregnancy. *Acta Diabetologica* (2020) 57(6):661–71. doi: 10.1007/s00592-019-01469-5

19. Luo J, Wang X, Yuan L, Guo L. Association of thyroid disorders with gestational diabetes mellitus: a meta-analysis. *Endocrine* (2021) 73(3):550–60. doi: 10.1007/s12020-021-02712-2

20. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* (2015) 44(2):512–25. doi: 10.1093/ije/dyv080

21. Jiang Y, Chen R, Xu S, Ding Y, Zhang M, Bao M, et al. Assessing causal associations of hyperparathyroidism with blood counts and biochemical indicators: a Mendelian randomization study. *Front Endocrinol (Lausanne)* (2023) 14. doi: 10.3389/fendo.2023.1295040

22. Chen R, Xu S, Ding Y, Li L, Huang C, Bao M, et al. Dissecting causal associations of type 2 diabetes with 111 types of ocular conditions: a Mendelian randomization study. *Front Endocrinol* (2023) 14. doi: 10.3389/fendo.2023.1307468

23. Fu Q, Chen R, Ding Y, Xu S, Huang C, He B, et al. Sodium intake and the risk of various types of cardiovascular diseases: A Mendelian randomization study. *Front Endocrinol (Lausanne)* (2023). doi: 10.3389/fnut.2023.1250509

24. Walker VM, Vujkovic M, Carter AR, Davies NM, Udler MS, Levin MG, et al. Separating the direct effects of traits on atherosclerotic cardiovascular disease from those mediated by type 2 diabetes. *Diabetologia* (2022) 65(5):790–9. doi: 10.1007/s00125-022-05653-1

25. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-Base platform supports systematic causal inference across the human phenome. *ELife* (2018) 7:e34408. doi: 10.7554/eLife.34408

26. Burgess S, Thompson SG. Avoiding bias from weak instruments in Mendelian randomization studies. *Int J Epidemiol* (2011) 40(3):755–64. doi: 10.1093/ije/dyr036

27. Habibi N, Mousa A, Tay CT, Khomami MB, Patten RK, Andraweera PH, et al. Maternal metabolic factors and the association with gestational diabetes: A systematic review and meta-analysis. *Diabetes Metab Res Rev* (2022) 38(5):e3532. doi: 10.1002/dmrr.3532

28. Hedderson MM, Gunderson EP, Ferrara A. Gestational weight gain and risk of gestational diabetes mellitus. *Obstetrics Gynecology* (2010) 115(3):597–604. doi: 10.1097/AOG.0b013e3181cfce4f

29. Saldana TM, Siega-Riz AM, Adair LS, Suchindran C. The relationship between pregnancy weight gain and glucose tolerance status among black and white women in central North Carolina. *Am J Obstetrics Gynecology* (2006) 195(6):1629–35. doi: 10.1016/j.ajog.2006.05.017

30. Torloni MR, Betrán AP, Horta BL, Nakamura MU, Atallah AN, Moron AF, et al. Prepregnancy BMI and the risk of gestational diabetes: a systematic review of the literature with meta-analysis. *Obes Rev* (2009) 10(2):194–203. doi: 10.1111/j.1467-789X.2008.00541.x

31. Yao D, Chang Q, Wu Q-J, Gao S-Y, Zhao H, Liu Y-S, et al. Relationship between maternal central obesity and the risk of gestational diabetes mellitus: A systematic review and meta-analysis of cohort studies. *J Diabetes Res* (2020) 2020:6303820. doi: 10.1155/2020/6303820

32. Madhavan A, Beena Kumari R, Sanal MG. A pilot study on the usefulness of body mass index and waist hip ratio as a predictive tool for gestational diabetes in Asian Indians. *Gynecological Endocrinol* (2008) 24(12):701–7. doi: 10.1080/09513590802444134

33. Martin AM, Berger H, Nisenbaum R, Lausman AY, MacGarvie S, Crerar C, et al. Abdominal visceral adiposity in the first trimester predicts glucose intolerance in later pregnancy. *Diabetes Care* (2009) 32(7):1308–10. doi: 10.2337/dc09-0290

34. Basraon SK, Mele L, Myatt L, Roberts JM, Hauth JC, Leveno KJ, et al. Relationship of early pregnancy waist-to-hip ratio versus body mass index with gestational diabetes mellitus and insulin resistance. *Am J Perinatology* (2016) 33(1):114–21. doi: 10.1055/s-0035-1562928

35. Hao M, Lin L. Fasting plasma glucose and body mass index during the first trimester of pregnancy as predictors of gestational diabetes mellitus in a Chinese population. *Endocr J* (2017) 64(5):561–9. doi: 10.1507/endocrj.EJ16-0359

36. Enquobahrie DA, Williams MA, Qiu C, Luthy DA. Early pregnancy lipid concentrations and the risk of gestational diabetes mellitus. *Diabetes Res Clin Pract* (2005) 70(2):134–42. doi: 10.1016/j.diabres.2005.03.022

37. Herrera E, Ortega-Senovilla H. Disturbances in lipid metabolism in diabetic pregnancy - Are these the cause of the problem? Best Practice & Research. *Clin Endocrinol Metab* (2010) 24(4):515–25. doi: 10.1016/j.beem.2010.05.006

38. Jin W-Y, Lin S-L, Hou R-L, Chen X-Y, Han T, Jin Y, et al. Associations between maternal lipid profile and pregnancy complications and perinatal outcomes: a population-based study from China. *BMC Pregnancy Childbirth* (2016) 16:60. doi: 10.1186/s12884-016-0852-9

39. Retnakaran R, Ye C, Connelly PW, Hanley AJ, Sermer M, Zinman B. Serum apoA1 (Apolipoprotein A-1), insulin resistance, and the risk of gestational diabetes mellitus in human pregnancy-brief report. *Arteriosclerosis Thrombosis Vasc Biol* (2019) 39(10):2192–7. doi: 10.1161/ATVBAHA.119.313195

40. Maged AM, Moety GAF, Mostafa WA, Hamed DA. Comparative study between different biomarkers for early prediction of gestational diabetes mellitus. *J Maternal-fetal Neonatal Med* (2014) 27(11):1108–12. doi: 10.3109/14767058.2013.850489

41. Smirnakis KV, Plati A, Wolf M, Thadhani R, Ecker JL. Predicting gestational diabetes: choosing the optimal early serum marker. *Am J Obstetrics Gynecology* (2007) 196(4):410.E1–410.E7. doi: 10.1016/j.ajog.2006.12.011

42. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA* (2006) 295(11):1288–99. doi: .1001/jama.295.11.1288

43. Hughes RCE, Moore MP, Gullam JE, Mohamed K, Rowan J. An early pregnancy HbA1c ≥5.9% (41 mmol/mol) is optimal for detecting diabetes and identifies women at increased risk of adverse pregnancy outcomes. *Diabetes Care* (2014) 37(11):2953–9. doi: 10.2337/dc14-1312

44. Renz PB, Cavagnolli G, Weinert LS, Silveiro SP, Camargo JL. HbA1c test as a tool in the diagnosis of gestational diabetes mellitus. *PloS One* (2015) 10(8):e0135989. doi: 10.1371/journal.pone.0135989

45. Xodo S, Londero AP, D'Agostin M, Novak A, Galasso S, Pittini C, et al. Is glycated hemoglobin A1c level associated with adverse pregnancy outcomes of women affected by pre-gestational diabetes? *Medicina (Kaunas)* (2021) 57(5):461. doi: 10.3390/medicina57050461

46. Bloomfield FH, Spiroski A-M, Harding JE. Fetal growth factors and fetal nutrition. *Semin In Fetal Neonatal Med* (2013) 18(3):118–23. doi: 10.1016/j.siny.2013.03.003

47. Schwartz R, Teramo KA. Effects of diabetic pregnancy on the fetus and newborn. *Semin In Perinatology* (2000) 24(2):120–35. doi: 10.1053/sp.2000.6363

48. Hankinson JL, Eschenbacher B, Townsend M, Stocks J, Quanjer PH. Use of forced vital capacity and forced expiratory volume in 1 second quality criteria for determining a valid test. *Eur Respir J* (2015) 45(5):1283–92. doi: 10.1183/09031936.00116814

49. Zhu LM, Zeng D, Lei XC, Huang J, Deng YF, Ji YB, et al. KLF2 regulates neutrophil migration by modulating CXCR1 and CXCR2 in asthma. *Biochim Biophys Acta Mol Basis Dis* (2020) 1866(12):165920. doi: 10.1016/j.bbadis.2020.165920

50. Peng Y, Zhong G-C, Wang L, Guan L, Wang A, Hu K, et al. Chronic obstructive pulmonary disease, lung function and risk of type 2 diabetes: a systematic review and meta-analysis of cohort studies. *BMC Pulmonary Med* (2020) 20(1):137. doi: 10.1186/s12890-020-1178-y

51. Wannamethee SG, Shaper AG, Rumley A, Sattar N, Whincup PH, Thomas MC, et al. Lung function and risk of type 2 diabetes and fatal and nonfatal major coronary heart disease events: possible associations with inflammation. *Diabetes Care* (2010) 33(9):1990–6. doi: 10.2337/dc10-0324

52. Zhang C, Solomon CG, Manson JE, Hu FB. A prospective study of pregravid physical activity and sedentary behaviors in relation to the risk for gestational diabetes mellitus. *Arch Internal Med* (2006) 166(5):543–8. doi: 10.1001/archinte.166.5.543

53. Richter EA. Is GLUT4 translocation the answer to exercise-stimulated muscle glucose uptake? *Am J Physiol Endocrinol Metab* (2021) 320(2):E240–e243. doi: 10.1152/ajpendo.00503.2020

54. Sjøberg KA, Frøsig C, Kjøbsted R, Sylow L, Kleinert M, Betik AC, et al. Exercise increases human skeletal muscle insulin sensitivity via coordinated increases in microvascular perfusion and molecular signaling. *Diabetes* (2017) 66(6):1501–10. doi: 10.2337/db16-1327

55. Sun Y, Li X, Benmarhnia T, Chen JC, Avila C, Sacks DA, et al. Exposure to air pollutant mixture and gestational diabetes mellitus in Southern California: Results from electronic health record data of a large pregnancy cohort. *Environ Int* (2022) 158:106888. doi: 10.1016/j.envint.2021.106888

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The impact of regional origin on the incidence of gestational diabetes mellitus in a multiethnic European cohort

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Introduction: Women with migration background present specific challenges related to risk stratification and care of gestational diabetes mellitus (GDM). Therefore, this study aims to investigate the role of ethnic origin on the risk of developing GDM in a multiethnic European cohort.

Methods: Pregnant women were included at a median gestational age of 12.9 weeks and assigned to the geographical regions of origin: Caucasian Europe ($n = 731$), Middle East and North Africa countries (MENA, $n = 195$), Asia ($n = 127$) and Sub-Saharan Africa (SSA, $n = 48$). At the time of recruitment maternal characteristics, glucometabolic parameters and dietary habits were assessed. An oral glucose tolerance test was performed in mid-gestation for GDM diagnosis.

Results: Mothers with Caucasian ancestry were older and had higher blood pressure and an adverse lipoprotein profile as compared to non-Caucasian mothers, whereas non-Caucasian women (especially those from MENA countries) had a higher BMI and were more insulin resistant. Moreover, we found distinct dietary habits. Non-Caucasian mothers, especially those from MENA and Asian countries, had increased incidence of GDM as compared to the Caucasian population (OR 1.87, 95%CI 1.40 to 2.52, $p < 0.001$). Early gestational fasting glucose and insulin sensitivity were consistent risk factors across different ethnic populations, however, pregestational BMI was of particular importance in Asian mothers.

Discussion: Prevalence of GDM was higher among women from MENA and Asian countries, who already showed adverse glucometabolic profiles at early gestation. Fasting glucose and early gestational insulin resistance (as well as higher BMI in women from Asia) were identified as important risk factors in Caucasian and non-Caucasian patients.

KEYWORDS

gestational diabetes mellitus, ethnicity, risk prediction, glucose levels, migration, risk stratification

1 Introduction

In parallel to the rising rates of metabolic disorders that affect the general and progressively more the reproductive-aged younger population, the prevalence of gestational diabetes mellitus (GDM) has increased over the last decades. Nowadays, the global prevalence of GDM ranges between 12 and 18% of pregnancies, with regional prevalence varying from 7% in North America to 27% in MENA countries (1–3). Thereby, hyperglycemia does not only elevate the risk of adverse outcome for women and offspring during pregnancy and at birth, but also the long-term risk of cardiovascular disease and type 2 diabetes (4).

It is known that the GDM prevalence markedly differs between different ethnic populations and hence, the region of origin has been recognized as a non-negligible risk factor. However, detailed data about each area and its specific role and importance are sparse and sometimes conflicting (4–9). Although Asian populations and women from the Sub-Saharan region have long been recognized as being at high risk of developing the disease, women from other regions are also often affected (3, 10, 11). For example, more recent evidence suggests an increased prevalence of GDM in pregnant women from the Middle East and North Africa countries as compared to other populations (3, 12). Migration has been steadily rising and the countries of origin have greatly diversified over the past years. As a consequence, women with migration background were reported to constitute up to 8–14% of the collective of pregnant women in two Nordic countries (8, 13). In this context, pregnant women with migration background (especially those from other continents) present specific challenges related to detection and care of GDM that need to be addressed (14). However, prospective studies, including information of clinical features, metabolic parameters and dietary habits in multiethnic cohorts are sparsely available.

Therefore, this study aims to investigate and refine the possible role of non-Caucasian ancestry for the development of GDM in a multiethnic European cohort. Moreover, ethnic specific differences in glucometabolic parameters and dietary habits at start of pregnancy as well as possible differences in their contribution for GDM development will be assessed in different ethnic groups.

2 Methods

2.1 Study design and patients

The study design is reported in detail elsewhere (15). In short, pregnant women who participated in this prospective cohort study were recruited among patients attending the pregnancy outpatient clinic at the Department of Obstetrics and Gynaecology, Division of Obstetrics and feto-maternal Medicine, Medical University of Vienna between 2016 and 2019. Patients with preexisting diabetes (such as type 1 or type 2 diabetes) or those with early gestational glycated haemoglobin A1c (HbA1c) equal or exceeding 6.5% at study entry were excluded. Study participants were included at a median gestational age of 12.9 weeks, interquartile range (IQR) 12.3 to 13.6 weeks. An assessment of maternal characteristics (e.g., maternal age, parity, obstetric history, family history of diabetes, as well as women's height and pregestational weight and body mass index (BMI)) were assessed at time of recruitment. Moreover, we collected detailed information about the country of origin of participating women and their parents. Therefore, we defined five geographical regions: Caucasian ($n = 731$), Middle East and North Africa (MENA, $n = 195$), Asia ($n = 127$), Sub Saharan African (SSA, $n = 48$) and Others and assigned each country present in our cohort to one region according to the definition of the NCD Risk Factors Collaboration (NCD-RisC) (16). We only included women whose parents' countries of birth were known and situated within the same region and hence excluded 22 women. In the non-Caucasian cohort, about 88.2% of women were first generation migrants and 11.8% were second generation migrants. Table 1 gives an overview of the number of women included and their regions and countries of origin. Thereafter, participants were followed-up until delivery to assess status of GDM and pregnancy outcomes.

2.2 Metabolic characterization

At time of recruitment, fasting plasma glucose (FPG), insulin and HbA1c as well as triglycerides, total-cholesterol, LDL-cholesterol and HDL-cholesterol were assessed and the homeostasis model assessment of insulin resistance (HOMA-IR) was evaluated (17). Universal Screening of GDM was routinely performed by use of a 75 g 2 h oral glucose tolerance test (OGTT) in the late second or early third trimester. Thereby, the diagnosis of GDM was based on the cut-offs after oral glucose load proposed by the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria (18). The standard laboratory methods at our certified Department of Medical and Chemical Laboratory Diagnostics[1] were used to determine all laboratory parameters in this study. According to the international and local guidelines, glucose measurements during the diagnostic OGTT were assessed by use of venous plasma blood samples at local public laboratories (19). Moreover, a food frequency questionnaire was used at time of inclusion to address dietary habits at the start of pregnancy (20).

2.3 Ethics approval

The Ethics Committee of the Medical University of Vienna approved the study (EK 1937/2015). The study was performed in accordance with the Declaration of Helsinki. All participants gave written informed consent.

2.4 Statistical analysis

Continuous variables were reported as mean ± standard deviation and in case of skewed distributed data as median and interquartile

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus; HbA1c, glycated haemoglobin A1c; HDL-cholesterol, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; IADPSG, International Association of the Diabetes and Pregnancy Study Groups; IQR, interquartile range; LDL-cholesterol, low density lipoprotein cholesterol; LGA, large for gestational age; MENA, Middle East and North Africa; NCD-RisC, Non-Communicable Diseases Risk Factors Collaboration; OGTT, oral glucose tolerance test; SSA, Sub Saharan African.

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TABLE 1 List of countries and study participants included for each region.

Region	Country	*n*	Region	Country	*n*	Region	Country	*n*
Europe	Albania	3	**Middle East and North Africa (MENA)**	Algeria	1	**Africa**	Cameroon	1
	Austria	272		Egypt	16		Congo	4
	Belarus	1		Iran	7		Cote d'Ivoire	1
	Bosnia	51		Iraq	7		Ethiopia	1
	Bulgaria	16		Jordan	1		Gambia	1
	Croatia	19		Lebanon	2		Ghana	1
	Czech Republic	9		Syria	32		Nigeria	13
	Denmark	1		Tunisia	2		Somalia	25
	Estonia	1		Turkey	127		Sudan	1
	Finland	1	**Asia**	Afghanistan	52	**Others**	Bolivia	1
	France	1		Armenia	2		Chile	1
	Germany	20		Azerbaijan	1		Ecuador	1
	Greece	2		Bangladesh	8		El Salvador	1
	Hungary	12		China	8		Haiti	1
	Italy	5		India	12		Peru	3
	Kosovo	28		Georgia	4		USA	1
	Latvia	1		Kazakhstan	2			
	Moldavia	1		Kyrgyzstan	1			
	North Macedonia	15		Mongolia	2			
	Poland	27		Pakistan	8			
	Romania	48		Philippines	17			
	Russia	28		Uzbekistan	5			
	Serbia	131		South/North Korea	1			
	Slovakia	25		Sri Lanka	2			
	Spain	5		Thailand	1			
	Switzerland	1		Vietnam	1			
	Ukraine	4						
	United Kingdom	3						

If both parents were born in different countries in the same region, the country of birth of the mother was considered in this table.

ranges (IQR). These were compared by either Welch's *t*-test (for two samples) and analysis of variance (for more than two samples), or rank based "nonparametric" inference such as the Kruskal Wallis Test, respectively. Categorical variables were summarized by counts and percentages and compared by binary logistic regression, whereby odds ratios and 95% Confidence Intervals (95%CI) were calculated for dichotomous outcomes (such as the development of GDM) by binary logistic regression. Multiple logistic regression was further used to identify a possible effect of non-Caucasian ethnicity on the risk of GDM adjusted for various confounders. Thereby, stepwise variable selection was used to identify the model with the lowest (i.e., best) Akaike's information criterion (AIC). For comparison of more than two groups with one reference group we used Dunnett's *post hoc* test to achieve a 95% coverage probability. We further used recursive partitioning to calculate variable importance metrics as the average difference in predictive accuracy before and after random permutation of the values of a predictor variable over 10^6 random decision trees (21). Statistical analysis was performed with R (version 4.2.2) and contributing packages (especially "multcomp," "nparcomp" and "randomForest" as well as "ggplot2" for visualisations) (22, 23). A two-sided *p*-value of ≤ 0.05 was considered statistically significant. All reported *p*-values were interpreted in an explorative manner aiming to generate new hypotheses.

3 Results

3.1 Characteristics of women with different ethnicity at early pregnancy

Characteristics of Caucasian and non-Caucasian study participants are provided in Table 2. Pregnant women of Caucasian origin were older, more often nulliparous and used assisted reproduction more frequently. Moreover, Caucasian mothers were more likely to smoke (or to be former smokers) and characterized

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TABLE 2 Early gestational characteristics of women with Caucasian and non-Caucasian origin.

	n	CAUC	*n*	NON-CAUC	*p*-value
Age (years)	731	32.0 ± 5.9	379	31.2 ± 5.6	0.022
Parity (≥1)	731	404 (55.3)	379	287 (75.7)	<0.001
Parity (≥2)	731	154 (21.1)	379	162 (42.7)	<0.001
Parity (≥3)	731	46 (6.3)	379	80 (21.1)	<0.001
GDM, previous pregnancy	731	67 (9.2)	379	51 (13.5)	0.029
Family history (1st degree)	731	164 (22.4)	379	134 (35.4)	<0.001
Assisted reproduction	731	93 (12.7)	379	28 (7.4)	0.008
Multiple pregnancy	731	95 (13.0)	379	32 (8.4)	0.025
Smoking status (actual smokers)	731	128 (17.5)	379	27 (7.1)	<0.001
Smoking status (former smokers)	731	232 (31.7)	379	41 (10.8)	<0.001
Smoking status (actual and former smokers)	731	360 (49.2)	379	68 (17.9)	<0.001
Pack years (a)	723	0 (0–4)	376	0 (0–0)	<0.001
Height (cm)	731	166 ± 6.6	379	162 ± 6.4	<0.001
Weight, before pregnancy (kg)	731	68 ± 16	379	67 ± 13	0.188
BMI, before pregnancy (kg/m^2)	731	24.7 ± 5.7	379	25.4 ± 4.9	0.018
RRS (mmHg)	731	120 ± 12	378	117 ± 13	<0.001
RRD (mmHg)	731	77 ± 10	378	75 ± 10	0.010
Triglycerides, early pregnancy (mg/dl)	692	119 ± 47	361	120 ± 47	0.850
Total-cholesterol, early pregnancy (mg/dl)	695	192 ± 35	364	183 ± 34	<0.001
LDL-cholesterol, early pregnancy (mg/dl)	695	96 ± 28	363	92 ± 27	0.008
HDL-cholesterol, early pregnancy (mg/dl)	695	72 ± 16	363	67 ± 15	<0.001
non-HDL-Cholesterol, early pregnancy (mg/dl)	695	120 ± 33	363	115 ± 31	0.026
FPG, early pregnancy (mg/dl)	690	81.2 ± 6.3	362	82.9 ± 7.1	<0.001
HbA1c, early pregnancy (mmol/mol)	700	30.7 ± 3.2	364	31.7 ± 3.5	<0.001
Fasting insulin, early pregnancy (μU/ml)	663	7.7 (5.3–11.6)	350	8.9 (6.3–13.1)	<0.001
HOMA-IR, early pregnancy (dimensionless)	656	1.6 (1.0–2.4)	347	1.8 (1.3–2.7)	<0.001

Data are number of available cases (*n*), mean ± SD or median (IQR) and count (%) for women with Caucasian (CAUC) and non-Caucasian (NON-CAUC) origin. GDM, gestational diabetes mellitus; BMI, body mass index; RRS, systolic blood pressure; RRD, diastolic blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; FPG, fasting plasma glucose; HbA1c, glycated haemoglobin A1c; HOMA-IR, homeostasis model assessment of insulin resistance.

by higher blood pressure and increased total-cholesterol, LDL-cholesterol but also HDL- and non-HDL-cholesterol as compared to non-Caucasian mothers at the beginning of pregnancy. In contrast, non-Caucasian women were more often multiparous, and showed higher BMI as well as a higher degree of insulin resistance associated with modestly higher HbA1c, fasting glucose and insulin levels. A more detailed comparison of early gestational metabolic parameters in pregnant women according to the regional origin is provided in the Supplementary Table S1, showing distinct differences between the investigated subgroups. Women from the MENA as well as those from the SSA region had higher BMI as compared to Caucasian mothers. Women from MENA countries were more insulin resistant with elevated fasting glucose and insulin levels, while women from SSA and Asia showed higher HbA1c but lower total- and non-HDL-cholesterol as compared to the Caucasian population. Distinct differences were also observed in dietary habits (Table 3). Non-Caucasian mothers consumed more rice, couscous or bulgur and legumes but less noodles, potatoes, vegetables, meat and fruits. However, a more detailed analysis of dietary habits according to the regional origin showed that especially women from the MENA regions consumed more carbohydrates such as bread, rice, couscous or bulgur, but were also eating more legumes as compared to Caucasian mothers. A higher rice, bulgur or couscous consumption was also observed in SSA and Asian mothers, whereas they were eating sweets less often.

3.2 Association of ethnicity and the development of GDM

The prevalence of GDM was higher in non-Caucasian mothers as compared to the Caucasian population ($n = 108$, 28.5% vs. $n = 128$, 17.5%, OR 1.87, 95%CI 1.40 to 2.52, $p < 0.001$) and comparable results were observed after adjustment for maternal age and BMI (adjusted OR 1.90, 95%CI 1.41 to 2.58, $p < 0.001$). The results remained unchanged in a fully adjusted logistic regression model including the variables provided in Tables 2, 3 (adjusted OR 2.95, 95%CI 1.32 to 6.60, $p = 0.008$) as well as in a reduced model using stepwise selection (adjusted OR 3.38, 95%CI 1.74 to 6.60, $p < 0.001$). Likewise, non-Caucasian mothers showed higher glucose concentrations within the diagnostic OGTT vs. Caucasian mothers (OGTT glucose baseline: 83 ± 9 vs. 81 ± 10 mg/dL, $p = 0.001$; OGTT glucose 60′: 142 ± 37 vs. 132 ± 33 mg/dL, $p < 0.001$; OGTT glucose

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TABLE 3 Dietary habits at early pregnancy of women with Caucasian and non-Caucasian origin.

	n	CAUC	*n*	NON-CAUC	*p*-value
Milk (ml/d)	627	100 (21–200)	291	43 (9–200)	<0.001
Water (l/d)	627	1.2 (0.9–4.8)	287	1.2 (0.6–3.6)	<0.001
Non-alcoholic beverages (ml/d)	597	200 (61–443)	261	104 (36–300)	<0.001
Coffee (ml/d)	632	32 (0–150)	283	3 (0–32)	<0.001
Tea (ml/d)	618	75 (8.0–182)	278	182 (67–581)	<0.001
Bread (g/d)	603	82 (48–150)	261	100 (50–200)	0.062
Rice, couscous, bulgur (g/d)	616	16 (7–32)	276	32 (13–75)	<0.001
Noodles (g/d)	615	27 (11–27)	275	11 (4–27)	<0.001
Potatoes (g/d)	614	49 (26–84)	259	40 (20–77)	0.003
Pizza (g/d)	617	13 (6–31)	273	13 (3–31)	<0.001
Breakfast cereals (g/d)	614	3 (0–10)	262	0 (0–5)	<0.001
Legumes (g/d)	627	7 (3–16)	276	13 (3–32)	0.005
Vegetables (g/d)	608	88 (34–182)	260	75 (20–150)	0.024
Fruits (g/d)	623	300 (130–450)	272	185 (150–320)	0.133
Butter and magarine (g/d)	626	4 (1–10)	286	1 (0–8)	<0.001
Cheese (g/d)	619	15 (5–30)	281	6 (1–30)	<0.001
Cream Cheese (g/d)	627	3 (1–13)	283	3 (0–15)	0.079
Curd cheese, soured milk, yoghurt (g/d)	617	43 (9–100)	285	43 (9–100)	0.379
Eggs (g/d)	624	13 (5–26)	287	26 (11–60)	<0.001
Meat (g/d)	624	26 (5–26)	283	13 (4–26)	0.013
Meat products (g/d)	616	12 (4–26)	276	0 (0–4)	<0.001
Poultry (g/d)	625	16 (13–32)	284	13 (3–32)	<0.001
Fish (g/d)	612	8 (2–19)	264	3 (0–10)	<0.001
Fast Food (g/d)	614	13 (0–25)	273	10 (0–25)	0.055
Sweat spreads (g/d)	628	3 (1–8)	283	4 (1–10)	0.283
Sweets (g/d)	595	38 (21–72)	257	27 (11–56)	<0.001
Salty snacks (g/d)	613	4 (2–10)	253	4 (2–11)	0.301

Data are number of available cases (*n*), mean ± SD or median (IQR) and count (%) for women with Caucasian (CAUC) and non-Caucasian (NON-CAUC) origin.

120′: 113 ± 28 vs. 106 ± 25 mg/dL, $p < 0.001$) and required glucose lowering medications more often ($n = 61$, 56.5% vs. $n = 53$, 41.4%, $p = 0.022$ for non-Caucasian vs. Caucasian GDM patients, respectively). A detailed analysis of regional origin showed that the increased incidence of GDM was especially observed in MENA (OR 1.95, 95%CI 1.35 to 2.79, $p < 0.001$) and Asian mothers (OR 2.09, 95%CI 1.36 to 3.17, $p < 0.001$). However, no differences were observed in pregnancy outcomes including cesarean section rate ($p = 0.919$), international birth weight percentiles ($p = 0.980$) or the incidence of LGA offspring ($p = 0.871$) and the results remained unchanged when women with normal glucose tolerance were excluded. Likewise, we found no significant difference in LGA delivery in previous pregnancy.

3.3 Risk factors for GDM stratified by ethnic origin

Ethnically stratified variable importance metrics were calculated for 396 Caucasian (64 with GDM) and 114 non-Caucasian women (MENA: 63, 20 with GDM; ASIA: 46, 15 with GDM; SSA: 5, 1 with GDM) with complete information about baseline characteristics (as summarized in Table 2) and dietary habits (as summarized in Table 3). As visualized in Figure 1, fasting glucose as well as HOMA-IR achieved high variable importance scores in both Caucasian and non-Caucasian mothers. Pregestational BMI was a more important predictor in non-Caucasian mothers, whereas history of pregnancy with GDM was more important in Caucasian mothers. In general, dietary habits were of minor importance in both groups. The estimated out of bag error (as a measurement of prediction error of the random forest) was lower in Caucasian patients as compared to non-Caucasian mothers (16.7% vs. 26.1%). In a further analysis non-Caucasian mothers were stratified according to their regional origin and showed that fasting glucose was especially important in women from the MENA regions, whereas maternal pregestational BMI and insulin sensitivity status was more important in Asian mothers (Figure 2). Due to the restricted sample size women from the SSA region were excluded from this analysis.

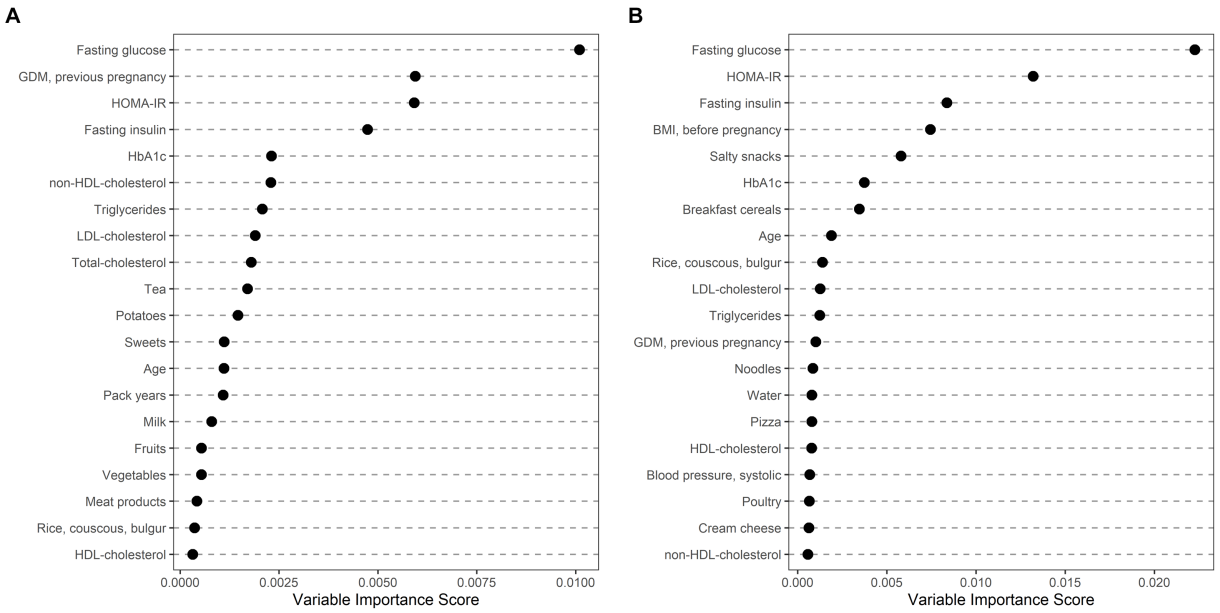

FIGURE 1
Ethnically stratified risk factors for GDM at early pregnancy showing variable importance scores for mothers with Caucasian **(A)** and non-Caucasian **(B)** origin. The first 20 variables with highest variable scores are shown.

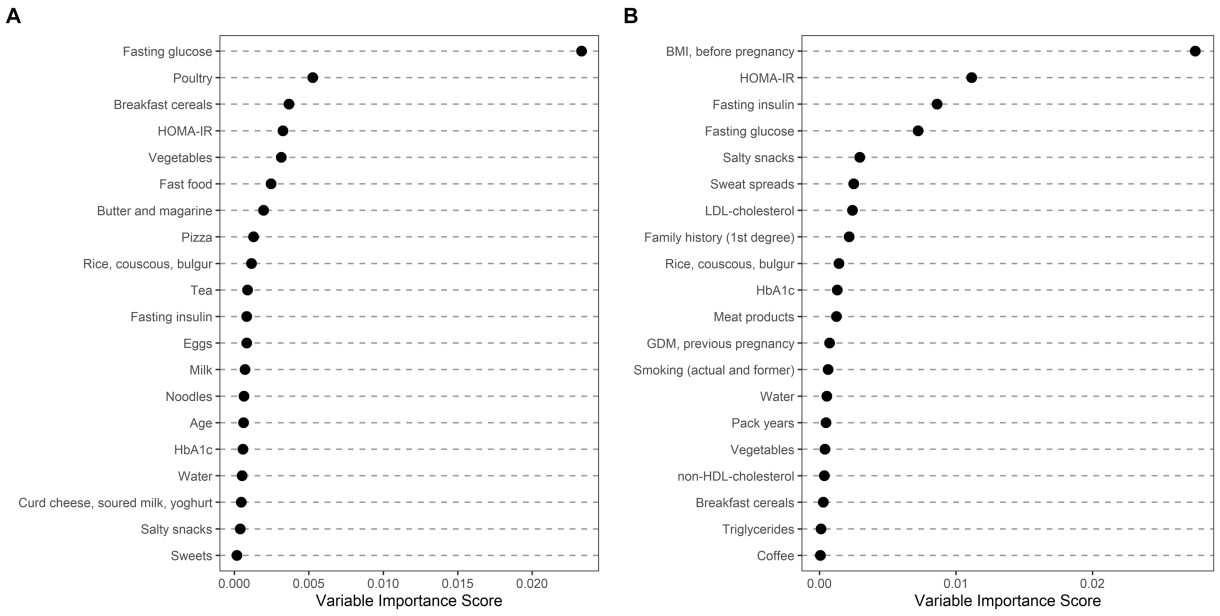

FIGURE 2
Ethnically stratified risk factors for GDM at early pregnancy showing variable importance scores for non-Caucasian mothers with Middle Eastern and Northern African **(A)** and Asian origin **(B)**. The first 20 variables with highest variable scores are shown.

4 Discussion

This study aimed to assess the role of ethnicity on the development of GDM in a prospectively assessed and well characterized multiethnic Central European cohort. We found that women with non-Caucasian ancestry, especially those with origin from the MENA and Asian countries have markedly increased risk as compared to Caucasian mothers. This is corroborated by results of some previous retrospective and register studies, suggesting a higher GDM incidence for specific ethnicities and minorities, such as South and East Asian, Indigenous Australian, African, Hispanics and Native Americans (24–28). Recently, Caputo et al. retrospectively analyzed data of 586 patients and found that despite being younger, GDM patients from "high migration pressure countries" required insulin treatment more often,

what is also indicated for non-Caucasian mothers in our study (29). Likewise, a population based Norwegian register study identified substantially increased GDM incidence in immigrant women, whereby the risk for hyperglycemia increased in parallel to the length of residence in certain immigrant groups (9), suggesting, that the elevated risk in these mothers is not only attributable to a genetic predisposition (24).

In the present study, we also observed distinct differences in patient characteristics and clinical-metabolic features between Caucasian and non-Caucasian women. Thereby, non-Caucasian mothers had higher pregestational BMI, were more insulin resistant and showed an adverse glucometabolic risk profile with higher fasting glucose, and HbA1c already at start of gestation. In another study of reproductive-aged Austrian women, we previously found that women with origin from the MENA countries undergoing infertility treatment were more obese and, despite being younger as compared to Caucasian patients, showed impaired ovarian function, possibly explained by a higher incidence of Polycystic Ovarian Syndrome – a disease markedly triggered by impaired insulin sensitivity (30). This is comparable to our present study, indicating that especially women with origin from the MENA region had higher BMI as well as a higher degree of insulin resistance and consequently an increased risk for GDM development. Aside from the MENA population, we additionally observed an increased risk for GDM in mothers with Asian ancestry. This is in line with another recent register study, indicating that the risk of GDM is increased in mothers with South Asian and Chinese ethnicity, who had lower BMI as compared to the general Canadian population (11). Interestingly, Sharma et al. observed, that glucose metabolism remained markedly impaired after GDM pregnancy, in particular in Asian mothers, who showed impaired β-cell function, insulin action and clearance as compared to Nordic women (31).

Ethnically specific differences are often explained by different lifestyle habits, especially dietary patterns. Thereby, some authors suggested that "nutrition transition" towards an energy dense Western diet may promote the development of metabolic disorders and the requirement of glucose lowering medication in GDM patients (29, 32, 33). Dietary habits can also directly affect fetal development and growth and this effect can be possibly modulated by ethnicity. For example, Zulyniak et al. found that consumption of plant-based diet reduced infant birth weight in white Europeans, whereas it increased the risk for LGA infants in South Asians living in Canada (34). Another meta-analysis recently assessed the effect of healthy diet on GDM incidence and indicated significant associations between dietary patterns and GDM risk markedly in white European mothers, whereas no consistent evidence was observed in non-Caucasian populations, what may be explained by heterogenous use of dietary assessment tools (35). Differences in dietary patterns were also observed in our study, whereby non-Caucasian immigrants differed markedly from the Caucasian population. However, a detailed analysis of GDM risk factors indicated that dietary patterns had inferior variable importance scores as compared to other clinical-metabolic features, such as fasting glucose and maternal insulin resistance, showing consistently high predictive performance in Caucasian and non-Caucasian mothers. Of note, maternal pregestational BMI achieved notably higher importance for GDM prediction in Asian mothers. In line with our findings, Read et al. found that BMI increased the risk for GDM at far lower levels in South Asian and Chinese mothers, possibly indicating that limiting excess weight gain may be particular effective for GDM prevention in Asian mothers (11). This effect may be mediated by distinct metabolic profiles of Asian and white European women as recently suggested by another study (36).

Some advantages and limitations need to be addressed: Clinical and metabolic risk factors as well as dietary patterns were only assessed once at start of pregnancy, what may be seen as a limitation. However, the large sample size with a high proportion of non-Caucasian study participants and GDM cases is a clear advantage. In addition, the prospective character of the cohort study design allowed us to assess detailed information about ancestry (i.e., the parental country of origin) and allowed us to accurately determine patient's ethnicity. Moreover, this is for our knowledge the first study including information of clinical features, metabolic parameters (such as early gestational insulin sensitivity) and dietary habits in a multiethnic cohort of pregnant women.

In summary, we identified distinct differences in dietary patterns and clinical metabolic features between Caucasian and non-Caucasian mothers, who showed a higher incidence of GDM and need of glucose lowering medications. GDM risk was highest in Asian mothers and those with origin from the MENA region. Fasting plasma glucose as well as maternal insulin resistance at start of pregnancy are important risk factors in both, Caucasian and non-Caucasian mothers, although, increased maternal BMI (even at lower levels) may be of particular importance in Asians. The information provided by our study is of clinical relevance to improve risk stratification and to provide "culturally appropriate care" (14) for non-Caucasian ethnicities indicating the need for further research in non-Caucasian populations.

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 the Ethics Committee of the Medical University of Vienna. 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.

Author contributions

GK: Writing – original draft, Writing – review & editing, Data curation, Project administration. CM: Writing – original draft, Writing – review & editing. TL: Data curation, Writing – review & editing. DE: Writing – review & editing. VS: Writing – review & editing. MF: Writing – review & editing. BM: Writing – review & editing. VF: Writing – review & editing. SW: Writing – review & editing. WH: Writing – review & editing. AT: Formal analysis, Writing – review & editing. CG: Conceptualization, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing.

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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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1286056/full#supplementary-material

References

1. Sacks DA, Hadden DR, Maresh M, Deerochanawong C, Dyer AR, Metzger BE, et al. Frequency of gestational diabetes mellitus at collaborating centers based on IADPSG consensus panel-recommended criteria: the hyperglycemia and adverse pregnancy outcome (HAPO) study. *Diabetes Care*. (2012) 35:526–8. doi: 10.2337/dc11-1641

2. Huhn EA, Massaro N, Streckeisen S, Manegold-Brauer G, Schoetzau A, Schulzke SM, et al. Fourfold increase in prevalence of gestational diabetes mellitus after adoption of the new International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria. *J Perinat Med*. (2017) 45:359–66. doi: 10.1515/jpm-2016-0099

3. 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

4. Hod M, Kapur A, Sacks DA, Hadar E, Agarwal M, Di Renzo GC, et al. The International Federation of Gynecology and Obstetrics (FIGO) initiative on gestational diabetes mellitus: a pragmatic guide for diagnosis, management, and care #. *Int J Gynecol Obstet*. (2015) 131:S173–211. doi: 10.1016/S0020-7292(15)30033-3

5. Shah NS, Wang MC, Freaney PM, Perak AM, Carnethon MR, Kandula NR, et al. Trends in gestational diabetes at first live birth by race and ethnicity in the US, 2011–2019. *J Am Med Assoc*. (2021) 326:660–9. doi: 10.1001/jama.2021.7217

6. Liu B, Lamerato LE, Misra DP. A retrospective analysis of the relationship between race/ethnicity, age at delivery and the risk of gestational diabetes mellitus. *J Matern Fetal Neonatal Med*. (2020) 33:2961–9. doi: 10.1080/14767058.2019.1566310

7. Webber J, Charlton M, Johns N. Diabetes in pregnancy: management of diabetes and its complications from preconception to the postnatal period (NG3). *Br J Diabetes Vasc Dis*. (2015) 15:107. doi: 10.15277/bjdvd.2015.029

8. Bastola K, Koponen P, Skogberg N, Gissler M, Kinnunen TI. Gestational diabetes among women of migrant origin in Finland-a population-based study. *Eur J Pub Health*. (2021) 31:784–9. doi: 10.1093/eurpub/ckab078

9. Strandberg RB, Iversen MM, Jenum AK, Sørbye LM, Vik ES, Schytt E, et al. Gestational diabetes mellitus by maternal country of birth and length of residence in immigrant women in Norway. *Diabet Med*. (2021) 38:e14493. doi: 10.1111/dme.14493

10. Gao C, Sun X, Lu L, Liu F, Yuan J. Prevalence of gestational diabetes mellitus in mainland China: a systematic review and meta-analysis. *J Diabetes Investig*. (2019) 10:154–62. doi: 10.1111/jdi.12854

11. Read SH, Rosella LC, Berger H, Feig DS, Fleming K, Ray JG, et al. BMI and risk of gestational diabetes among women of south Asian and Chinese ethnicity: a population-based study. *Diabetologia*. (2021) 64:805–13. doi: 10.1007/s00125-020-05356-5

12. Al-Rifai RH, Abdo NM, Paulo MS, Saha S, Ahmed LA. Prevalence of gestational diabetes mellitus in the Middle East and North Africa, 2000–2019: a systematic review, meta-analysis, and meta-regression. *Front Endocrinol (Lausanne)*. (2021) 12:668447. doi: 10.3389/fendo.2021.668447

13. Kragelund Nielsen K, Andersen GS, Damm P, Andersen AMN. Gestational diabetes risk in migrants. A Nationwide, register-based study of all births in Denmark 2004 to 2015. *J Clin Endocrinol Metab*. 105:2020, e692–e703. doi: 10.1210/clinem/dgaa024

14. Tzotzis L, Hooper M-E, Douglas A, Kurz E, Atchan M, Spiller S, et al. The needs and experiences of women with gestational diabetes mellitus from minority ethnic backgrounds in high-income nations: a systematic integrative review. *Women Birth*. (2023) 36:205–16. doi: 10.1016/j.wombi.2022.08.006

15. Kotzaeridi G, Blätter J, Eppel D, Rosicky I, Mittlböck M, Yerlikaya-Schatten G, et al. Performance of early risk assessment tools to predict the later development of gestational diabetes. *Eur J Clin Investig*. (2021) 51:e13630. doi: 10.1111/eci.13630

16. NCD Risk Factor Collaboration. Worldwide trends in diabetes since. A pooled analysis of 751 population-based studies with 4·4 million participants NCD risk factor collaboration (NCD-RisC) Europe PMC funders group. *Lancet*. (1980) 2016:387.

17. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. (1985) 28:412–9. doi: 10.1007/BF00280883

18. International Association of D, Pregnancy Study Groups Consensus P. International Association of Diabetes and Pregnancy Study Groups Recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care*. (2010) 33:676–82. doi: 10.2337/dc09-1848

19. Schäfer-Graf UM, Gembruch U, Kainer F, Groten T, Hummel S, Hösli I, et al. Gestational diabetes mellitus (GDM) – diagnosis, treatment and follow-UpGuideline of the DDG and DGGG (S3 level, AWMF registry number 057/008, February 2018). *Geburtshilfe Frauenheilkd*. (2018) 78:1219–31. doi: 10.1055/a-0659-2596

20. Haftenberger M, Heuer T, Heidemann C, Kube F, Krems C, Mensink GBM. Relative validation of a food frequency questionnaire for national health and nutrition monitoring. *Nutr J*. (2010) 9:36. doi: 10.1186/1475-2891-9-36

21. Breiman L. Random forests. *Mach Learn*. (2001) 45:5–32. doi: 10.1023/A:1010933404324

22. R Development Core Team. R Core Team. (2020). *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/. R Foundation for Statistical Computing. 2019; 2.

23. Konietschke F, Placzek M, Schaarschmidt F, Hothorn LA. Nparcomp: an R software package for nonparametric multiple comparisons and simultaneous confidence intervals. *J Stat Softw*. (2015) 64:1–17. doi: 10.18637/jss.v064.i09

24. Yuen L, Wong VW, Simmons D. Ethnic disparities in gestational diabetes. *Curr Diab Rep*. (2018) 18:68. doi: 10.1007/s11892-018-1040-2

25. Xiang AH, Li BH, Black MH, Sacks DA, Buchanan TA, Jacobsen SJ, et al. Racial and ethnic disparities in diabetes risk after gestational diabetes mellitus. *Diabetologia*. (2011) 54:3016–21. doi: 10.1007/s00125-011-2330-2

26. Dornhorst A, Paterson CM, Nicholls JS, Wadsworth J, Chiu DC, Elkeles RS, et al. High prevalence of gestational diabetes in women from ethnic minority groups. *Diabet Med*. (1992) 9:820–5. doi: 10.1111/j.1464-5491.1992.tb01900.x

27. Berkowitz GS, Lapinski RH, Wein R, Lee D. Race/ethnicity and other risk factors for gestational diabetes. *Am J Epidemiol*. (1992) 135:965–73. doi: 10.1093/oxfordjournals.aje.a116408

28. Jaffe A, Giveon S, Rubin C, Novikov I, Ziv A, Kalter-Leibovici O. Gestational diabetes risk in a multi-ethnic population. *Acta Diabetol.* (2020) 57:263–9. doi: 10.1007/s00592-019-01404-8

29. Caputo M, Bullara V, Mele C, Samà MT, Zavattaro M, Ferrero A, et al. Gestational diabetes mellitus: clinical characteristics and perinatal outcomes in a multiethnic population of North Italy. *Int J Endocrinol.* (2021) 2021:1–10. doi: 10.1155/2021/9474805

30. Feichtinger M, Göbl C, Weghofer A, Feichtinger W. Reproductive outcome in European and middle eastern/north African patients. *Reprod Biomed Online.* (2016) 33:684–9. doi: 10.1016/j.rbmo.2016.09.003

31. Sharma A, Lee-Ødegård S, Qvigstad E, Sommer C, Sattar N, Gill JMR, et al. β-Cell function, hepatic insulin clearance, and insulin sensitivity in south Asian and Nordic women after gestational diabetes mellitus. *Diabetes.* (2022) 71:2530–8. doi: 10.2337/db22-0622

32. Popkin BM. Nutrition transition and the global diabetes epidemic. *Curr Diab Rep*. (2015) 15:64. doi: 10.1007/s11892-015-0631-4

33. Popkin BM, Ng SW. The nutrition transition to a stage of high obesity and noncommunicable disease prevalence dominated by ultra-processed foods is not inevitable. *Obes Rev*. (2022) 23:e13366. doi: 10.1111/obr.13366

34. Zulyniak MA, De Souza RJ, Shaikh M, Desai D, Lefebvre DL, Gupta M, et al. Does the impact of a plant-based diet during pregnancy on birth weight differ by ethnicity? A dietary pattern analysis from a prospective Canadian birth cohort alliance. *BMJ Open*. (2017) 7:e017753. doi: 10.1136/bmjopen-2017-017753

35. Fuller H, Moore JB, Iles MM, Zulyniak MA. Ethnic-specific associations between dietary consumption and gestational diabetes mellitus incidence: a meta-analysis. *PLOS Glob Public Health*. (2022) 2:e0000250. doi: 10.1371/journal.pgph.0000250

36. Fuller H, Iles M, Moore JB, Zulyniak MA. Unique metabolic profiles associate with gestational diabetes and ethnicity in low- and high-risk women living in the UK. *J Nutr*. (2022) 152:2186–97. doi: 10.1093/jn/nxac163

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Relationships between triglyceride-glucose index and incident gestational diabetes mellitus: a prospective cohort study of a Korean population using publicly available data

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Background: The connection between the triglyceride-glucose index (TyG index) and gestational diabetes mellitus (GDM) is currently debated. Our study aimed to investigate the connection between the TyG index and GDM within the Korean population.

Methods: Using publically accessible data in Korea, we performed a secondary study on a sample of 589 pregnant women who were carrying a single fetus. The analysis employed a binary logistic regression model, some sensitivity analyses, and subgroup analysis to investigate the association between the TyG index and the occurrence of GDM. To assess the TyG index's potential to predict GDM, a receiver operating characteristic (ROC) study was also carried out.

Results: The mean age of the pregnant women was 32.065 ± 3.798 years old, while the mean TyG index was 8.352 ± 0.400. The prevalence rate of GDM was found to be 6.112%. Upon adjusting for potential confounding variables, a positive association was detected between the TyG index and incident GDM (OR = 12.923, 95%CI: 3.581–46.632, $p = 0.00009$). The validity of this connection was further confirmed by subgroup analysis and sensitivity analyses. With an area under the ROC curve of 0.807 (95%CI: 0.734–0.879), the TyG index showed strong predictive power for GDM. The TyG index's ideal cutoff value for detecting GDM was found to be 8.632, with a sensitivity of 78.7% and a specificity of 72.2%.

Conclusion: The findings of our study provide evidence that an increased TyG index is significantly associated with the occurrence of GDM. Utilizing the TyG index during the 10–14 week gestational period may be a valuable tool in identifying pregnant individuals at a heightened risk for developing GDM. Early detection enables timely and efficacious interventions, thereby enhancing the prognosis of affected individuals.

KEYWORDS

triglyceride-glucose index, triglyceride, gestational diabetes mellitus, logistic regression model, insulin resistance, receiver operating characteristic curve

Introduction

Gestational diabetes mellitus (GDM) refers to varying degrees of glucose intolerance that occur or are identified for the first time during pregnancy, irrespective of pre-existing diabetes (1). During pregnancy, GDM is a prevalent complication, with its incidence steadily rising in recent decades (2–4). The etiology of GDM is multifaceted, encompassing obesity/pre-gravidic weight, maternal age, and history of polycystic ovary syndrome (5, 6). Notably, GDM is associated with an increased likelihood of adverse perinatal outcomes, such as pre-eclampsia, gestational hypertension, miscarriage, cesarean section, and macrosomia (2, 7, 8). Furthermore, GDM has been acknowledged as a significant predisposing factor for maternal cardiovascular disease and diabetes (9), as well as obesity and insulin resistance (IR) in the offspring (10). The conventional approach for the clinical ascertainment of GDM is conducted within the 24–28th weeks of gestation, employing a 75 g oral glucose tolerance test (OGTT) delineated in the literature (11). However, empirical evidence suggests that by the time GDM is diagnosed at this stage, both the mother and fetus may have already been adversely affected to varying degrees despite the potential benefits of symptom management (12, 13). Therefore, the timely recognition of women at heightened risk for gestational diabetes mellitus is of paramount importance for mitigating the potential adverse outcomes and stemming the tide of transgenerational metabolic sequelae.

Previous studies have indicated that insulin resistance (IR) is a critical element in both the onset and progression of GDM. It is characterized by an impaired response to insulin in peripheral tissues, which becomes particularly problematic during pregnancy as the demand for insulin escalates (14). The insidious nature of IR often means that it is well established by the time GDM is clinically recognized, contributing to the challenge of timely diagnosis and management (15). The interaction between maternal IR and β-cell dysfunction is a central component in the pathophysiology of GDM (16). However, there is a scarcity of previous studies examining the potential predictive value of IR for GDM; previous studies likely lack a dependable and practical surrogate marker for IR (17). Traditionally, the definitive test for insulin sensitivity is the hyperinsulinemic-euglycemic clamp test (18). Nevertheless, this method is time-consuming and expensive, significantly limiting its use in clinical practice (19). In recent research, the triglyceride-glucose index (TyG index), a metric generated from fasting blood glucose and triglyceride levels, has been recommended as a trustworthy and practical diagnostic of IR (20, 21). A greater TyG index has been linked to a higher risk of type 2 diabetes mellitus (T2DM) in the adult population, according to prior studies (22). Based on the results of earlier investigations, it is not yet obvious if the TyG index can predict the risk of GDM (23–26). Consequently, the objective of this investigation was to comprehensively assess the prospective predictive ability of the TyG index for GDM within a cohort study of the Korean population, utilizing publicly available data.

Methods

Data source

The primary data utilized in this research were generously provided by Lee SM et al. (27). The primary data are available to the public. They are published under the Creative Commons Attribution License, which allows free use, distribution, and reproduction in any format as long as the author and source are properly acknowledged. We express our gratitude to the data contributors for their invaluable contributions.

Study population

Between November 2014 and July 2016, the initial study encompassed 663 singleton pregnant women who had sought antenatal care at two prominent medical institutions, namely the Incheon Seoul Women Hospital and Seoul Metropolitan Government Seoul National University Boramae Medical Center, both located in Seoul, Korea. These participants were included if they had commenced prenatal care before reaching 14 weeks gestation. These women were recruited within the ongoing "Fatty Liver in Pregnancy" registry framework. Notably, before their inclusion, all singleton pregnant women provided written informed consent (27). The original professional staff employed a comprehensive and non-selective approach to meticulously collect cases for the original study.

The research ethics of this study were approved by the committee of the Seoul Metropolitan Government Seoul National University Boramae Medical Center and the committee of the Ministry of Health and Welfare of Korea (27). Therefore, given this prior ethical approval, no additional ethical clearance was required for this secondary analysis. Additionally, the primary research complied with the principles outlined in the Declaration of Helsinki.

Abbreviations: TyG index, low-density lipoprotein cholesterol to high-density lipoprotein cholesterol ratio; GDM, gestational diabetes mellitus; IR, insulin resistance; GCT, glucose challenge screening test; BMI, body mass index; LDL-C, low-density lipid cholesterol; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; TC, total cholesterol; FPG, fasting plasma glucose; TG, triglycerides; HOMA-IR, homeostasis model assessment-insulin resistance; GAM, Generalized additive models; OR, odds ratios; SD, standard deviation; CI, confidence interval; TG/HDL-C, high-density lipoprotein cholesterol ratio; T2DM, type 2 diabetes mellitus.

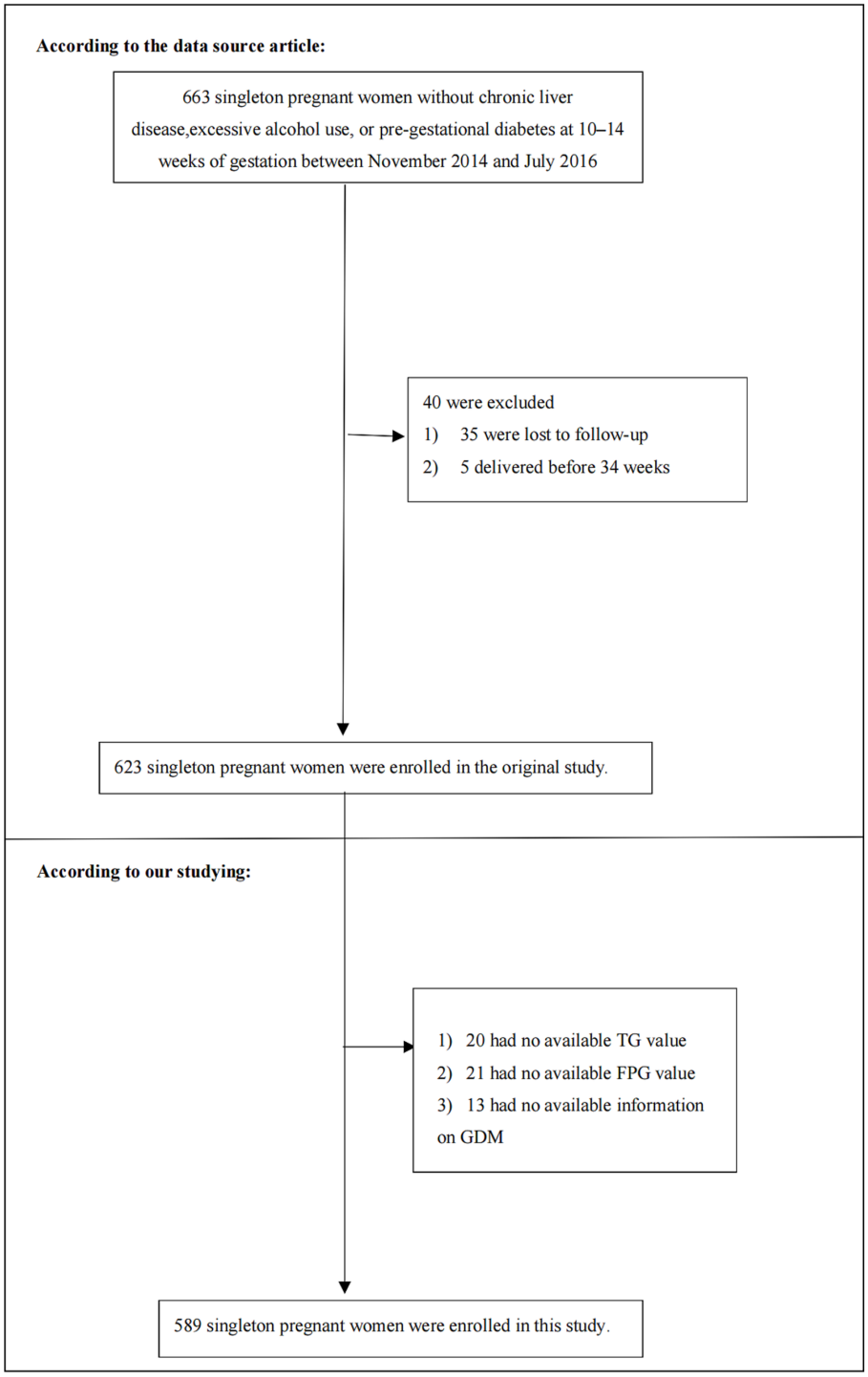

FIGURE 1
Flowchart of study participants. Figure showed the inclusion of participants. Six hundred and twenty-three participants were assessed for eligibility in the original study. We excluded patients with missing values of FPG ($n = 21$), TG ($n = 20$), GDM ($n = 13$). The final analysis included 589 subjects in the present study.

For the initial study, patients were excluded if they had (1) previous diagnosis of GDM, high alcohol consumption (more than 20 grams of alcohol per day), or chronic liver disease; (2) preterm delivery occurring before 34 weeks; or (3) were lost to follow-up. As a result, the initial study comprised 623 participants. Subsequently, we further excluded participants with missing data for GDM ($n = 13$), fasting plasma glucose (FPG) ($n = 21$), and triglyceride (TG) ($n = 20$). The final analysis included 589 singleton pregnant women. Figure 1 in the manuscript illustrates the study's design and the flow of participants.

Variables

TyG index

Venous blood samples from the subjects were taken between 10 and 14 weeks of pregnancy following a minimum 8-h fast. These specimens were subsequently subjected to centrifugation at an acceleration of 2000 *g* for a temporal span of 10 min, then partitioned into aliquots for preservation at a temperature of −70°C until the assay could be conducted. The intra-coefficient variation and inter-coefficient variation for FPG measured with the Roche/Hitachi 911 chemistry analyzer (Roche Diagnostics) were 1.75 and 2.33%, respectively. Similarly, the intra-coefficient variation and inter-coefficient variation for TG using the same analyzer were 3.50 and 4.66%, respectively. The precise method for calculating the TyG index is Ln[(TG (mg/dL) × FPG (mg/dL)/2)] (28).

Diagnosis of incident GDM

All participants were diagnosed with GDM in the two-step method during 24–28 weeks (27). For the initial screening, serum glucose levels were measured after a non-fasting 50 g oral glucose challenge (GCT) test, taken 1 h after consuming a 50 g oral glucose load. A blood glucose level of ≥7.8 mmol/L indicated a positive result on the GCT. An additional 100 g OGTT was administered to people who had a positive result on the GCT. GDM was established when two or more glucose levels were elevated: FPG ≥5.3 mmol/L, one-hour glucose ≥10 mmol/L, two-hour glucose ≥8.6 mmol/L, and three-hour glucose ≥7.8 mmol/L.

Covariates

In selecting risk variables for this study, a comprehensive approach was undertaken, drawing insights from clinical expertise, the original research, and existing literature on risk factors associated with GDM. Therefore, based on the above considerations, the following variables were adopted as covariates: (1) continuous variables: high-density lipoprotein cholesterol (HDL-C), age, insulin, aspartate aminotransferase (AST), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), pre-pregnancy body mass index (BMI), total cholesterol (TC), gamma-glutamyl transferase (GGT); (2) categorical variables: parity, hepatic steatosis.

The general clinical and demographic data collection encompassed maternal age, prior history of GDM, height, parity, and pre-gestational weight. These details were gathered using a standardized questionnaire. Venous blood samples were obtained during the 10–14 weeks of pregnancy, ensuring an 8-h fasting period, to assess hematological markers, including GGT, TG, ALT, insulin, FPG, TC, and AST levels. Hepatic steatosis severity was determined using a previously established semiquantitative grading system (grades 0–3) (29). The homeostasis model assessment-insulin resistance (HOMA-IR) was determined using the formula [insulin (IU/mL) × FPG (mmol/L)/22.5], following established methodologies (27).

Statistical analysis

We initially assessed the baseline data distribution by categorizing it into tertiles based on the TyG index. Continuous data were reported as medians with interquartile ranges (25th-75th percentile) or means with standard deviations (SD), while categorical data were expressed as frequencies and percentages. To assess disparities between TyG index groups, The Kruskal-Wallis H test, chi-square test, and one-way ANOVA were employed. Cumulative incidence rates were used to express incidence rates.

The study employed both univariate and multivariate logistic regression to establish three models. Model 1 did not incorporate any covariates, while Model 2 adjusted only for sociodemographic factors, including parity, age, and pre-pregnancy BMI. In contrast, Model 3 encompassed all factors, including parity, age, hepatic steatosis, pre-pregnancy BMI, AST, HDL-C, GGT, LDL-C, insulin, ALT, and TC. Adjusted odds ratios (OR) and their corresponding 95% confidence intervals (CI) were computed to assess GDM risk. Adjustments were made for covariates, and when the inclusion of a covariate in the model resulted in an OR change of at least 10% (30), it was deemed necessary to include that covariate for adjustment.

The current research applied some sensitivity analyses to assess robust results. To assess the relationship of the TyG index as a continuous variable and explore potential non-linearity, we categorized the TyG index into tertiles and calculated the *p* value for trend. The presence of obesity and nonalcoholic fatty liver disease was connected to GDM risk (31, 32). In other sensitivity analyses, we excluded individuals with a grade of hepatic steatosis >0 or pre-pregnancy BMI ≥ 25 kg/m^2 to assess the connection between the TyG index and GDM. The present study employed a generalized additive model (GAM) to incorporate the continuity variables into the equation as a curve to examine the robustness of our findings (Model 4) (33). Furthermore, we computed E-values to evaluate the potential impact of unmeasured confounding between the TyG index and GDM (34).

Moreover, we applied the stratified logistic regression model to the subgroup analysis, including HOMA-IR, hepatic steatosis, pre-pregnancy BMI, age, and parity. Initially, continuous variables such as HOMA-IR (≤2, >2), pre-pregnancy BMI (<25, ≥25 kg/m^2), and age (<35, ≥35 years) were discretized according to clinical cutoff points. Subsequently, apart from the stratification factor, we introduced adjustments for all variables (parity, age, hepatic steatosis, pre-pregnancy BMI, AST, HDL-C, GGT, LDL-C, insulin, ALT, and TC) within each stratification. To validate interactions among subgroups, we executed a likelihood ratio test.

Moreover, we conducted a receiver operating characteristic (ROC) analysis to assess the predictive capacity of the TyG index for GDM. The area under the curve (AUC) of the ROC and the optimal threshold were calculated. For all results, the STROBE declaration was followed (30). R software version 3.6 and EmpowerStats (R) version 4.0 were used for all statistical analyses. *P*-values of 0.05 were used to determine statistical significance.

Results

Characteristics of participants

This study involved 589 pregnant women with no previous diagnosis of GDM. The average age of the participants was 32.065 ± 3.798 years. The mean TyG index was 8.352 ± 0.400. Between the 24th and 28th weeks of pregnancy, 36 (6.112%) women experienced GDM.

TABLE 1 The baseline characteristics of participants.

TyG index	T1 (≤8.181)	T2 (8.181–≤8.514)	T3 (>8.514)	*P*-value
Participants	196	196	197	
Age(years)	31.612 ± 3.591	31.888 ± 3.638	32.690 ± 4.081	0.014
Pre-pregnancy BMI (kg/m^2)	21.026 ± 2.757	21.765 ± 3.491	23.265 ± 3.758	<0.001
Parity				0.072
No	116 (59.184%)	99 (50.510%)	95 (48.223%)	
Yes	80 (40.816%)	97 (49.490%)	102 (51.777%)	
Hepatic steatosis				<0.001
Grade 0	171 (87.245%)	169 (86.224%)	139 (70.558%)	
Grade 1	25 (12.755%)	22 (11.224%)	38 (19.289%)	
Grade 2	0 (0.000%)	4 (2.041%)	13 (6.599%)	
Grade 3	0 (0.000%)	1 (0.510%)	7 (3.553%)	
HDL-C (mg/dL)	66.210 ± 12.574	65.372 ± 13.805	63.119 ± 14.079	0.064
TG (mg/dL)	77.852 ± 14.441	111.061 ± 15.868	167.503 ± 46.743	<0.001
TC (mg/dL)	161.138 ± 21.966	173.469 ± 24.963	183.756 ± 29.325	<0.001
LDL-C (mg/dL)	79.357 ± 18.486	85.518 ± 20.407	87.135 ± 25.225	<0.001
ALT (IU/L)	11 (8–13.25)	11 (8–15)	12 (8–18)	0.095
AST (IU/L)	16 (14–18.25)	16 (14–19)	17 (14–21)	0.161
GGT(IU/L)	11 (10–14)	11 (10–15)	13 (10–18)	0.002
FPG (mg/dL)	73.260 ± 8.010	76.847 ± 8.850	80.964 ± 10.600	<0.001
Insulin (μIU/mL)	7.172 ± 4.030	8.725 ± 4.363	12.646 ± 8.987	<0.001

Values were n(%) or mean ± SD or median (quartile). TyG index, triglyceride-glucose index; BMI: body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipid cholesterol; FPG, fasting plasma glucose.

TABLE 2 Incidence rate of incident gestational diabetes mellitus.

TyG index	Participants (*n*)	GDM events (*n*)	Cumulative incidence rate (95% CI) (%)
Total	589	36	6.112 (4.172–8.052)
T1	196	2	1.020 (−0.399–2.440)
T2	196	6	3.061 (0.628–5.494)
T3	197	28	14.213 (9.294–19.132)
P for trend			<0.001

TyG index, triglyceride-glucose index; CI, confidence interval; GDM, gestational diabetes mellitus.

Table 1 lists the baseline characteristics of the pregnant women. Based on the tertiles of the TyG index values, the individuals were split into three groups (T1 ≤ 8.181; 8.181 < T2 ≤ 8.514; T3 > 8.514). It was shown that individuals in the T3 group tended to be older, have higher LDL-C, insulin, GGT, TC, FPG, TG, pre-pregnancy BMI, and a lower prevalence of grade 0 hepatic steatosis.

The prevalence rate of GDM

Table 2 displays the prevalence rate of GDM. Specifically, the prevalence rates of GDM were 6.112% (4.172–8.052%), 1.020% (−0.399–2.440%), 3.061% (0.628–5.494%), and 14.213% (9.294–19.132%) for the overall population of women and for the three TyG index groups (T1groups, T2groups, T3groups). Participants in T3 exhibited a significantly higher prevalence rate of GDM than those in the T1 group ($p < 0.001$ for trend).

The results of univariate analyses

The outcomes of the univariate analysis have been presented in Table 3. The univariate analysis results indicate that pre-pregnancy BMI, TG, grade of hepatic steatosis, insulin, GGT, FPG, TyG index, and ALT were positively correlated with the occurrence of GDM. Additionally, an inverse association was observed between HDL-C and incident GDM.

The results of multivariate analyses

Table 4 demonstrates the application of a multivariate logistic regression model to explore the link between the TyG index and incident GDM. In Model 1, a positive connection was observed between the TyG index and incident GDM (OR: 30.230, 95%CI: 10.535–86.746, $p < 0.00001$). Model 2, which incorporated adjustments for age, pre-pregnancy BMI, and parity, yielded consistent outcomes with no significant alterations (OR: 17.816, 95%CI: 5.511–57.588, $p < 0.00001$). Moreover, even after accounting for variables including parity, age, hepatic steatosis, pre-pregnancy BMI, AST, HDL-C, GGT, LDL-C, insulin, ALT, and TC in Model 3, a noticeable connection between the TyG index and incident GDM persisted (OR: 12.923,

TABLE 3 The results of the univariate analysis.

	Statistics	OR (95% CI)	*P*-value
Participants			
Age (years)	32.065 ± 3.798	1.037 (0.949, 1.134)	0.42325
Pre-pregnancy BMI (kg/m^2)	22.019 ± 3.483	1.275 (1.175, 1.384)	<0.00001
Parity			
No	310 (52.632%)	ref	
Yes	279 (47.368%)	0.994 (0.506, 1.952)	0.98553
Hepatic steatosis			
Grade 0	479 (81.324%)	ref	
Grade 1	85 (14.431%)	3.427 (1.462, 8.033)	0.00460
Grade 2	17 (2.886%)	25.722 (8.780, 75.359)	<0.00001
Grade 3	8 (1.358%)	17.362 (3.814, 79.042)	0.00022
HDL-C (mg/dL)	64.897 ± 13.543	0.964 (0.938, 0.989)	0.00602
TG (mg/dL)	118.888 ± 47.482	1.018 (1.012, 1.025)	<0.00001
TC (mg/dL)	172.806 ± 27.185	1.010 (0.998, 1.022)	0.09210
LDL-C (mg/dL)	84.009 ± 21.789	1.000 (0.985, 1.016)	0.99692
ALT (IU/L)	13.414 ± 9.587	1.037 (1.014, 1.061)	0.00172
AST (IU/L)	17.802 ± 8.101	1.019 (0.992, 1.046)	0.17401
GGT(IU/L)	13.963 ± 8.455	1.034 (1.008, 1.062)	0.01130
FPG (mg/dL)	77.031 ± 9.728	1.069 (1.037, 1.103)	0.00002
Insulin (μIU/mL)	9.524 ± 6.632	1.116 (1.066, 1.169)	<0.00001
TyG index	8.352 ± 0.400	30.230 (10.535, 86.746)	<0.00001

Values are n(%) or mean ± SD. TyG index, triglyceride-glucose index; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipid cholesterol; FPG, fasting plasma glucose.

TABLE 4 Relationship between TyG index and the incident GDM in different models.

Variable	Model 1 (OR.,95% CI, P)	Model 2 (OR,95% CI, P)	Model 3 (OR, 95% CI, P)	Model 4 (OR, 95% CI, P)
TyG index	30.230 (10.535, 86.746) <0.00001	17.816 (5.511, 57.588) <0.00001	12.923 (3.581, 46.632) 0.00009	19.836 (4.699, 83.743) 0.00005
TyG index (tertile)				
T1	Ref	Ref	Ref	1.0
T2	3.063 (0.611, 15.367) 0.17369	2.276 (0.435, 11.900) 0.32978	1.811 (0.342, 9.606) 0.48520	1.222 (0.210, 7.105) 0.82297
T3	16.071 (3.772, 68.465) 0.00017	8.543 (1.913, 38.153) 0.00496	5.618 (1.194, 26.438) 0.02896	5.586 (1.159, 26.922) 0.03202
P for trend	<0.00001	0.00056	0.00687	0.00520

Model 1: we did not adjust other covariants. Model 2: we adjusted age, pre-pregnancy BMI, parity. Model 3: we adjusted age, pre-pregnancy BMI, parity, hepatic steatosis, AST, GGT, ALT, TC, HDL-C, LDL-C, insulin. Model 4: we adjusted age(smooth), pre-pregnancy BMI(smooth), parity, hepatic steatosis, AST (smooth), GGT (smooth), ALT (smooth), TC (smooth), TC (smooth), HDL-C (smooth), insulin (smooth). OR, odds ratios; CI, confidence interval; Ref, Reference; TyG index, triglyceride-glucose index.

95%CI: 3.581–46.632, $p = 0.00009$). These findings imply that a 12-fold increase in the likelihood of getting GDM is associated with each unit rise in the TyG index.

Sensitive analysis

We reintroduced the TyG index after categorically transforming it from a continuous variable. Compared to the reference category (T1) of the TyG index, the multivariate-adjusted model exhibited an HR of 1.811 (95%CI: 0.342–9.606) in the T2 group and 5.618 (95% CI: 1.194–26.438) in the T3 group (Table 4).

The continuity covariate was introduced into the equation as a curve using a GAM. According to the results of model 4, the TyG index is positively correlated with the risk of GDM (HR:19.836 ， 95%CI: 4.699–83.743) (Table 4). Notably, the E value for this study was 25.34, surpassing the relative risk of the TyG index and potential unmeasured confounders. This outcome suggested that the association between the TyG index and incident GDM remained largely unaffected by unmeasured or unknown confounders.

Furthermore, we conducted sensitivity analyses on subjects with BMI < 25 kg/m^2. The TyG index was found to be positively correlated with the risk of GDM after adjusting for parity, age, hepatic steatosis, pre-pregnancy BMI, AST, HDL-C, GGT, LDL-C, insulin, ALT, and TC (OR: 13.204, 95%CI: 2.547–68.446, $p = 0.00211$) (Table 5). Similarly, even when individuals with grade 0 hepatic steatosis were included in additional sensitivity analyses, the positive relationship between the TyG index and the likelihood of developing GDM persisted after

adjusting for confounding covariates (OR: 10.524, 95%CI: 1.925–57.547, $p = 0.00662$) (Table 5). The sensitivity analysis supported the robustness of our conclusions. Notably, the E value for this study was 25.34, surpassing the relative risk of the TyG index and potential unmeasured confounders. This outcome suggested that the connection between the TyG index and GDM risk remained largely unaffected by unmeasured or unknown confounders.

The results of the subgroup analysis

The connection between the TyG index and GDM risk was examined using subgroup analysis (Table 6) to find potential confounding factors that may have impacted the results. HOMA-IR, parity, pre-pregnancy BMI, hepatic steatosis, and age were selected as stratification factors. It was determined that those mentioned above potential confounding variables did not impact the association between the TyG index and the risk of GDM. The results of the subgroup analysis underscore the robustness of our conclusions.

TABLE 5 Relationship between TyG index and incident GDM in different sensitivity analyses.

Exposure	Model 5 (OR, 95%CI, P)	Model 6 (OR, 95%CI, P)
TyG index	13.204 (2.547, 68.446) 0.00211	10.524 (1.925, 57.547) 0.00662
TyG index (tertile)		
Q1	Ref	Ref
Q2	1.916 (0.342, 10.720) 0.45939	1.498 (0.256, 8.768) 0.65374
Q3	3.579 (0.666, 19.235) 0.13722	4.221 (0.809, 22.023) 0.08749
P for trend	0.11446	0.04806

Model 5 was sensitivity analysis after excluding those with pre-pregnancy BMI ≥ 25 kg/m^2. We adjusted age, pre-pregnancy BMI, parity, hepatic steatosis, AST, GGT, ALT, TC, HDL-C, LDL-C, insulin. Model 6 was sensitivity analysis after including those with grade 0 hepatic steatosis. We adjusted age, pre-pregnancy BMI, parity, AST, GGT, ALT, TC, HDL-C, LDL-C, insulin. OR, odds ratios; CI, confidence; Ref: reference; TyG index, triglyceride-glucose index.

ROC analysis

ROC analysis was performed to assess the predictive capacity of the TyG index for GDM. The results revealed an AUC of 0.807 (95% CI: 0.734–0.879), as presented in Table 7 and Figure 2. In comparison to other factors, including TG, triglyceride to high-density lipoprotein cholesterol ratio (TG/HDL-C), FPG, HDL-C, HOMA-IR, TC, insulin, and LDL-C, the TyG index exhibited the highest AUC for GDM prediction. Using Youden's index, the optimal cutoff point for the TyG index to predict GDM was determined to be 8.632. This threshold corresponded to a specificity of 78.7% and a sensitivity of 72.2%.

Discussion

This study explored the relationship between the TyG index and GDM risk within the Korean population. Our findings unveiled a positive connection between the TyG index and incident GDM. Notably, a 12-fold increase in the likelihood of getting GDM is associated with each unit rise in the TyG index. Our findings revealed a higher diagnostic efficiency with an AUC of 0.807 (95%CI: 0.734–0.879) for the TyG index in predicting GDM, which is significantly superior to the AUC values reported in similar studies, ranging from 0.57 to 0.69 (25, 26, 35). These similar studies rely on FPG, a one-step 75 g OGTT, or self-reported diagnosis of GDM. The use of a two-step testing procedure for GDM diagnosis in our study

TABLE 6 Effect size of TyG index on GDM in prespecified and exploratory subgroups.

Characteristic	No of patients	Effect size (95%CI)	*P*-value	*P* for interaction
Age (years)				0.9935
<35	452	21.926 (4.544, 105.803)	0.0001	
≥35	137	22.256 (0.881, 562.024)	0.0597	
Pre-pregnancy BMI (kg/m^2)				0.3923
<25	493	11.807 (2.559, 54.487)	0.0016	
≥25	95	41.965 (2.979, 591.166)	0.0056	
Parity				0.3476
No	310	24.258 (3.849, 152.869)	0.0007	
Yes	279	7.246 (1.162, 45.207)	0.0340	
Hepatic steatosis				0.8196
Grade 0	479	10.765 (2.025, 57.236)	0.0053	
Grade 1–3	110	14.552 (1.965, 107.793)	0.0088	
HOMA-IR				0.5168
≤2	388	9.991 (1.484, 67.258)	0.0180	
>2	201	22.912 (4.235, 123.956)	0.0003	

Above model adjusted for we adjusted for age, pre-pregnancy BMI, parity, hepatic steatosis, AST, GGT, ALT, TC, HDL-C, LDL-C, insulin. The model is not adjusted for the stratification variable in each case.

TABLE 7 Areas under the receiver operating characteristic curves (AUROC) for each evaluated parameters in identifying GDM.

Test	AUROC	95%CI	Best threshold	Specificity	Sensitivity	Youden Index
TyG index	0.807	0.734–0.879	8.632	0.787	0.722	0.509
TG	0.780	0.704–0.856	121.500	0.642	0.833	0.475
HDL-C	0.602	0.496–0.709	49.200	0.884	0.361	0.245
TG/HDL-C ratio	0.786	0.707–0.866	2.268	0.751	0.722	0.473
TC	0.573	0.473–0.672	181.500	0.662	0.500	0.162
LDL-C	0.505	0.400–0.611	77.550	0.620	0.472	0.092
FPG	0.658	0.555–0.762	90.500	0.957	0.306	0.263
Insulin	0.764	0.675–0.853	13.900	0.866	0.611	0.477
HOMA-IR	0.765	0.679–0.851	2.7500	0.875	0.583	0.458

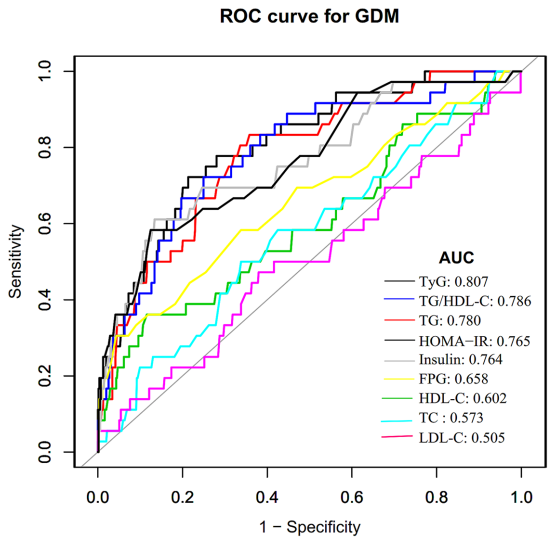

FIGURE 2
The TyG index for predicting DM in all participants by ROC analyses. ROC analysis was further conducted to explore the ability of the TyG index to predict GDM. The results showed that the AUC of the TyG index was 0.807. Compared to TG, HDL-C, TG/HDL-C ratio, TC, LDL-C, FPG, insulin, and HOMA-IR, the AUC of the TyG index for predicting DM was the highest.

may have contributed to the higher diagnostic accuracy of the TyG index. In addition, this disparity in diagnostic performance may also be related to study design, population characteristics, and sample size.

The prevalence of GDM has seen an uptick to 12.70% within the broader Korean population in recent times (36). Interestingly, the prevalence of GDM within the scope of this study was found to be 6.112%, which is comparatively lower than the documented rates. This current study used stricter exclusion criteria (excessive alcohol consumption, chronic liver disease, or previous diagnosis of GDM) as well as diagnostic criteria for GDM (using the two-step test), all of which would have led to a decrease in the prevalence of GDM in this current study. Consequently, the lower GDM incidence among research participants finds validation within this context. However, it's worth highlighting that the GDM prevalence still stands at 6.112% within this population. This emphasizes the continued importance of exploring potential additional risk factors for GDM.

Impaired insulin sensitivity or insulin secretion is widely recognized as the main underlying pathology of gestational diabetes mellitus. Women with dominant insulin resistance and GDM are more likely to experience negative effects. Conventional indicators of IR, such as the euglycemic-hyperinsulinemic clamp, face limitations due to invasiveness and complexity in clinical settings. Accessibility issues and a lack of clear cutoff values additionally hamper these techniques. Additionally, GDM is often detected between 24 and 28 weeks of pregnancy, giving little opportunity to prevent it from developing and causing damage. Thus, it becomes imperative to identify women susceptible to GDM early in pregnancy, aiming to reduce its impact using a proxy marker of insulin resistance. According to several findings, the TyG index could serve as a valuable indicator of insulin resistance. It has demonstrated potential in foretelling the beginning and development of diabetes in the general population. In two separate studies involving 352 Chinese women and 954 Iranian (23, 26), those in the highest tertile of the first-trimester TyG index were found to be 3.54-fold and 4.91-fold more likely to develop GDM, respectively. The Korean National Health Screening Exam study further highlights that an increase in the TyG index of just one standard deviation 2 years before conception increases the risk of gestational diabetes by 33% (25). A subsequent meta-analysis has confirmed and reinforced these findings (37). This study emphasizes that the risk of confirmed GDM within the Korean population increases with a rising TyG index, even after accounting for confounding variables. In our sensitivity analysis, we observed that the connection between the TyG index and GDM risk remains significant among Korean women with a BMI of less than 25 kg/m^{2}or with grade 0 hereditary steatosis. Moreover, we expanded our adjustments to include additional covariates like insulin, AST, hepatic steatosis, and GGT, which are all recognized risk factors for GDM (2, 32). Further analyses stratified by HOMA-IR, parity, pre-pregnancy BMI, hepatic steatosis, and age yielded consistent results, underscoring the stability of the relationship between the TyG and GDM risk. Consequently, this study broadens the applicability of the association between the TyG and GDM to the wider population. As such, this research holds substantial clinical significance. The implications of this study may serve as a stepping stone for future endeavors in developing predictive models for GDM.

The predictive capacity of the TyG index for GDM or T2DM has been extensively investigated, with consistent threshold values

found across various studies (24–26, 35, 38, 39). Notably, Wang et al. (38) conducted a 15-year prospective study in Chinese adults, revealing a threshold of around 8.51 for the TyG index's impact on incident T2DM risk. Similarly, Lee et al. (40) established a TyG index cutoff of 8.52 for predicting T2DM in more than 7,000 Korean adults. Kim et al. (25) reported a TyG index cutoff of 8.15 (AUC 0.60, specificity 68.2%, sensitivity 47.0%) for forecasting GDM 2 years before pregnancy. Regarding the diagnostic performance of the TyG index in detecting GDM during pregnancy, an AUC of 0.686 (95%CI: 0.615–0.756) was obtained by Liu et al. (26) in their evaluation of the TyG index's diagnostic capacity to predict GDM during the first prenatal visit. At the same time, no specific threshold value was specified. Similarly, Sanchez-Garcia et al. (39) identified a relatively low cutoff value of 4.69 (specificity 50%, sensitivity 89.0%). In addition, Zeng Y et al. found limited diagnostic efficacy of the TyG index for GDM (AUC = 0.57, 95% CI: 0.50–0.63) (35). In the current study, the TyG index demonstrated robust predictive capability for GDM, with an AUC of 80.7% and an optimal predictive cutoff value of around 8.632. Furthermore, the TyG index outperformed TG, HDL-C, TG/HDL-C, TC, LDL-C, FPG, insulin, and HOMA-IR indices in predicting GDM. Remarkably, the TyG index's diagnostic accuracy in GDM surpassed that of the HOMA-IR, suggesting its potential as an early biomarker for insulin resistance in early pregnancy and a reliable indicator for GDM detection.

However, the mechanism by which TyG associates with GDM is unclear. Firstly, the TyG index is a useful marker for insulin resistance (41–43), a core pathophysiological feature of GDM. Insulin resistance leads to reduced glucose uptake by peripheral tissues and increased hepatic glucose production, contributing to hyperglycemia during pregnancy (44). Secondly, high triglyceride levels, as part of the TyG index, suggest a disturbance in lipid metabolism as a consequence of hyperglycemia. This dyslipidemia can lead to an accumulation of fatty acids in tissues such as muscle and liver, which can interfere with insulin signaling and exacerbate insulin resistance, thereby increasing the risk of GDM (35). Furthermore, FPG levels reflect insulin sensitivity of the liver and insulin secretion by pancreatic β-cells, which are key factors in the pathogenesis of GDM (2, 32). Thus, the underlying mechanism of the TyG index's association with GDM risk can be attributed to the interplay between FPG and TG, both of which are associated with insulin resistance.

Our study presents several notable strengths. Firstly, we utilized tertiles of the TyG index as a categorical and continuous variable in our independent variables, enabling a comprehensive examination of its association with GDM risk. Secondly, meticulous statistical adjustments were employed to minimize the impact of residual confounding factors. Thirdly, subgroup analyses were conducted to evaluate the influence of other potential risk factors on the relationship between the TyG index and GDM.

However, certain limitations of our study should be acknowledged. Firstly, the association between the TyG index and GDM might exhibit variations across different ethnicities, underscoring the need for validation in diverse ethnic groups. Secondly, as a secondary analysis, our research could not adjust for variables like uric acid, hypertension, and renal function, which were not originally present in the dataset. Thirdly, the original study did not account for the fluctuations in FPG and TG over time. As previously reported (45), serum triglycerides are increased 2–3 times by late pregnancy, although they progressively increase from the first phases. Besides, triglycerides are subject to considerable analytical variability and, to an even greater extent, biological variability, exhibiting fluctuations that may range between 20 and 40% (46, 47). Future iterations of our investigation could encompass these additional confounding variables and track changes in FPG and TG throughout the follow-up period. Fourthly, there may be an impact on the results due to the existence of intra-coefficient variation and inter-coefficient variation for TG and FPG. In the future, we can consider designing our study with multiple measurements of TG and FPG on the same specimen to avoid influencing our results.

Conclusion

In conclusion, this study underscores the independent and positive correlation between an elevated TyG index and the risk of developing incident GDM within the Korean population. As such, the abnormal TyG index could be a valuable predictor for GDM. Consequently, it aids in identifying individuals in Korea who are at a heightened risk of GDM development. This finding holds the potential to aid healthcare practitioners in formulating and applying effective care strategies. Additionally, it might function as an early screening and monitoring tool to curtail the onset and advancement of GDM within clinical settings.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found here: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0221400.

Ethics statement

The studies involving humans were approved by the committee of the Seoul Metropolitan Government Seoul National University Boramae Medical Center and the committee of the Ministry of Health and Welfare of Korea. 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

ZM: Writing – original draft. CC: Supervision, Writing – original draft. YHa: Writing – original draft. HH: Writing – review & editing. YHe: Writing – review & editing. XZ: Writing – review & editing.

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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. Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy: a World Health Organization guideline. *Diabetes Res Clin Pract*. (2014) 103:341–63. doi: 10.1016/j.diabres.2013.10.012

2. Szmuilowicz ED, Josefson JL, Metzger BE. Gestational diabetes mellitus. *Endocrinol Metab Clin N Am*. (2019) 48:479–93. doi: 10.1016/j.ecl.2019.05.001

3. Chatzakis C, Cavoretto P, Sotiriadis A. Gestational diabetes mellitus pharmacological prevention and treatment. *Curr Pharm Des*. (2021) 27:3833–40. doi: 10.2174/1381612827666210125155428

4. Johns EC, Denison FC, Norman JE, Reynolds RM. Gestational diabetes mellitus: mechanisms, treatment, and complications. *Trends Endocrinol Metab*. (2018) 29:743–54. doi: 10.1016/j.tem.2018.09.004

5. Mirabelli M, Tocci V, Donnici A, Giuliano S, Sarnelli P, Salatino A, et al. Maternal preconception body mass index overtakes age as a risk factor for gestational diabetes mellitus. *J Clin Med*. (2023) 12:2830. doi: 10.3390/jcm12082830

6. Toulis KA, Goulis DG, Kolibianakis EM, Venetis CA, Tarlatzis BC, Papadimas I. Risk of gestational diabetes mellitus in women with polycystic ovary syndrome: a systematic review and a meta-analysis. *Fertil Steril*. (2009) 92:667–77. doi: 10.1016/j.fertnstert.2008.06.045

7. Franzago M, Fraticelli F, Stuppia L, Vitacolonna E. Nutrigenetics, epigenetics and gestational diabetes: consequences in mother and child. *Epigenetics-US*. (2019) 14:215–35. doi: 10.1080/15592294.2019.1582277

8. Zehravi M, Maqbool M, Ara I. Correlation between obesity, gestational diabetes mellitus, and pregnancy outcomes: an overview. *Int J Adolesc Med Health*. (2021) 33:339–45. doi: 10.1515/ijamh-2021-0058

9. Lorenzo-Almoros A, Hang T, Peiro C, Soriano-Guillen L, Egido J, Tunon J, et al. Predictive and diagnostic biomarkers for gestational diabetes and its associated metabolic and cardiovascular diseases. *Cardiovasc Diabetol*. (2019) 18:140. doi: 10.1186/s12933-019-0935-9

10. Bianco ME, Josefson JL. Hyperglycemia during pregnancy and long-term offspring outcomes. *Curr Diab Rep*. (2019) 19:143. doi: 10.1007/s11892-019-1267-6

11. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care*. (2010) 33:676–82. doi: 10.2337/dc09-1848

12. Li P, Lin S, Li L, Cui J, Zhou S, Fan J. First-trimester fasting plasma glucose as a predictor of gestational diabetes mellitus and the association with adverse pregnancy outcomes. *Pak J Med Sci*. (2019) 35:95–100. doi: 10.12669/pjms.35.1.216

13. Abokaf H, Shoham-Vardi I, Sergienko R, Landau D, Sheiner E. In utero exposure to gestational diabetes mellitus and long term endocrine morbidity of the offspring. *Diabetes Res Clin Pract*. (2018) 144:231–5. doi: 10.1016/j.diabres.2018.09.003

14. Bengtson AM, Ramos SZ, Savitz DA, Werner EF. Risk factors for progression from gestational diabetes to postpartum type 2 diabetes: a review. *Clin Obstet Gynecol*. (2021) 64:234–43. doi: 10.1097/GRF.0000000000000585

15. Sharma AK, Singh S, Singh H, Mahajan D, Kolli P, Mandadapu G, et al. Deep insight of the pathophysiology of gestational diabetes mellitus. *Cell*. (2022) 11:2672. doi: 10.3390/cells11172672

16. Fu J, Retnakaran R. The life course perspective of gestational diabetes: an opportunity for the prevention of diabetes and heart disease in women. *EClinicalMedicine*. (2022) 45:101294. doi: 10.1016/j.eclinm.2022.101294

17. Gerstein HC, Ferrannini E, Riddle MC, Yusuf S. Insulin resistance and cardiovascular outcomes in the ORIGIN trial. *Diabetes Obes Metab*. (2018) 20:564–70. doi: 10.1111/dom.13112

18. Rebelos E, Honka MJ. PREDIM index: a useful tool for the application of the euglycemic hyperinsulinemic clamp. *J Endocrinol Investig*. (2021) 44:631–4. doi: 10.1007/s40618-020-01352-z

19. Tura A, Chemello G, Szendroedi J, Gobl C, Faerch K, Vrbikova J, et al. Prediction of clamp-derived insulin sensitivity from the oral glucose insulin sensitivity index. *Diabetologia*. (2018) 61:1135–41. doi: 10.1007/s00125-018-4568-4

20. Khan SH, Sobia F, Niazi NK, Manzoor SM, Fazal N, Ahmad F. Metabolic clustering of risk factors: evaluation of triglyceride-glucose index (TyG index) for evaluation of insulin resistance. *Diabetol Metab Syndr*. (2018) 10:74. doi: 10.1186/s13098-018-0376-8

21. Brito A, Hermsdorff H, Filgueiras MS, Suhett LG, Vieira-Ribeiro SA, Franceschini S, et al. Predictive capacity of triglyceride-glucose (TyG) index for insulin resistance and cardiometabolic risk in children and adolescents: a systematic review. *Crit Rev Food Sci Nutr*. (2021) 61:2783–92. doi: 10.1080/10408398.2020.1788501

22. Da SA, Caldas A, Rocha D, Bressan J. Triglyceride-glucose index predicts independently type 2 diabetes mellitus risk: a systematic review and meta-analysis of cohort studies. *Prim Care Diabetes*. (2020) 14:584–93. doi: 10.1016/j.pcd.2020.09.001

23. Pazhohan A, Rezaee MM, Pazhohan N. Association of first-trimester maternal lipid profiles and triglyceride-glucose index with the risk of gestational diabetes mellitus and large for gestational age newborn. *J Matern Fetal Neonatal Med*. (2019) 32:1167–75. doi: 10.1080/14767058.2017.1402876

24. Sanchez-Garcia A, Rodriguez-Gutierrez R, Saldivar-Rodriguez D, Guzman-Lopez A, Mancillas-Adame L, Gonzalez-Nava V, et al. Early triglyceride and glucose index as a risk marker for gestational diabetes mellitus. *Int J Gynaecol Obstet*. (2020) 151:117–23. doi: 10.1002/ijgo.13311

25. Kim JA, Kim J, Roh E, Hong SH, Lee YB, Baik SH, et al. Triglyceride and glucose index and the risk of gestational diabetes mellitus: a nationwide population-based cohort study. *Diabetes Res Clin Pract*. (2021) 171:108533. doi: 10.1016/j.diabres.2020.108533

26. Liu PJ, Liu Y, Ma L, Yao AM, Chen XY, Hou YX, et al. The predictive ability of two triglyceride-associated indices for gestational diabetes mellitus and large for gestational age infant among Chinese pregnancies: a preliminary cohort study. *Diabetes Metab Syndr Obes*. (2020) 13:2025–35. doi: 10.2147/DMSO.S251846

27. Lee SM, Kim BJ, Koo JN, Norwitz ER, Oh IH, Kim SM, et al. Nonalcoholic fatty liver disease is a risk factor for large-for-gestational-age birthweight. *PLoS One*. (2019) 14:e0221400. doi: 10.1371/journal.pone.0221400

28. Simental-Mendia LE, Rodriguez-Moran M, Guerrero-Romero F. The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. *Metab Syndr Relat Disord*. (2008) 6:299–304. doi: 10.1089/met.2008.0034

29. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology*. (2002) 123:745–50. doi: 10.1053/gast.2002.35354

30. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Int J Surg*. (2014) 12:1495–9. doi: 10.1016/j.ijsu.2014.07.013

31. Lee SM, Kwak SH, Koo JN, Oh IH, Kwon JE, Kim BJ, et al. Non-alcoholic fatty liver disease in the first trimester and subsequent development of gestational diabetes mellitus. *Diabetologia*. (2019) 62:238–48. doi: 10.1007/s00125-018-4779-8

32. McIntyre HD, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm P. Gestational diabetes mellitus. *Nat Rev Dis Primers*. (2019) 5:47. doi: 10.1038/s41572-019-0098-8

33. Zhu F, Chen C, Zhang Y, Chen S, Huang X, Li J, et al. Elevated blood mercury level has a non-linear association with infertility in U.S. women: data from the NHANES 2013-2016. *Reprod Toxicol*. (2020) 91:53–8. doi: 10.1016/j.reprotox.2019.11.005

34. Haneuse S, VanderWeele TJ, Arterburn D. Using the E-value to assess the potential effect of unmeasured confounding in observational studies. *JAMA*. (2019) 321:602–3. doi: 10.1001/jama.2018.21554

35. Zeng Y, Yin L, Yin X, Zhao D. Association of triglyceride-glucose index levels with gestational diabetes mellitus in the US pregnant women: a cross-sectional study. *Front Endocrinol (Lausanne)*. (2023) 14:1241372. doi: 10.3389/fendo.2023.1241372

36. Kim KS, Hong S, Han K, Park CY. The clinical characteristics of gestational diabetes mellitus in Korea: a National Health Information Database Study. *Endocrinol Metab*. (2021) 36:628–36. doi: 10.3803/EnM.2020.948

37. Song T, Su G, Chi Y, Wu T, Xu Y, Chen C. Triglyceride-glucose index predicts the risk of gestational diabetes mellitus: a systematic review and meta-analysis. *Gynecol Endocrinol*. (2022) 38:10–5. doi: 10.1080/09513590.2021.1940932

38. Wang Z, Zhao L, He S. Triglyceride-glucose index as predictor for future type 2 diabetes mellitus in a Chinese population in Southwest China: a 15-year prospective study. *Endocrine*. (2021) 72:124–31. doi: 10.1007/s12020-020-02589-7

39. Sanchez-Garcia A, Rodriguez-Gutierrez R, Saldivar-Rodriguez D, Guzman-Lopez A, Castillo-Castro C, Mancillas-Adame L, et al. Diagnostic accuracy of the triglyceride-glucose index for gestational diabetes screening: a practical approach. *Gynecol Endocrinol*. (2020) 36:1112–5. doi: 10.1080/09513590.2020.1742687

40. Lee JW, Lim NK, Park HY. The product of fasting plasma glucose and triglycerides improves risk prediction of type 2 diabetes in middle-aged Koreans. *BMC Endocr Disord*. (2018) 18:33. doi: 10.1186/s12902-018-0259-x

41. Lopez-Jaramillo P, Gomez-Arbelaez D, Martinez-Bello D, Abat M, Alhabib KF, Avezum A, et al. Association of the triglyceride glucose index as a measure of insulin resistance with mortality and cardiovascular disease in populations from five continents (PURE study): a prospective cohort study. *Lancet Healthy Longev*. (2023) 4:e23–33. doi: 10.1016/S2666-7568(22)00247-1

42. Song K, Park G, Lee HS, Choi Y, Oh JS, Choi HS, et al. Prediction of insulin resistance by modified triglyceride glucose indices in youth. *Life (Basel)*. (2021) 11:286. doi: 10.3390/life11040286

43. Selvi N, Nandhini S, Sakthivadivel V, Lokesh S, Srinivasan AR, Sumathi S. Association of Triglyceride-Glucose Index (TyG index) with HbA1c and insulin resistance in type 2 diabetes mellitus. *Maedica*. (2021) 16:375–81. doi: 10.26574/maedica.2021.16.3.375

44. Liu Y, Chi R, Jiang Y, Chen B, Chen Y, Chen Z. Triglyceride glycemic index as a biomarker for gestational diabetes mellitus: a systemic review and meta-analysis. *Endocr Connect*. (2021) 10:1420–7. doi: 10.1530/EC-21-0234

45. Piechota W, Staszewski A. Reference ranges of lipids and apolipoproteins in pregnancy. *Eur J Obstet Gynecol Reprod Biol*. (1992) 45:27–35. doi: 10.1016/0028-2243(92)90190-a

46. Yang D, Cai Q, Qi X, Zhou Y. Postprandial lipid concentrations and daytime biological variation of lipids in a healthy Chinese population. *Ann Lab Med*. (2018) 38:431–9. doi: 10.3343/alm.2018.38.5.431

47. Miller M, Stone NJ, Ballantyne C, Bittner V, Criqui MH, Ginsberg HN, et al. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. *Circulation*. (2011) 123:2292–333. doi: 10.1161/CIR.0b013e3182160726

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Retinol-binding protein 4 (RBP4) circulating levels and gestational diabetes mellitus: a systematic review and meta-analysis

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Background: Gestational diabetes mellitus (GDM) is a prevalent condition where diabetes is diagnosed during pregnancy, affecting both maternal and fetal outcomes. Retinol-binding protein 4 (RBP4) is a circulating adipokine which belongs to the lipocalin family and acts as a specific carrier protein that delivers retinol (vitamin A) from the liver to the peripheral tissues. Growing data indicate that circulating RBP4 levels may positively correlate with GDM. Thus, this systematic review and meta-analysis aimed to investigate the potential relationship between circulating RBP4 levels and GDM when measured at various stages of pregnancy.

Methods: MEDLINE, CINAHL, EMCARE, EMBASE, Scopus, and Web of Science databases were searched to identify studies comparing pregnant women with and without GDM, whose circulating RBP4 levels were measured in at least one pregnancy trimester. Findings were reported using standardized mean difference (SMD) and random-effects models were used to account for variability among studies. Furthermore, the risk of bias was assessed using the RoBANS tool.

Results: Out of the 34 studies identified, 32 were included in the meta-analysis (seven with circulating RBP4 levels measured in the first trimester, 19 at 24–28 weeks, and 14 at >28 weeks of pregnancy). RBP4 levels were statistically higher in the GDM group than in controls when measured during all these pregnancy stages, with the noted RBP4 SMD being 0.322 in the first trimester (95% CI: 0.126–0.517; $p < 0.001$; 946 GDM cases vs. 1701 non-GDM controls); 0.628 at 24–28 weeks of gestation (95% CI: 0.290–0.966; $p < 0.001$; 1776 GDM cases vs. 1942 controls); and 0.875 at >28 weeks of gestation (95% CI: 0.252–1.498; $p = 0.006$; 870 GDM cases vs. 1942 non-GDM controls). Significant study heterogeneity was noted for all three pregnancy timepoints.

Conclusion: The present findings indicate consistently higher circulating RBP4 levels in GDM cases compared to non-GDM controls, suggesting the potential relevance of RBP4 as a biomarker for GDM. However, the documented substantial study heterogeneity, alongside imprecision in effect estimates, underscores the need for further research and standardization of measurement methods to elucidate whether RBP4 can be utilized in clinical practice as a potential GDM biomarker.

Systematic review registration: PROSPERO (CRD42022340097: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022340097).

KEYWORDS

retinol-binding protein 4, RBP4, gestational diabetes mellitus, GDM, pregnancy, systematic review, meta-analysis

1 Introduction

Diabetes diagnosed during pregnancy, i.e., gestational diabetes mellitus (GDM), is a highly prevalent condition that is typically characterized by hyperglycemia, glucose intolerance, and insulin resistance, potentially resulting in adverse effects for both the mother and the fetus (1). The reported GDM prevalence rates range from 1 to 14% depending on the studied population, with Asia, Latin America, and the Middle East regions exhibiting higher prevalence rates, whilst inconsistencies in the testing protocols and diagnostic criteria further contribute to the varying GDM prevalence rates reported worldwide (2). In the United Kingdom, approximately 1 in 23 pregnancies is affected by GDM (3). GDM frequently resolves soon after delivery, but these women are more likely to experience GDM in subsequent pregnancies and have an increased risk of later developing type 2 diabetes (4, 5).

Several factors contribute to a higher risk of developing GDM, including an increased body mass index (BMI) at overweight or obesity levels, excessive weight gain during pregnancy, specific ethnic backgrounds (e.g., women from South Asia), genetic factors, a personal or family history of GDM, and polycystic ovary syndrome (PCOS) (6–8). Currently, to diagnose GDM, most pregnant women are offered an oral glucose tolerance test (OGTT) between 24 and 28 weeks of gestation or earlier for those considered at high risk (9). However, using pre-diagnostic risk factor screening alone is not always an effective method of identifying women at risk of GDM, as shown by meta-analysis data (9). This highlights that there is still a need for novel biomarkers to more accurately identify women at high GDM risk. As such, recent research in the field of GDM has focused on studying an array of biomarkers which can be measured in the circulation of pregnant women and are linked to the complex pathophysiology of the condition, such as biomarkers associated with obesity-related inflammation, insulin resistance, and those derived from the adipose tissue (i.e., adipokines) or the placenta.

Retinol-binding protein 4 (RBP4) is a 21-kDa protein (10), which is secreted mainly by the liver and adipose tissue, and was initially identified as a transport protein for retinol (vitamin A) and other retinoid derivatives in the bloodstream (11). A 2005 study showed for the first time the potential involvement of RBP4 in the pathogenesis of type 2 diabetes (11), with the expression of RBP4 playing a regulatory role in glucose metabolism in both the liver and skeletal muscle. Indeed, the decreased expression of glucose transporter-4 (GLUT4) is linked to increased RBP4 secretion from the adipose tissue, which leads to increased hepatic gluconeogenesis and reduced glucose uptake in the muscle, ultimately resulting in increased blood glucose levels, impaired glucose tolerance, and diabetes (12). Furthermore, recent studies have also revealed close associations between RBP4 and cardiovascular disease (CVD) and related risk factors, such as obesity, hypertension, dyslipidemia, heart failure, and coronary heart disease (10).

In this context, there has been increasing interest in investigating the potential role of RBP4 as a novel biomarker for GDM. However, the reported results have been inconsistent, with previous meta-analyses suggesting that serum RBP4 levels in early pregnancy show an independent positive association with GDM risk (13), and that Asian women with GDM had increased circulating RBP4 levels during the second/third pregnancy trimester (14). Although such data support the hypothesis that circulating RBP4 may be linked to GDM (15), there is still a need for a comprehensive systematic analysis and an updated meta-analysis of the relevant published studies examining the association between GDM and circulating RBP4 levels measured during all pregnancy stages/trimesters. Therefore, the present systematic review and meta-analysis aimed to explore this potential relationship across the pregnancy duration, providing an up-to-date critical synthesis of the relevant available data.

2 Materials and methods

The present systematic review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) (16) guidelines (Supplementary Table S1.1), and was prospectively registered on PROSPERO (International Prospective Register of Systematic Reviews – University of York), with the registration number CRD42022340097.

2.1 Search strategy and data sources

A search was conducted based on a predefined search strategy and was adapted to the syntax and appropriate subject headings of the following databases: MEDLINE, CINAHL, EMCARE, EMBASE via Ovid, Scopus, and Web of Science. Reference lists were also browsed to ensure literature saturation. Final searches were completed in June 2023, and the main search strategy for MEDLINE

TABLE 1 MEDLINE search string.

(Retinol binding proteins[MeSH Terms]) OR (retinol binding protein 4) OR (retinol-binding protein-4) OR (retinol binding protein-4) OR RBP4 OR (RBP 4) OR (RBP-4))
AND
(Pregnancy[MeSH Terms]) OR pregnan*))

is presented in Table 1, whilst all other search strategies are detailed in Supplementary data and Supplementary material 1.2.

2.2 Eligibility criteria

Eligible articles included those conducted in adult (age > 18 years old) pregnant women with and without GDM, whose circulating levels of RBP4 were measured during at least one pregnancy trimester. No restrictions were imposed regarding the year of publication, type of setting, language, or timing of RBP4 measurement during the pregnancy. All observational study designs were included, while single case reports, expert opinion manuscripts, commentaries, animal studies, and review articles were excluded.

2.3 Study selection and data extraction

The study selection and data extraction processes were conducted independently by two reviewers (BML and LL), and any discrepancies or disagreements were resolved through consultation with a third reviewer (CK).

The initial selection of potentially eligible studies was based on title and abstract screening and was performed using the Rayyan software (17), following a predefined protocol. Papers considered eligible progressed to a full-text review.

A standardized data extraction form was developed to extract relevant information from the included eligible studies. The extracted data included country of origin, study design, patient demographics, number of participants, and relevant study outcomes/findings (e.g., circulating RBP4 levels). In addition, attempts were made to contact the corresponding study investigators in cases where relevant data on circulating RBP4 levels were missing or reported as median and interquartile range (IQR). Where relevant responses were not received (18–25), median and IQR data were transformed using the formulas provided by Luo et al. (26) and Wan et al. (27). Furthermore, for one study (28) these values were extracted from figures using a plot digitizer,[1] as previously reported (29).

Herein, data on circulating RBP4 levels are reported as mean and standard deviations (SDs) (30). For certain included studies (25, 31–33), it was necessary to combine study groups; this was done using recommended formulae (34).

When multiple methods were used to measure circulating RBP4 levels (23, 24), the enzyme linked immunosorbent assay (ELISA) result was chosen as the most commonly utilized method. Additionally, for Tepper et al. (35), a sensitivity analysis was conducted by switching the data to Western Blot due to the differences observed between measurements.

1 https://apps.automeris.io/wpd/

2.4 Quality assessment

The risk of bias for each included study was assessed independently by two reviewers (BML and LL) using the Risk of Bias Assessment Tool for Nonrandomized Studies (RoBANS) (36), which covers six domains, namely: selection of participants, confounding variables, exposure measurement, blinding of outcome assessment, incomplete outcome data, and selective outcome reporting. For each domain, the risk of bias was assessed as low, high, or unclear. Any disagreements were resolved through discussion between reviewers and if needed, consultation with a third reviewer (CK).

2.5 Statistical analysis

The statistical analysis was performed using Comprehensive Meta-Analysis Version 4.0 (37). The results were reported using the standardized mean difference (SMD) to quantify the magnitude of the effect and 95% confidence intervals (CI) as a measure of precision around effect estimates. The effect size represents the SMD between circulating RBP4 levels in the GDM group and the pregnant control group at different timepoints (i.e., at the first trimester, 24–28 weeks of gestation, and > 28 weeks of gestation).

A random-effects model was used for the performed meta-analysis, and the effect size for each timepoint was calculated. Heterogeneity among studies was assessed using Cochran's Q and I^2 statistics, and was considered significant if $p < 0.1$ in the Q-test whilst for the I^2: 0–40% heterogeneity might not be important; 30–60% may represent moderate heterogeneity; 50–90% may represent substantial heterogeneity; and 75–100% represents considerable heterogeneity (30).

To investigate heterogeneity, we sub-grouped studies based upon the country in which they were conducted, the diagnostic criteria used to identify GDM cases, and the RBP4 measurement method/assay. It was not possible to sub-group based upon any other variable due to the incompleteness of reporting. Supplementary Table S2.1 presents the summary of effect estimates and heterogeneity for the sub-groups at each pregnancy stage.

For the studies where mean and SDs were calculated (18–25), sensitivity analysis was performed, removing studies that contained data significantly skewed away from the normal distribution (19, 21, 22, 24).

Where analyses included ten or more studies (30), publication bias was assessed using the Egger's test and regression intercept. Additionally, a Duval and Tweedie's trim-and-fill analysis was conducted to obtain an adjusted summary effect that accounts for publication bias.

3 Results

3.1 Study selection

A total of 354 articles were initially identified from the searched databases. Following deduplication in RefWorks, this number was refined to 155 unique records that required screening. Out of these, 101 records were excluded during the title and abstract screening process. The remaining 54 were successfully retrieved and the full texts were assessed for eligibility, resulting in the exclusion of 20 reports for various reasons, i.e., one was a duplicate, five had the wrong outcome,

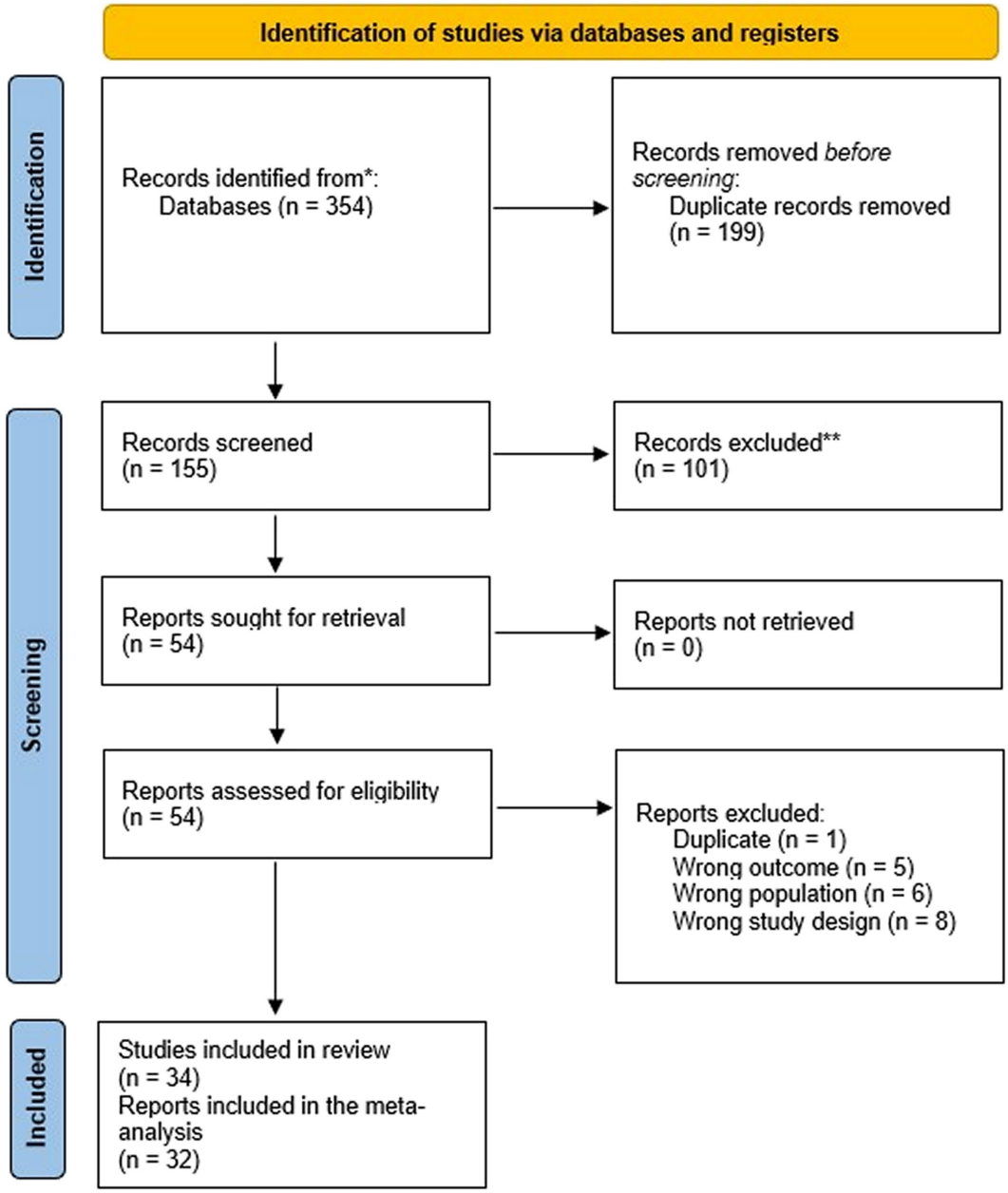

FIGURE 1
PRISMA flow diagram of the present systematic review.

six involved the wrong population, and eight had the wrong study design (Figure 1). Furthermore, two studies (38, 39) were included in the review, but excluded from the meta-analysis because the reported data on RBP4 levels could not be extracted/converted for meta-analysis and repeated attempts to contact the authors were unsuccessful.

3.2 Risk of bias assessment

The risk of bias assessment of the included studies is presented in Figure 2 and in Supplementary Figure S2.2. Most studies ($n = 24$; 70.5%) had a low risk of bias in participant selection, although some lacked clarity in their selection methods (eight studies with high risk of bias, and two with unclear; Supplementary Figure S2.2). When it came to controlling for confounding variables, 27 studies (77%) were rated as having a low risk of bias, with four having an unclear risk, and three having a high risk in this regard. When assessing the exposure measurement, in five studies the exact criteria used to diagnose GDM were unclear, while the rest of the studies were classified as having a low risk of bias (87.1%). In terms of utilizing a valid measurement method for RBP4, 32 studies (94.1%) had a low risk of bias, but two had unclear measurement methods. Given that none of the studies were interventional, and therefore did not report on assessor blinding, all had an unclear risk of bias in blinding the outcome assessment. Concerning handling incomplete outcome data, one study was at a high risk of bias, while one other had an unclear risk in this category; the remaining

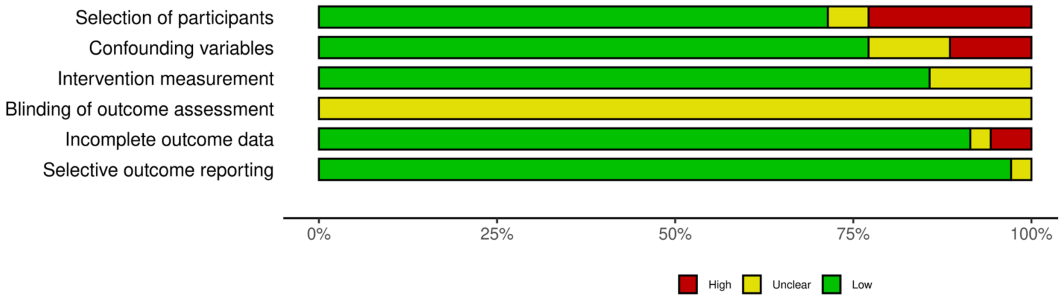

FIGURE 2
Risk of bias assessment - summary plot.

studies ($n = 32$; 91%) were judged to have a low risk of bias. In the selective outcome reporting domain, all studies apart from one had a low risk of bias (13, 18–25, 28, 31–33, 35, 39–57); Zhu et al. (58) was judged to have an unclear risk.

3.3 Main characteristics of the included studies

The main characteristics of the included studies are presented in Table 2, and reported circulating RBP4 levels are presented in Supplementary Table S1.3. Of the 34 eligible studies, nine measured circulating RBP4 levels in the first trimester, 21 at 24–28 weeks, and 14 at >28 weeks of gestation. However, two studies did not report the measured RBP4 levels in a way that could be extracted (38, 39), so were not included in the meta-analysis. When sensitivity analyses were conducted by removing the studies with skewed data, the effect on estimates was negligible, therefore they were included in the analysis. The final selected studies included a total of 3,595 GDM cases and 4,544 non-GDM controls.

3.4 Circulating RBP4 levels in the first trimester of pregnancy

From the nine studies that examined the relationship between circulating RBP4 levels during the first pregnancy trimester and GDM, seven were meta-analyzed (13, 18–20, 32, 41, 54) (946 GDM vs. 1701 non-GDM controls). Based on these, circulating RBP4 levels were statistically higher in pregnant women with GDM compared to pregnant controls (SMD: 0.322; 95% CI: 0.126 to 0.517; $p < 0.001$) (Figure 3). Moreover, there was substantial heterogeneity among these studies ($I^2 = 80\%$), although it is essential to acknowledge that the low number of eligible studies may limit the reliability of heterogeneity estimates (30). Additionally, removal of the study with skewed data in a sensitivity analysis (19) slightly reduced the SMD (0.309, 95% CI: 0.078–0.539; $p = 0.009$) (Supplementary Table S2.3).

3.5 Circulating RBP4 levels at 24–28 weeks of gestation

A total of 19 studies investigated the relationship between circulating RBP4 and GDM at 24–28 weeks of gestation and reported the corresponding RBP4 levels, with 1776 GDM cases and 1942 non-GDM controls in the performed meta-analysis. When compared to controls, circulating RBP4 levels at 24–28 weeks of gestation were significantly higher in women with GDM (SMD: 0.628; 95% CI: 0.290–0.966; $p < 0.001$) (Figure 4). Heterogeneity among these studies was considerable ($I^2 = 95\%$). Furthermore, when switching the reported RBP4 data from the Tepper et al. study (35) to their Western Blot reported data, the effect estimate remained similar at 0.620 (95% CI: 0.282–0.959; $p < 0.001$). Additionally, removal of the skewed studies (19, 21, 22) increased the effect size (SMD: 0.702; 95% CI: 0.289–1.115; $p = 0.001$). A one-study-removed analysis was also performed, as presented in Supplementary Figure S2.4.

3.6 Circulating RBP4 levels at more than 28 weeks of gestation

In total, 14 eligible studies compared circulating RBP4 at >28 weeks of gestation and reported the corresponding RBP4 levels (870 GDM cases vs. 901 non-GMD controls). Based on these, circulating RBP4 levels at >28 weeks of pregnancy were statistically higher in women with GDM compared to non-GDM controls (SMD: 0.875; 95% CI: 0.252–1.498; $p = 0.006$) (Figure 5). Considerable heterogeneity was noted among these studies ($I^2 = 97\%$), suggesting potential differences in the true effect sizes among the populations under investigation. Furthermore, removal of the study with skewed data (24) slightly increased the SMD to 0.984 (95% CI: 0.348–1.620). A one-study-removed analysis was also performed, as presented in Supplementary Figure S2.5.

3.7 Sub-group analysis

During the first trimester, sub-group analyses were completed for GDM diagnosis, RBP4 measurement method/assay, and country of study (Supplementary Table S2.1). Regarding the applied GDM diagnostic criteria, only one sub-group (IADPSG criteria) had more than one study in; in this group, the effect estimate was increased (SMD: 0.347, 95% CI: 0.073–0.621), but so too was the degree of heterogeneity ($I^2 = 85.4\%$). For RBP4 measurement, there was also only one subgroup with more than one study included. Three studies used an R&D Systems ELISA (SMD: 0.305, 95% CI 0.203–0.406) which reduced the I^2 in that sub-group to 0%. Similarly for country in

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TABLE 2 General characteristics of the eligible studies included in the present systematic review and meta-analysis.

Authors (Country)	Group Characteristics	GDM diagnosis made by	Assay for RBP4	Unit	Data measured at (weeks of gestation)	Key outcome(s)
Abetew DF et al., 2013 (41), (United States)	GDM ($N = 173$, age = 34.15 ± 4.56); Controls ($N = 187$, age = 32.95 ± 4.32)	ADA	ELISA (Catalog number DRB400, Quantikine TM, R&D Systems, Minneapolis, MN, United States)	μg/mL	16	The mean serum RBP4 level was significantly higher among GDM cases than controls. There was modest evidence of a positive association of early pregnancy elevated RBP4 concentration with increased GDM risk, particularly among women of advanced age.
Chan TF et al., 2007 (42), (Taiwan)	GDM ($N = 20$, age = 32.7 ± 5, BMI = 26.1 ± 4.7); Controls ($N = 20$, age = 32.7 ± 5, BMI = 25.9 ± 2.9)	NDDG	ELISA (Immundiagnostik AG, Bensheim, Germany)	ng/mL	24–28, upon delivery	Serum RBP4 concentrations at glucose challenge test were significantly higher in the GDM group than in the healthy control group. BMI was significantly correlated to serum RBP4 concentrations by multiple linear regression analysis.
Chen and Du, 2011 (31), (China)	GDM (Obesity: age = 32 ± 4.8, normal weight: age = 31.7 ± 3.5, $N = 52$); Controls (Obesity: age = 28.4 ± 3.1, normal weight: age = 28.3 ± 3, $N = 46$)	N/A	ELISA	μg/L	37–39	Serum RBP4 levels were higher in obese pregnant women than in non-obese women. RBP4 levels in GDM with obesity were higher than in other groups.
Du M et al., 2016 (43), (China)	GDM ($N = 38$, age = 28.79 ± 4.04); Controls ($N = 38$, age = 28.92 ± 3.02)	NDDG	ELISA (R&D Company, United States)	μg/mL	37–42	RBP4 levels were higher in women with GDM. In healthy controls, RBP4 concentrations were positively correlated with HOMA-IR and TG.
Du X et al., 2019 (44), (China)	GDM ($N = 194$, age = 31.71 ± 3.63); Controls ($N = 67$, age = 31 ± 3.43)	FIGO	ELISA (R&D Systems in the United States of America)	μg/mL	24–28, 37–40	RBP4 levels were significantly higher in the GDM group compared to control group. RBP4 is related to GDM, and its levels increase with the increase of gestational weeks.
Ping F et al., 2012 (45), (China)	GDM ($N = 488$); GIGT ($N = 235$); NGT ($N = 582$); Normal (GCT−) ($N = 290$)	ADA	ELISA (Phoenix, Belmont, CA, United States EK-028-28)	μg/mL	13–15, 24–28	The estimated indices of IR gradually increased from NGT to GDM. RBP4 mRNA expression in adipose tissue of GDM patients was significantly increased.
Francis E et al., 2020 (39), (United States)	GDM ($N = 107$, age = 30.5 ± 5.7); Controls ($N = 214$, age = 30.4 ± 5.4)	Carpenter-Coustan	Quantikine Human RBP4 Immunoassay (R&D Systems)	N/A	10–14, 15–26, 23–31, 33–39	Adipokines, including FABP4, chemerin, and sOB-R may be implicated in the pathogenesis of GDM, with significant associations detected approximately 10–18 weeks before typical GDM screening. Chemerin and RBP4 were associated with a worse lipid profile.

(Continued)

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TABLE 2 (Continued)

Authors (Country)	Group Characteristics	GDM diagnosis made by	Assay for RBP4	Unit	Data measured at (weeks of gestation)	Key outcome(s)
Fruscalzo A et al., 2015 (32), (Italy)	GDM (iGDM: age = 33.55 ± 4.06, dGDM: age = 33.43 ± 4.03) (*N* = 32); Controls (AGA: age = 37.18 ± 4.44, LGA: age = 32.85 ± 3.47) (*N* = 64)	IADPSG	Non-commercial ELISA using polyclonal rabbit anti-human antibodies (Biozol, Eching, Germany)		11–13	GDM patients were characterised by reduced RBP4 compared to controls.
Gashlan H et al., 2017 (46), (Saudi Arabia)	GDM (*N* = 51, age = 32.4 ± 0.98, BMI = 33.8 ± 1.01); Controls (*N* = 37, age = 34 ± 1.52, BMI = 33.4 ± 0.81)	WHO	Assay from Elabscience Company (Wuhan, China)	ng/mL	2nd trimester, 3rd trimester	RBP4 was significantly decreased in GDM compared to control and was significantly correlated with IR in the GDM group only.
Gorkem U et al., 2016 (21), (Turkey)	GDM (*N* = 76, age = 29 (24–28), BMI = 33.25 (22.8–52.2)); Controls (*N* = 82, age = 26 (18–35), BMI = 26.43 (19.1–47))	Carpenter-Coustan	ELISA (Immundiagnostik, Immundiagnostik AG; Bensheim, Germany)	mg/mL	24–28	Serum RBP4 did not demonstrate significant differences between GDM and controls.
Hou W et al., 2018 (18), (China)	GDM (*N* = 131, age = 31.4 ± 3.8); Controls (*N* = 138, age = 30.4 ± 3.8)	IADPSG	N/A	mg/L	12	Multivariate models combining clinical markers and metabolites can potentially differentiate GDM subjects from healthy controls. Pre-pregnancy BMI was higher in GDM participants, as were ChE, RBP4, CysC and TG.
Jia X et al., 2022 (47), (China)	GDM (*N* = 62, age = 29.38 ± 4.65, BMI = 22.79 ± 2.93); Controls (*N* = 58, age = 28.93 ± 3.31, BMI = 25.8 ± 3.04)	People's Republic of China Health Industry Standards	ELISA (American RD Company, San Francisco, CA, United States)	μg/mL	24–28	There were no statistically significant differences in RBP4 levels in GDM compared to healthy pregnancies. There were higher serum FGF-21 levels in GDM, which might be related to pre-pregnancy BMI, weight gain during pregnancy, leptin, RBP4, and adiponectin.
Jin C et al., 2020 (19), (China)	GDM (*N* = 135, age = 29 (28–33)); Controls (*N* = 135, age = 29 (28–33))	IADPSG	ELISA (R&D Systems China, Shanghai)	μg/L	< 14, 24–28	The GDM cases had significantly higher levels of RBP4 in the first trimester than controls. With adjustment for diet, physical activity, and other risk factors for GDM, the risk of GDM increased with every 1-log μg/L increment of RBP4 level.
Khovidhunkit W et al., 2012 (33), (Thailand)	GDM (*N* = 171, age = 33 (29–37)); Non-GDM (GIGT, NGT) (*N* = 361, age = 33 (28–36)); GCT– (*N* = 22, age = age = 32 (26–39))	Carpenter-Coustan	ELISA (R&D Systems Minneapolis, MN)	μg/mL	24–28	The degree of IR was higher in the GDM group than the non-GDM group, but serum RBP4 levels between the 2 groups were not different. Serum RBP4 levels in pregnancy are not associated with IR.

(Continued)

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TABLE 2 (Continued)

Authors (Country)	Group Characteristics	GDM diagnosis made by	Assay for RBP4	Unit	Data measured at (weeks of gestation)	Key outcome(s)
Kim SH et al., 2007 (48), (South Korea)	GDM ($N = 10$, age = 32.6 ± 3); Controls ($N = 9$, age = 32.6 ± 3.3)	ADA	ELISA (Immundiagnostik, Bensheim, Germany)	μg/mL	24–28	Women with GDM had higher RBP4 concentrations than those seen in healthy women during pregnancy, but short-term rise in serum insulin did not modulate circulating RBP4 concentrations.
Klein K et al., 2010 (49), (Austria)	GDM ($N = 63$, age = 33.3 ± 4.8, BMI = 28.1 ± 6.2); Controls ($N = 38$, age = 32.7 ± 5.2, BMI = 27.7 ± 5.6)	German & Austrian Society for Diabetes (modified Carpenter Coustan)	ELISA (DRG Instruments, Marburg, Germany)	mg/L	24–28, 33	Serum RBP4 levels increased significantly between the two measurements in patients with GDM. In patients with GDM, RBP4 concentrations at 33 weeks of gestation correlated positively with mean blood glucose and HbA1c values.
Krzyzanowska K et al., 2008 (24), (Austria)	GDM ($N = 41$, age = 33 (29–35), BMI = 34 (29–38); Controls ($N = 45$, age = 28 (24–34), BMI = 29 (25–31))	4th Workshop Conference of GDM	ELISA (RBP4 EIA kit; Phoenix Pharmaceuticals, Belmont, CA, United States)	μg/mL	29, 30, 8 weeks after delivery	Women with GDM had lower RBP4 levels than controls. The RBP4: retinol ratio and the RBP4:TTR ratio are more informative than RBP4 levels alone when assessing insulin–glucose homeostasis during pregnancy.
Kuzmicki M et al., 2011 (22), (Poland)	GDM ($N = 88$, age = 29.5 (27–33), BMI = 27.2 (25.2–30.1)); Controls ($N = 86$, age = 29.5 (27–31.5), BMI = 27.3 (23.1–29.4))	WHO	ELISA (Phoenix Pharmaceuticals, Inc., United States)	mg/L	24–30, 36–40	Serum RBP4 concentration and its expression in SAT were higher in the women with GDM than in the controls. No association between serum or tissue RBP4 and the indices of IR was noted.
Lewandowski KC et al., 2008 (50), (Poland)	GDM (GCT+, OGTT+) ($N = 15$, age = 34 (29–36), BMI = 26.3 (29.4–30.1)); IGT (GCT+, OGTT−) ($N = 15$, age = 32 (32–36), BMI = 25.1 (23.7–28.4)); Controls (GCT−, OGTT−) ($N = 20$, age = 32 (29–35), BMI = 25.1 (23.5–28.2))	WHO	Commercial RBP4 assay kit (Phoenix Pharmaceuticals Inc.: Burlingame, California, United States)	μg/mL	28	RBP4 levels were higher in women with GDM than in controls but did not correlate with IR.
Liu M et al., 2020 (51), (China)	GDM ($N = 50$, age = 33.88 ± 4.22, BMI = 27.69 ± 4.47); Controls ($N = 47$, age = 33.66 ± 3.97, BMI = 27.39 ± 2.32)	IADPSG	ELISA (Cusabio Biotech, Wuhan, Hubei, China)	μg/mL	37–42	GDM subjects had a lower RBP4/TTR ratio than the control subjects.
Maghbooli Z et al., 2010 (52), (Iran)	GDM ($N = 92$, age = 32.48 ± 5.23); Controls ($N = 100$, age = 27.88 ± 7.07)	O'Sullivan and Mahan criteria	ELISA (AdipoGen Kit, AdipoGen, Seoul, Korea)	μg/mL	24–28	RBP4 concentrations in GDM patients were significantly higher than in controls.

(Continued)

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TABLE 2 (Continued)

Authors (Country)	Group Characteristics	GDM diagnosis made by	Assay for RBP4	Unit	Data measured at (weeks of gestation)	Key outcome(s)
Mazaki-Tovi S et al., 2010 (25), (United States)	GDM (AGA: age = 34 (28–39), LGA: age = 32 (30–38)) (*N* = 97); Controls (AGA: age = 26 (22–29), LGA: age = 28 (22–32)) (*N* = 108)	WHO	Sensitive ELISA (Millipore Corporation, St. Charles, MO, United States)	ng/mL	>37	Patients with GDM had a higher median plasma concentration of RBP4 than normal pregnant women. GDM is characterized by alterations in maternal circulating RBP4 concentrations.
Nanda S et al., 2013 (20), (United Kingdom)	GDM (*N* = 60, age = 32 (28.5–35.6), BMI = 28.6 (24.6–4.2)); Controls (*N* = 240, age = 33 (27.3–35.9), BMI = 23.8 (21.7–26.2)); Pre-eclampsia (*N* = 60); LGA (*N* = 60); SGA (*N* = 60)	WHO	ELISA (Immundiagnostik, Stubenwaldallee, Bensheim, Germany	ng/mL	11–13	The serum concentration of RBP4 in the first trimester was not significantly different between the groups.
Ortega-Senovilla H et al., 2011 (28), (Spain)	GDM (*N* = 98, age = 30.9 ± 0.5); Controls (*N* = 86, age = 28.7 ± 0.5)	Carpenter-Coustan	Sandwich ELISA (AdipoGen, Seoul, Korea)	μg/mL	1 week before delivery	Maternal serum insulin, insulin-to-glucose ratio, HOMA-IR and RBP4 were higher, and adiponectin was lower in GDM than in control subjects.
Saucedo R et al., 2011 (40), (Mexico)	GDM (*N* = 60, age = 31.9 ± 5.6, BMI = 30.2 ± 4.9); Controls (*N* = 60, age = 24.8 ± 6.4, BMI = 28.4 ± 7.3)	ADA	RIA, using reagents from Phoenix Pharmaceuticals (Belmont, CA)	μg/mL	30, 6 weeks postpartum, 6 months postpartum	Women with GDM showed higher IR than controls. There was no difference in adipokines between the two groups, but in women with a healthy pregnancy, RBP4 was associated with IR.
Skvarca A et al., 2012 (23), (Slovenia)	GDM (*N* = 30, age = 30.33 ± 4.86, BMI = 27.57 (24.88–29.76)); IGT (*N* = 19, age = 30.84 ± 4.51, BMI = 27.61 (23.78–31.18)); Controls (*N* = 25, age = 31.2 ± 3.34, BMI = 25.39 (23.18–27.43))	4th Workshop Conference of GDM	Commercially available ELISA	mg/L	24–28	Significant differences in HOMA–IR were found, but no significant differences in serum adipokine levels. Adiponectin, leptin, resistin, visfatin and RBP4 were not associated with the degree of glucose intolerance in pregnancy.
Su YX et al., 2010 (53), (China)	NP-NGT (*N* = 65, age = 28.1 ± 3.4); GDM (*N* = 63, age = 28.8 ± 1.8, BMI = 25.5 ± 2.6); Controls (*N* = 58, age = 28.4 ± 2.4, BMI = 24.9 ± 2.1)	ADA	Sandwich ELISA (a protocol developed in-house) using affinity chromatography-purified polyclonal and monoclonal antibodies generated against recombinant human RBP4 protein	mg/L	24–28	Serum RBP4 levels in the pregnant NGT and GDM groups were significantly higher than in the non-pregnant. RBP4 levels were much higher in the GDM vs. pregnant NGT group. Serum RBP4 levels significantly increase in pregnancy, independent of age and BMI. RBP4 levels appear to be a valuable marker of IR and dysfunctional lipid metabolism in pregnancy.

(Continued)

TABLE 2 (Continued)

Authors (Country)	Group Characteristics	GDM diagnosis made by	Assay for RBP4	Unit	Data measured at (weeks of gestation)	Key outcome(s)
Tepper BJ et al., 2010 (35), (United States)	GDM ($N = 12$, age = 28.6 ± 4.9, BMI = 31.1 ± 0.6); Controls ($N = 10$, age = 28.8 ± 6.2, BMI = 31.1 ± 0.9)	Carpenter-Coustan	ELISA	μmol/L	24–28	RBP4, retinol and RBP4/retinol molar ratio were not different between the groups; GDM is not associated with RBP4 or retinol among borderline-obese pregnant women.
Wu P et al., 2021 (13), (China)	GDM ($N = 332$, age = 28 (25–30)); Controls ($N = 664$, age = 28 (25–30))	IADPSG	ELISA (R&D Quantikine)	μg/mL	9–12	RBP4 was associated with a 1.39-fold higher risk of GDM. Serum RBP4 levels in early pregnancy, independent of metabolic risk factors, are positively associated with the risk of GDM.
Yuan X et al., 2017 (54), (China)	GDM ($N = 86$, age = 29 (27–33), BMI = 24.58 (21.72–26.98)); Controls ($N = 273$, age = 26 (24–28.25), BMI = 22.32 (20.66–28.25))	IADPSG	Automatic biochemical analyzer (Hitachi 7,180; Hitachi, Ibaraki-ken, Japan) using commercial kits (Wako Pure Chemical Industries, Osaka, Japan)	μg/mL	16–18	The group that developed GDM had statistically significantly higher concentrations of ficolin-3, CRP, RBP4 and FFAs than the control group. The elevated ratios of RBP4/adiponectin were also observed in participants who developed GDM.
Zhang H et al., 2022 (55), (China)	GDM ($N = 70$, age = 25.68 ± 4.27); Controls ($N = 70$, age = 27.02 ± 3.54)	Obstetrics and Gynecology Section of the Chinese Medical Association	ELISA (R&D System, United States)	mg/L	35–40	Glucose metabolism and islet function in women with GDM are significantly correlated with serum RBP4.
Zhang Y et al., 2016 (56), (China)	GDM ($N = 40$, age = 32.24 ± 3.81, BMI = 27.55 ± 3.4); Controls ($N = 240$, age = 28.21 ± 4.12, BMI = 24.31 ± 2.92)	IADPSG	ELISA (R&D Systems, China, Shanghai)	mg/L	24–28, >37	The GDM group showed greater levels of AFABP, leptin and RBP4 and a decreased adiponectin level.
Zhaoxia L et al., 2014 (57), (China)	GDM ($N = 35$, age = 29 ± 2.53); Controls ($N = 35$, age = 29.3 ± 3.06)	NDDG	Double antibody sandwich ELISA (Phoenix Pharmaceutical Company, Saint Joseph, MO)	μg/mL	24–28	Serum RBP4 levels in the GDM group were significantly higher than in the control group. Serum RBP4 levels in the GDM group were correlated with HOMA-IR, TG and blood glucose levels.
Zhu JP et al., 2014 (58), (China)	GDM ($N = 177$); Controls ($N = 354$)	N/A	N/A	mg/L	24–28	The plasma glucose, serum insulin, HOMA-IR, HbA1C and TG levels were significantly higher in the GDM group than in the controls. RBP4 levels of GDM women were significantly and positively correlated with the BMI.

Units: age: years; BMI: kg/m^2. ADA, American Diabetes Association; AFABP, adipocyte fatty acid-binding protein; AGA, appropriate for gestational age; BMI, body mass index; ChE, chemerin E; CRP, C-reactive protein; CysC, cystatin C; dGDM, diet treated gestational diabetes mellitus; ELISA, enzyme-linked immunosorbent assay; FABP4, fatty acid binding protein 4; FGF-21, fibroblast growth factor 21; FIGO, International Federation of Gynecology and Obstetrics; GCT−, normal glucose challenge test; GCT+: abnormal glucose challenge test; GDM, gestational diabetes mellitus; GIGT, gestational impaired glucose tolerance; HbA1c, glycated hemoglobin; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; IADPSG, International Association of Diabetes and Pregnancy Study Groups; iGDM, insulin treated gestational diabetes mellitus; IGT, impaired glucose tolerance; IR, insulin resistance; LGA, large for gestational age; mRNA, messenger ribonucleic acid; NDDG, National Diabetes Data Group; NGT, normal glucose tolerance; OGTT−, normal oral glucose tolerance test; OGTT+, abnormal oral glucose tolerance test; PAI-1, plasminogen activator inhibitor-1; RBP4, retinol-binding protein 4; RIA, radioimmunoassay; SAT, subcutaneous adipose tissue; sOB-R, soluble leptin receptor; TG, triglycerides; TTR, transthyretin; WHO, World Health Organization.

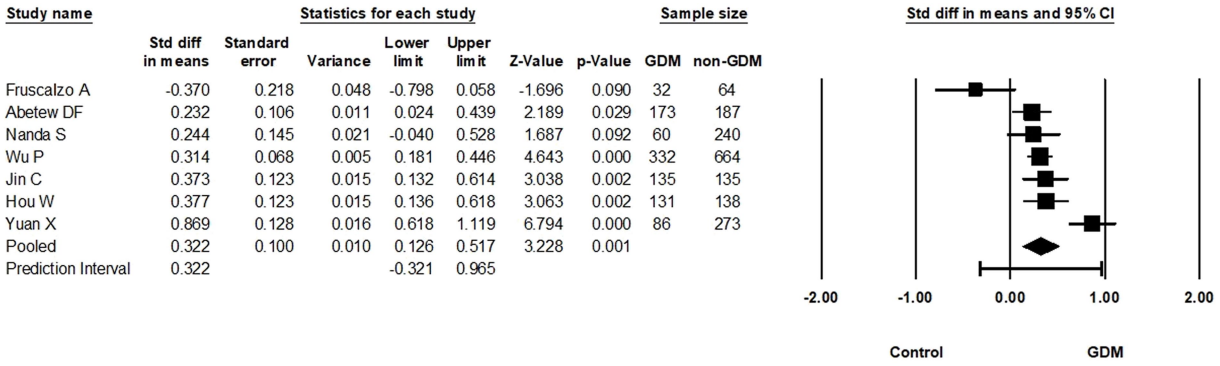

FIGURE 3
Forest plot of circulating RBP4 levels: gestational diabetes mellitus (GDM) compared to control in the first trimester of pregnancy. Std diff in means: standardized mean difference; CI: Confidence intervals.

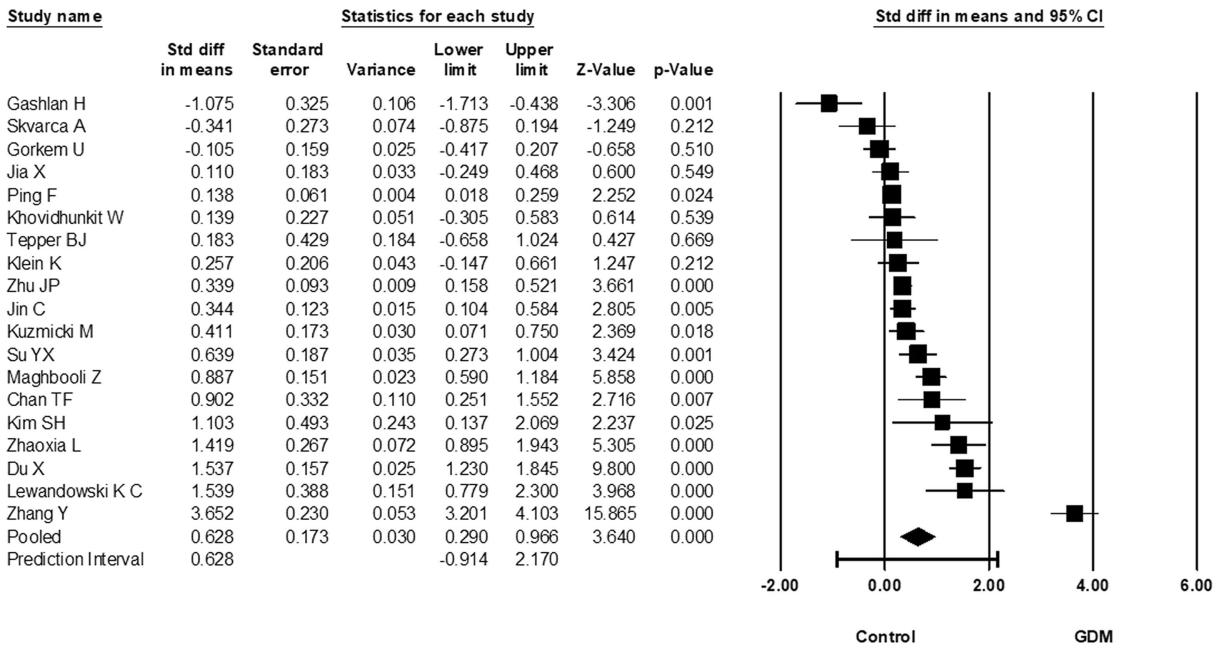

FIGURE 4
Forest plot of circulating RBP4 levels: gestational diabetes mellitus (GDM) compared to control at 24-28 weeks of gestation. Std diff in means: standardized mean difference; CI: Confidence intervals.

which included studies were conducted, it was only studies from China that constituted a group including multiple studies; the effect estimate was larger (SMD: 0.473, 95% CI: 0.237–0.708) in this sub-group, but the I^2 was practically unchanged.

For 24–28 weeks of gestation, the sub-group analysis based upon the applied GDM diagnostic criteria identified four sub-groups that contained more than one study (Supplementary Table S2.1). As such, studies using the ADA, or Carpenter-Coustan, or 4th Workshop conference criteria were grouped together based on the applied GDM diagnostic cut-offs/criteria specified in the corresponding papers (7 studies, 1894 participants). For these, the statistical effect was lost, whilst the I^2 was reduced to 63.5%. For the sub-group of studies using the IADPSG, or FIGO, or People's Republic of China Health Industry Standards criteria (4 studies, 931 participants), the SMD increased to 1.402 (95% CI: 0.084 to 2.721) and so too did the I^2 (98.5%). When the studies using the WHO criteria were grouped there was still considerable heterogeneity (93.0%), and the statistical effect was lost. Finally, for the two studies using the NDDG GDM criteria, the effect estimate retained statistical significance (SMD: 1.198, 95% CI: 0.696–1.699), whilst heterogeneity was reduced to an amount that may not be important ($I^2 = 32.1\%$). When RBP4 measurement method/assay was sub-grouped, three sub-groups were created (Supplementary Table S2.1). The sub-groups which used either an R&D Systems (five studies; SMD: 1.151, 95% CI: 0.042–2.260) or a Phoenix Pharmaceuticals (five studies; SMD: 0.699, 95% CI: 0.167–1.232) ELISA retained statistical effects, but with considerable heterogeneity ($I^2 = 98.1$ and 88.3%, respectively). For the third sub-group which used an Immundiagnostik AG ELISA, the statistical effect estimate was lost, whilst there was still evidence of

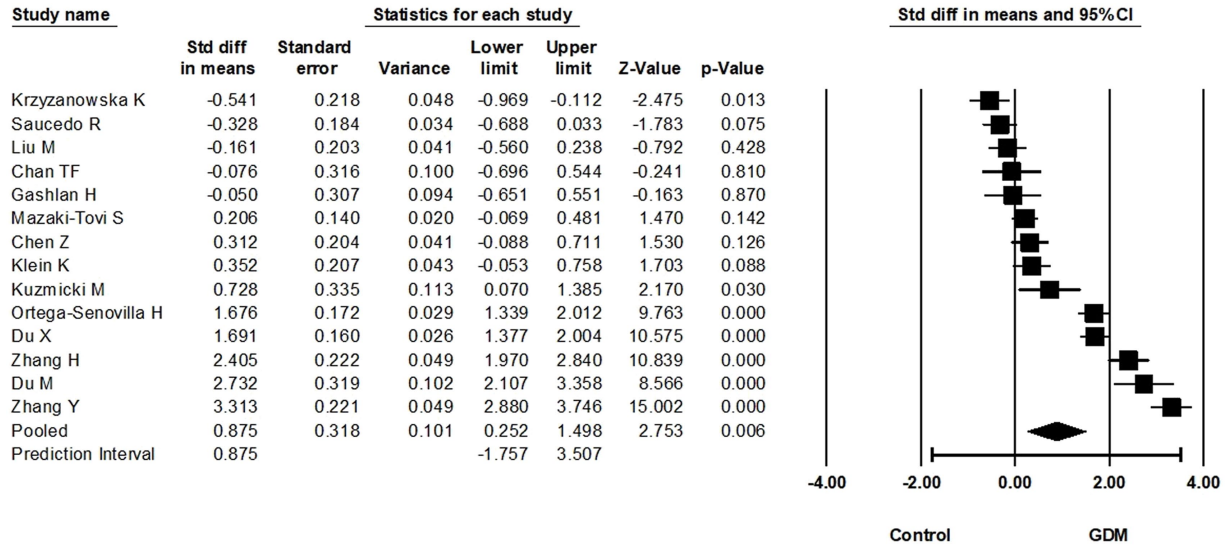

FIGURE 5
Forest plot of circulating RBP4 levels: gestational diabetes mellitus (GDM) compared to control at more than 28 weeks of gestation. Std diff in means: standardized mean difference; CI: Confidence intervals.

substantial heterogeneity ($I^2 = 78.8\%$). Finally, for sub-group analysis based upon country in which included studies were conducted, only two countries had more than one study, namely China (8 studies, SMD: 1.001; $I^2 = 97.6\%$) and Poland (2 studies, SMD: 0.922; $I^2 = 85.8\%$). A considerable degree of heterogeneity was apparent in both these sub-groups, while a statistical effect was retained for the studies from China only (Supplementary Table S2.1).

When sub-group analysis was completed based upon the applied GDM diagnostic criteria for the >28 weeks of gestation timepoint (Supplementary Table S2.1), a statistical effect was not retained for any of these sub-groups. The heterogeneity remained at a considerable level ($I^2 > 95\%$) for all but one of these sub-groups, namely the sub-group of studies that applied the WHO criteria for which the heterogeneity was reduced to a level that may not be important (3 studies; $I^2 = 34.7\%$). When RBP4 measurement methods/assays were sub-grouped, only two sub-groups were formed. For the four studies which used the R&D Systems ELISA (SMD: 2.337, 95% CI: 2.130–2.544) a statistical effect was retained, whereas for the two studies which used the Phoenix Pharmaceuticals ELISA the statistical effect was lost. Both these sub-groups demonstrated a considerable amount of heterogeneity ($I^2 \geq 90\%$). Finally, based upon country in which included studies were conducted, sub-group analysis was possible only for China (six studies) and Austria (two studies). A statistical effect was retained for the studies from China (SMD: 1.708, 95% CI: 0.634–2.782), but not for the studies from Austria. Both these sub-groups had a considerable degree of heterogeneity ($I^2 > 88\%$).

3.8 Publication bias

Egger's regression intercept test indicated that publication was not present at 24–28 weeks of gestation ($t = 1.3$, $p = 0.2$) (Supplementary Figure S2.6) nor at >28 weeks of gestation ($t = 0.2$, $p = 0.8$) (Supplementary Figure S2.7).

4 Discussion

The pathogenesis of GDM remains a subject of intense research interest due to the increasing GDM prevalence and the potential significant health implications for both mothers and offspring. In this context, recent research has further focused on novel factors (e.g., circulating adipokines such as RBP4) which appear implicated in the pathogenesis of GDM and may be utilized as GDM biomarkers (59). Therefore, this systematic review and meta-analysis aimed to offer up-to-date, comprehensive evidence on the relationship between circulating RBP4 levels and GDM at various timepoints across the pregnancy. The present meta-analyses included data from seven eligible studies examining circulating RBP4 levels in the first trimester, 19 studies at 24–28 weeks, and 13 studies at >28 weeks of pregnancy. Overall, the results showed statistically higher RBP4 levels in women with GDM compared to non-GDM controls at these different pregnancy timepoints.

Indeed, such a statistical difference in the circulating RBP4 levels was evident during the first trimester when women with and without GDM were compared. This finding suggests that circulating RBP4 levels in early pregnancy may be an early biomarker for GDM; although, the limited number of eligible existing studies for this early pregnancy timepoint warrants caution in interpreting this finding. Nevertheless, this is in accord to that from a previous meta-analysis from Wu et al. (13) on the association between RBP4 levels in early pregnancy and GDM risk. However, the paucity of relevant data for this pregnancy trimester/timepoint was also noted in this previous meta-analysis, together with potential ethnic-related differences; hence, further research is clearly required to determine if circulating RBP4 has potential as a GDM-related biomarker during the first trimester.

The present meta-analysis also revealed statistically higher circulating RBP4 levels in GDM cases compared to non-GDM controls at 24–28 weeks of gestation. The noted moderate effect size during this pregnancy period suggests that such elevated circulating RBP4 levels may be associated to GDM. This finding is in accord with

previous research indicating the potential role of RBP4 in insulin resistance and glucose metabolism regulation after the first trimester of pregnancy (13–15). Thus, monitoring circulating RBP4 levels in pregnant women during the second trimester could be further explored as a potential GDM biomarker.

Finally, at >28 weeks of pregnancy, our meta-analysis also revealed higher circulating RBP4 levels in patients with GDM compared to non-GDM controls. The relatively large effect size noted for this gestation period indicates a potential relationship between these RBP4 levels in late pregnancy and GDM. Indeed, it is plausible that elevated circulating RBP4 levels at this stage may reflect an intensified insulin-resistant state, a hallmark of GDM, although further research is also required to establish this link.

Collectively, the findings of the present systematic review and meta-analysis offer updated evidence, which is also in line with Huang et al. (14) who conducted the first reported meta-analysis of observational studies aiming to investigate the relationship between circulating RBP4 levels and GDM. Indeed, their data included a total of 14 studies with 884 women with GDM and 1,251 normoglycemic pregnant women. Similar to the present meta-analysis, their overall results showed that circulating RBP4 levels were significantly higher in women with GDM compared to the studied controls. However, their stratified results indicated that this significant difference was observed only in the second/third trimester and was limited to Asian populations. This may be, at least in part, attributed to the lower number of eligible studies analyzed by Huang et al. (14), whilst potential ethnic differences in circulating RBP4 levels in pregnancy and GDM merits further targeted research. Another meta-analysis by Hu et al. (15) also included 14 case–control studies on serum RBP4 levels and GDM risk, involving a total of 647 GDM cases and 620 controls. This showed that high serum RBP4 levels represent a risk factor for GDM, with a pooled SMD of 0.758 (95% CI: 0.387–1.128). Their subgroup analyses based on gestational age at blood sampling and diagnostic criteria were consistent with the overall results, supporting the hypothesis that elevated RBP4 is a modest independent risk factor for GDM. However, in contrast with our present findings, no association was found by Hu et al. between circulating RBP4 levels and GDM in the first trimester. This may be partly due to changing insulin resistance levels during pregnancy; however, it should be noted that our meta-analysis included seven studies which assessed circulating RBP4 levels during the first trimester, while only one such study was included in the analyses by Hu et al. (15), potentially reducing the reliability of their stratified analysis on this point. Finally, another meta-analysis (60) that focused on the association of leptin and RBP4 with GDM risk included six studies with a total of 2,715 participants and 841 cases of GDM. In that meta-analysis, serum RBP4 levels also showed a significant positive association with the overall GDM risk, and pregnant women with the highest serum RBP4 levels were 2.04-fold more prone to GDM than those with the lowest levels. However, as with our findings, significant heterogeneity of the included studies was also noted (60). Overall, the exiting evidence supports the association of higher circulating RBP4 levels during pregnancy in patients with GDM, whilst this association appears to be more consistent in later pregnancy stages (second/third trimester), as was also documented in the aforementioned previous meta-analyses (14, 15). While this growing evidence is promising, further research is still required to advance our understanding, validate previous findings, and better explore the clinical implications of circulating RBP4 in the context of GDM.

The present meta-analysis has several strengths, including a comprehensive study selection process, thorough risk of bias assessment, and a relatively large sample size of 32 included studies with 3,595 GDM cases and 4,544 non-GDM controls, which is larger than previous meta-analyses on this topic. Indeed, by including detailed temporal analyses at different (early, mid, and late) pregnancy stages, the present work adds to the understanding of the potential association between circulating RBP4 levels and GDM. Moreover, the performed sensitivity analyses, addressing skewed data and the impact of specific studies, enhance the robustness of the present findings. Finally, our systematic review and meta-analysis addresses and evaluates potential publication bias, contributing to the overall reliability of the reported results.

However, certain limitations of the present work should also be acknowledged. Firstly, the total number of existing eligible studies included in some analyses was limited, which may have affected the robustness of the results. In addition, the study designs, participant characteristics, and laboratory methods for measuring RBP4 varied among the included studies, contributing to the observed high heterogeneity which may affect the reliability of the meta-analysis results, whilst inconsistencies in how relevant data are reported across the included studies might affect the accuracy of the present meta-analysis. A meta-regression would have been useful to help explain the high degree of heterogeneity in the analyses, but this was not performed due to inconsistencies with how continuous variables were reported across the identified studies (i.e., not all studies reported all variables). Moreover, variation in the methods used to measure circulating RBP4 levels across the identified studies could impact on the comparability of the results. Notably, most of the included studies were retrospective case–control studies, thus causality could not be established, whilst this may also introduce bias. The generalizability of the findings may be also limited by the small sample sizes of some of the included eligible studies. Furthermore, the identified statistically significant differences between GDM and non-GMD pregnancies cannot be necessarily considered as clinically significant, particularly given the proximity of the lower bound CI to zero. As is also common in systematic reviews, the possibility of publication bias, where studies with significant findings are more likely to be published, may have introduced a bias regarding the eligible studies which are published and are subsequently included in the searched databases. Finally, although multiple established biomedical databases were searched, the present systematic review identified only articles with English-language abstracts and main text written in either English or Chinese, which may have introduced a potential language bias.

5 Conclusion

The present systematic review and meta-analysis offers updated and comprehensive data which suggest that circulating RBP4 levels measured at different pregnancy timepoints/stages are higher in patients with GDM compared to non-GDM controls. Taken together with previous findings, this suggests that circulating RBP4 could be considered as a potential biomarker associated with GDM. Given that circulating levels of RBP4 are not routinely measured in the clinical practice, it is plausible that standardizing the method/assay for measuring circulating RBP4 in routine

practice and adding this measurement as part of the GDM risk assessment visit/protocol in the context of antenatal care may be helpful to promptly identify those at high risk. However, the scarcity of relevant data particularly for early pregnancy and the noted high study heterogeneity, as well as factors relating to variability in RBP4 measurement methods and GDM diagnostic criteria/protocols, highlight the need for additional research in this field. Particularly, prospective (including the first trimester) and large-scale cohort studies across diverse populations and with standardized measurements of circulating RBP4 are needed to validate the present findings and confirm the generalizability of existing evidence. Future studies should also explore the potential underlying biological mechanisms which may link RBP4 to GDM, considering key pathophysiologic factors, such as insulin resistance and obesity-related inflammation.

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

BL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. CK: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. LL: Data curation, Formal analysis, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. ADav: Data curation, Writing – original draft, Writing – review & editing. ADal: Data curation, Formal analysis, Software, Writing – original draft, Writing – review & editing. KC: Data curation, Writing – original draft, Writing – review & editing. HR: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. IK: Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Formal analysis, Investigation.

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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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1348970/full#supplementary-material

References

1. Plows JF, Stanley J, Baker P, Reynolds C, Vickers M. The pathophysiology of gestational diabetes mellitus. *Int J Mol Sci*. (2018) 19:3342. doi: 10.3390/ijms19113342

2. Khambule L, George JA. The role of inflammation in the development of GDM and the use of markers of inflammation in GDM screening. *Adv Exp Med Biol*. (2019) 1134:217–42. doi: 10.1007/978-3-030-12668-1_12

3. National Institute for Health and Care Excellence (NICE). *Diabetes in Pregnancy: Management from Preconception to the Postnatal Period*. London: National Institute for Health and Care Excellence (NICE) (2020).

4. Getahun D, Fassett MJ, Jacobsen SJ. Gestational diabetes: risk of recurrence in subsequent pregnancies. *Am J Obstet Gynecol*. (2010) 203:467.e1–6. doi: 10.1016/j.ajog.2010.05.032

5. Noctor E, Dunne FP. Type 2 diabetes after gestational diabetes: the influence of changing diagnostic criteria. *World J Diabetes*. (2015) 6:234–44. doi: 10.4239/wjd.v6.i2.234

6. Durnwald C. Gestational diabetes: linking epidemiology, excessive gestational weight gain, adverse pregnancy outcomes, and future metabolic syndrome. *Semin Perinatol*. (2015) 39:254–8. doi: 10.1053/j.semperi.2015.05.002

7. Jenum AK, Mørkrid K, Sletner L, Vange S, Torper JL, Nakstad B, et al. Impact of ethnicity on gestational diabetes identified with the WHO and the modified International Association of Diabetes and Pregnancy Study Groups criteria: a population-based cohort study. *Eur J Endocrinol*. (2012) 166:317–24. doi: 10.1530/EJE-11-0866

8. Anghebem-Oliveira MI, Martins BR, Alberton D, Ramos EAS, Picheth G, Rego FGM. Type 2 diabetes-associated genetic variants of FTO, LEPR, PPARg, and TCF7L2 in gestational diabetes in a Brazilian population. *Arch Endocrinol Metab*. (2017) 61:238–48. doi: 10.1590/2359-3997000000258

9. Farrar D, Simmonds M, Bryant M, Lawlor DA, Dunne F, Tuffnell D, et al. Risk factor screening to identify women requiring oral glucose tolerance testing to diagnose gestational diabetes: a systematic review and meta-analysis and analysis of two pregnancy cohorts. *PLoS One*. (2017) 12:e0175288. doi: 10.1371/journal.pone.0175288

10. Ji Y, Song J, Su T, Gu X. Adipokine retinol binding protein 4 and cardiovascular diseases. *Front Physiol*. (2022) 13:856298. doi: 10.3389/fphys.2022.856298

11. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature*. (2005) 436:356–62. doi: 10.1038/nature03711

12. Saucedo R, Valencia J, Basurto L, Hernandez M, Puello E, Zarate A, et al. The retinol binding Protein-4 (RBP4) gene and Gestational diabetes In: R Rajendram, VR Preedy and VB Patel, editors. *Nutrition and Diet in Maternal Diabetes: An Evidence-Based Approach*. Cham: Springer International Publishing (2018). 135–45.

13. Wu P, Wang Y, Ye Y, Yang X, Lu Q, Liu Y, et al. Serum retinol-binding protein 4 levels and risk of gestational diabetes mellitus: a nested case-control study in Chinese women and an updated meta-analysis. *Diabetes Metab Res Rev*. (2022) 38:e3496. doi: 10.1002/dmrr.3496

14. Huang QT, Huang Q, Luo W, Li F, Hang LL, Yu YH, et al. Circulating retinol-binding protein 4 levels in gestational diabetes mellitus: a meta-analysis of observational studies. *Gynecol Endocrinol*. (2015) 31:337–44. doi: 10.3109/09513590.2015.1005594

15. Hu S, Liu Q, Huang X, Tan H. Serum level and polymorphisms of retinol-binding protein-4 and risk for gestational diabetes mellitus: a meta-analysis. *BMC Pregnancy Childbirth*. (2016) 16:52. doi: 10.1186/s12884-016-0838-7

16. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. (2021) 372:n71. doi: 10.1136/bmj.n71

17. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev*. (2016) 5:210. doi: 10.1186/s13643-016-0384-4

18. Hou W, Meng X, Zhao A, Zhao W, Pan J, Tang J, et al. Development of multimarker diagnostic models from metabolomics analysis for gestational diabetes mellitus (GDM). *Mol Cell Proteomics*. (2018) 17:431–41. doi: 10.1074/mcp.RA117.000121

19. Jin C, Lin L, Han N, Zhao Z, Liu Z, Luo S, et al. Plasma retinol-binding protein 4 in the first and second trimester and risk of gestational diabetes mellitus in Chinese women: a nested case-control study. *Nutr Metabolism*. (2020) 17:1–7. doi: 10.1186/s12986-019-0425-9

20. Nanda S, Nikoletakis G, Markova D, Poon LCY, Nicolaides KH. Maternal serum retinol-binding protein-4 at 11-13 weeks' gestation in normal and pathological pregnancies. *Metab Clin Exp*. (2013) 62:814–9. doi: 10.1016/j.metabol.2012.12.011

21. Gorkem U, Kucukler FK, Togrul C, Gungor T. Are adipokines associated with gestational diabetes mellitus? *J Turk German Gynecol Assoc*. (2016) 17:186–90. doi: 10.5152/jtgga.2016.16112

22. Kuzmicki M, Telejko B, Wawrusiewicz-kurylonek N, Nikolajuk A, Zwierz-gugala D, Jelski W, et al. Retinol-binding protein 4 in adipose and placental tissue of women with gestational diabetes. *Gynecol Endocrinol*. (2011) 27:1065–9. doi: 10.3109/09513590.2011.579651

23. Skvarca A, Tomazic M, Krhin B, Blagus R, Janez A. Adipocytokines and insulin resistance across various degrees of glucose tolerance in pregnancy. *J Int Med Res*. (2012) 40:583–9. doi: 10.1177/147323001204000220

24. Krzyzanowska K, Zemany L, Krugluger W, Schernthaner GH, Mittermayer F, Schnack C, et al. Serum concentrations of retinol-binding protein 4 in women with and without gestational diabetes. *Diabetologia*. (2008) 51:1115–22. doi: 10.1007/s00125-008-1009-9

25. Mazaki-Tovi S, Romero R, Vaisbuch E, Kusanovic JP, Chaiworapongsa T, Kim SK, et al. Retinol-binding protein 4: a novel adipokine implicated in the genesis of LGA in the absence of gestational diabetes mellitus. *J Perinat Med*. (2010) 38:147–55. doi: 10.1515/jpm.2010.044

26. Luo D, Wan X, Liu J, Tong T. Optimally estimating the sample mean from the sample size, median, mid-range, and/or mid-quartile range. *Stat Methods Med Res*. (2018) 27:1785–805. doi: 10.1177/0962280216669183

27. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol*. (2014) 14:135. doi: 10.1186/1471-2288-14-135

28. Ortega-Senovilla H, Schaefer-Graf U, Meitzner K, Abou-Dakn M, Graf K, Kintscher U, et al. Gestational diabetes mellitus causes changes in the concentrations of adipocyte fatty acid-binding protein and other adipocytokines in cord blood. *Diabetes Care*. (2011) 34:2061–6. doi: 10.2337/dc11-0715

29. Jelicic Kadic A, Vucic K, Dosenovic S, Sapunar D, Puljak L. Extracting data from figures with software was faster, with higher interrater reliability than manual extraction. *J Clin Epidemiol*. (2016) 74:119–23. doi: 10.1016/j.jclinepi.2016.01.002

30. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJet al eds. *Cochrane Handbook for Systematic Reviews of Interventions Version 6.4 (Updated August 2023)*. Chichester, United Kingdom: Cochrane. (2023).

31. Chen Zy, Du J. *Change of Serum Retinol-Binding Protein-4 Level in Pregnancy Obese Subjects and GDM and Related Factors* Journal of China Medical University (2011). 405:465–468.

32. Fruscalzo A, Londero AP, Driul L, Henze A, Tonutti L, Ceraudo M, et al. First trimester concentrations of the TTR-RBP4-retinol complex components as early markers of insulin-treated gestational diabetes mellitus. *Clin Chem Lab Med*. (2015) 53:1643–51. doi: 10.1515/cclm-2014-0929

33. Khovidhunkit W, Pruksakorn P, Plengpanich W, Tharavanij T. Retinol-binding protein 4 is not associated with insulin resistance in pregnancy. *Metab Clin Exp*. (2012) 61:65–9. doi: 10.1016/j.metabol.2011.05.019

34. Liengme B, Hekman K. *Liengme's Guide to Excel 2016 for Scientists and Engineers:(Windows and Mac)*. London, United Kingdom: Academic Press (2019).

35. Tepper BJ, Kim YK, Shete V, Shabrova E, Quadro L. Serum retinol-binding protein 4 (RBP4) and retinol in a cohort of borderline obese women with and without gestational diabetes. *Clin Biochem*. (2010) 43:320–3. doi: 10.1016/j.clinbiochem.2009.10.013

36. Kim SY, Park JE, Lee YJ, Seo HJ, Sheen SS, Hahn S, et al. Testing a tool for assessing the risk of bias for nonrandomized studies showed moderate reliability and promising validity. *J Clin Epidemiol*. (2013) 66:408–14. doi: 10.1016/j.jclinepi.2012.09.016

37. Borenstein M. Comprehensive meta-analysis software In: JPT Higgins, M Egger and GD Smith, editors. *Systematic Reviews in Health Research: Meta-Analysis in Context*. 3rd ed (2022). Chichester, United Kingdom: BMJ Books, Wiley Blackwell, John Wiley & Sons Ltd.

38. Du C, Kong F. A prospective study of maternal plasma concentrations of retinol-binding protein 4 and risk of gestational diabetes mellitus. *Ann Nutr Metab*. (2019) 74:1–8. doi: 10.1159/000494888

39. Francis EC, Li M, Hinkle SN, Cao Y, Chen J, Wu J, et al. Adipokines in early and mid-pregnancy and subsequent risk of gestational diabetes: a longitudinal study in a multiracial cohort. *BMJ Open Diabetes Res Care*. (2020) 8:8. doi: 10.1136/bmjdrc-2020-001333

40. Saucedo R, Zarate A, Basurto L, Hernandez M, Puello E, Galvan R, et al. Relationship between circulating adipokines and insulin resistance during pregnancy and postpartum in women with gestational diabetes. *Arch Med Res*. (2011) 42:318–23. doi: 10.1016/j.arcmed.2011.06.009

41. Abetew DF, Qiu C, Fida NG, Dishi M, Hevner K, Williams MA, et al. Association of retinol binding protein 4 with risk of gestational diabetes. *Diabetes Res Clin Pract*. (2013) 99:48–53. doi: 10.1016/j.diabres.2012.10.023

42. Chan TF, Chen HS, Chen YC, Lee CH, Chou FH, Chen IJ, et al. Increased serum retinol-binding protein 4 concentrations in women with gestational diabetes mellitus. *Reprod Sci*. (2007) 14:169–74. doi: 10.1177/1933719106298407

43. du M, Wang B, Liang Z, Dong M, Chen D. The relationship between retinol-binding protein 4 concentrations and gestational diabetes mellitus in Chinese women. *Gynecol Obstet Investig*. (2016) 81:174–80. doi: 10.1159/000398794

44. du X, Dong Y, Xiao L, Liu GH, Qin W, Yu H. Association between retinol-binding protein 4 concentrations and gestational diabetes mellitus (A1GDM and A2GDM) in different pregnancy and postpartum periods. *Ann Transl Med*. (2019) 7:479. doi: 10.21037/atm.2019.08.45

45. Ping F, Xiang HD, Li M, Li W, Liu JT, Nie M, et al. Effects of variation in retinol binding protein 4 gene and adipose specific expression of gestational diabetes in Beijing, China. *Diabetes Res Clin Pract*. (2012) 97:283–9. doi: 10.1016/j.diabres.2012.02.017

46. Gashlan HM. Relationship between levels of retinol binding protein 4, Vaspin and Chemerin and insulin resistance in gestational diabetes mellitus. *Int J Pharm Res Allied Sci*. (2017) 6:236–50.

47. Jia X, Bai L, Ma N, Lu Q. The relationship between serum adipokine fibroblast growth factor-21 and gestational diabetes mellitus. *J Diabetes Invest*. (2022) 13:2047–53. doi: 10.1111/jdi.13889

48. Kim SH, Choi HJ, Im JA. Retinol-binding protein 4 responses during an oral glucose tolerance testing in women with gestational diabetes mellitus. *Clin Chim Acta*. (2008) 391:123–5. doi: 10.1016/j.cca.2008.01.030

49. Klein K, Bancher-Todesca D, Leipold H, Knöfler M, Haslinger P, Handisurya A, et al. Retinol-binding protein 4 in patients with gestational diabetes mellitus. *J Women's Health*. (2010) 19:517–21. doi: 10.1089/jwh.2009.1615

50. Lewandowski KC, Stojanovic N, Bienkiewicz M, Tan BK, Prelevic GM, Press M, et al. Elevated concentrations of retinol-binding protein-4 (RBP-4) in gestational diabetes mellitus: negative correlation with soluble vascular cell adhesion molecule-1 (sVCAM-1). *Gynecol Endocrinol*. (2008) 24:300–5. doi: 10.1080/09513590802141052

51. Liu MT, Chen YM, Chen DQ. Association between transthyretin concentrations and gestational diabetes mellitus in Chinese women. *Arch Gynecol Obstet*. (2020) 302:329–35. doi: 10.1007/s00404-020-05599-y

52. Maghbooli Z, Hossein-nezhad A, Mirzaei K, Karimi F, Besharati A, Omidfar K, et al. Association between retinol-binding protein 4 concentrations and gestational diabetes mellitus and risk of developing metabolic syndrome after pregnancy. *Reprod Sci*. (2010) 17:196–201. doi: 10.1177/1933719109351097

53. Su YX, Hong J, Yan Q, Xu C, Gu WQ, Zhang YF, et al. Increased serum retinol-binding protein-4 levels in pregnant women with and without gestational diabetes mellitus. *Diabetes Metab*. (2010) 36:470–5. doi: 10.1016/j.diabet.2010.06.006

54. Yuan XS, Shi H, Wang HY, Yu B, Jiang J. Ficolin-3/adiponectin ratio for the prediction of gestational diabetes mellitus in pregnant women. *J Diabetes Invest*. (2018) 9:403–10. doi: 10.1111/jdi.12688

55. Zhang H, Sun T. Correlation of blood glucose and pancreatic islet function with serum retinol-binding protein 4, serum cystatin C, and human new satiety molecule Protein-1 in pregnant women with gestational diabetes mellitus. *Evid Based Complement Alternat Med*. (2022) 2022:4247412. doi: 10.1155/2022/4247412

56. Zhang Y, Zhang HH, Lu JH, zheng SY, Long T, Li YT, et al. Changes in serum adipocyte fatty acid-binding protein in women with gestational diabetes mellitus and

normal pregnant women during mid- and late pregnancy. *J Diabetes Invest*. (2016) 7:797–804. doi: 10.1111/jdi.12484

57. Zhaoxia L, Mengkai D, Qin F, Danqing C. Significance of RBP4 in patients with gestational diabetes mellitus: a case-control study of Han Chinese women. *Gynecol Endocrinol*. (2014) 30:161–4. doi: 10.3109/09513590.2013.871515

58. Zhu JP, Ji Y, Tan M, Teng Y. Variations of retinol-binding protein 4 level of patients with non-obese gestational diabetes mellitus and the influence factors. *J Shanghai Jiaotong Univ (Med Sci)*. (2014) 34:1506–10. doi: 10.3969/j.issn.1674-8115.2014.10.018

59. Valencia-Ortega J, González-Reynoso R, Ramos-Martínez EG, Ferreira-Hermosillo A, Peña-Cano MI, Morales-Ávila E, et al. New insights into Adipokines in gestational diabetes mellitus. *Int J Mol Sci*. (2022) 23:6279. doi: 10.3390/ijms23116279

60. Mousavi SN, Bahramfard T, Rad EY, Hosseinikia M, Saboori S. Association of Leptin and Retinol Binding Protein 4 with the risk of gestational diabetes: a systematic review and Meta-analysis of observational studies. *Indian J Endocrinol Metab*. (2023) 27:96–104. doi: 10.4103/ijem.ijem_385_22

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RNA-seq analysis-based study on the effects of gestational diabetes mellitus on macrosomia

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Background: Both the mother and the infant are negatively impacted by macrosomia. Macrosomia is three times as common in hyperglycemic mothers as in normal mothers. This study sought to determine why hyperglycemic mothers experienced higher macrosomia. Methods: Hematoxylin and Eosin staining was used to detect the placental structure of normal mother(NN), mothers who gave birth to macrosomia(NM), and mothers who gave birth to macrosomia and had hyperglycemia (DM). The gene expressions of different groups were detected by RNA-seq. The differentially expressed genes (DEGs) were screened with DESeq2 R software and verified by qRT-PCR. The STRING database was used to build protein-protein interaction networks of DEGs. The Cytoscape was used to screen the Hub genes of the different group.

Results: The NN group's placental weight differed significantly from that of the other groups. The structure of NN group's placenta is different from that of the other group, too. 614 and 3207 DEGs of NM and DM, respectively, were examined in comparison to the NN group. Additionally, 394 DEGs of DM were examined in comparison to NM. qRT-PCR verified the results of RNA-seq. Nucleolar stress appears to be an important factor in macrosomia, according on the results of KEGG and GO analyses. The results revealed 74 overlapped DEGs that acted as links between hyperglycemia and macrosomia, and 10 of these, known as Hub genes, were key players in this process. Additionally, this analysis believes that due of their close connections, non-overlapping Hubs shouldn't be discounted.

Conclusion: In diabetic mother, ten Hub genes (RPL36, RPS29, RPL8 and so on) are key factors in the increased macrosomia in hyperglycemia. Hyperglycemia and macrosomia are linked by 74 overlapping DEGs. Additionally, this approach contends that non-overlapping Hubs shouldn't be ignored because of their tight relationships.

KEYWORDS

macrosomia, hyperglycemia, placenta, differentially expressed genes, hub genes

1 Introduction

Macrosomia is typically defined as a birth weight above the 90th percentile for gestational age or >4,000 g. Gestational diabetes mellitus is a state of hyperglycemia that occurs during pregnancy. Macrosomia has a number of negative impacts on both moms and infants (1). Hyperglycemia can result in serious maternal and newborn problems, which are a growing source of health anxiety (2). When compared to controls with normal glucose levels, about 15–45% of infants born to diabetic moms may develop macrosomia, which is a 3-fold greater rate. More studies are proving that aberrant placenta development and function are related to pregnancy problems and poor fetal outcomes associated with hyperglycemia (3). In order to reduce macrosomia, it is necessary to understand the process through which hyperglycemia causes macrosomia.

Previous research has attempted to identify the reason why gestational diabetes mellitus (GDM) suffers from higher macrosomia. According to metabolic profile of carnitine metabolism in second trimester GDM women, Carnitine metabolism aberration could predict macrosomia complicated with GDM (4). Reduced maternal adiponectin and higher IGF-1 levels in the placenta of GDM women may have increased GLUT-1 expression through enhanced insulin/IGF-1 signaling, which may have affected fetal growth (5). With a normal pre-pregnancy BMI, the development of GDM-induced macrosomia is tightly correlated with fasting plasma glucose and placenta. The mechanism may be hyperglycemia promotes trophoblast cell proliferation via ERK1/2 signaling (6).

By examining the gene expression of macrosomia and hyperglycemia combined with macrosomia, we intended to investigate the reason why there is greater macrosomia in hyperglycemia in the current study. Three groups of clinic samples were created. We collected the placentas from NN, NM, and DM. H&E staining was used to reveal their structural details. The placentas from the three groups were subjected to RNA-seq. In order to identify the DEGs between NN and NM, NN and DM, and NM and DM, the following thresholds were used: $p<0.05$ and $|\log2(\text{fold change})|>1$. qRT-PCR was used to validate several DEGs. We examined the functional and route enrichment of DEGs to better investigate the connection between hyperglycemia and macrosomia. The CytoHubba in Cytoscape plug-in was used to aid in the selection of the Hub genes. To investigate the mechanism of macrosomia brought on by hyperglycemia, the overlapping DEGs and the protein-protein interaction (PPI) between Hub genes from different comparisons were investigated.

2 Materials and methods

2.1 Patients

The department of obstetrics and gynecology of Maternal and Child Health Centre in Dezhou recruited the subjects. After receiving informed consent, placental tissue samples were collected for this investigation, which was authorized by the institutional review board. Ages of the expectant mothers ranged from 25 to 40. The chosen control women had no relevant medical history and no difficulties from pregnancy. Newborns were weighed right after delivery. According to Endocrine Society standards, hyperglycemia was diagnosed when fasting blood glucose was > 5.1 mmol/L. When the birth weight exceeded 4,000 grams, macrosomia was identified.

2.2 Tissue collection

Placentas were obtained from 30 healthy women, 15 women with macrosomia and 15 women with hyperglycemia and macrosomia immediately after caesarean section, some immediately frozen in liquid nitrogen for RNA extraction, and some immobilized in formaldehyde for H&E staining.

2.3 Placentas morphology

Placentas tissue (n=10) from each group were fixed in 4% formaldehyde, embedded in paraffin, and sectioned at 4 μm thickness for visualization. H&E staining was used to observe the sections under a microscope (Nikon, Eclipse).

2.4 RNA library construction and high-throughput sequencing

Following the manufacturer's instructions, total RNA was extracted from placentas using Trizol Reagent (Invitrogen: 15596018). Utilizing the Agilent RNA Nano 6000 Assay Kit and the 2100 Bioanalyzer instrument, RNA purity and quantity were assessed (Agilent Technologies, Santa Clara, CA, USA). Following the manufacturer's instructions, total RNA was used as input material for the RNA sample preparations. Briefly, mRNA was purified from total RNA by using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in First Strand Synthesis Reaction Buffer(5X). First strand cDNA was synthesized using random hexamer primer and M-MuLV Reverse Transcriptase, then use RNaseH to degrade the RNA. Second strand cDNA synthesis was subsequently performed using DNA PolymeraseI and dNTP. Remaining overhangs were converted into blunt ends via exonuclease/ polymerase activities. After adenylation of 3' ends of DNA fragments, Adaptor with hairpin loop structure were ligated to prepare for hybridization. In order to select cDNA fragments of preferentially 370~420bp in length, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA). Then PCR amplification, the PCR product was purified by AMPure XP beads, and the library was finally obtained. In order to ensure the quality of the library, the library needs to be tested. After the construction of the library, the library was initially quantified by Qubit2.0 Fluorometer, then diluted to 1.5ng/ul, and the insert size of the library is detected by Agilent 2100 bioanalyzer. After insert

size meets the expectation, qRT-PCR is used to accurately quantify the effective concentration of the library (the effective concentration of the library is higher than that of 2nM) to ensure the quality of the library.

After the library is qualified, the different libraries are pooling according to the effective concentration and the target amount of data off the machine, then being sequenced by the Illumina NovaSeq 6000. The end reading of 150bp pairing is generated. The basic principle of sequencing is to synthesize and sequence at the same time (Sequencing by Synthesis). Four fluorescent labeled dNTP, DNA polymerase and splice primers were added to the sequenced flow cell and amplified. When the sequence cluster extends the complementary chain, each dNTP labeled by fluorescence can release the corresponding fluorescence. The sequencer captures the fluorescence signal and converts the optical signal into the sequencing peak by computer software, so as to obtain the sequence information of the fragment to be tested.

2.5 Transcriptome data analysis

The image data measured by the high-throughput sequencer are converted into sequence data (reads) by CASAVA base recognition. Raw data (raw reads) of fastq format were firstly processed through in-house perl scripts. In this step, clean data (clean reads) were obtained by removing reads containing adapter, reads containing Nbase and low quality reads from raw data. At the same time, Q20, Q30 and GC content the clean data were calculated. All the downstream analyses were based on the clean data with high quality.

Reference genome and gene model annotation files were downloaded from genome website directly. Index of the reference genome was built using Hisat2(v2.0.5) and paired-end clean reads were aligned to the reference genome usingHisat2 (v2.0.5). We selected Hisat2 as the mapping tool for that Hisat2 can generate a database of splice junctions based on the gene model annotation file and thus a better mapping result than other non-splice mapping tools.

The mapped reads of each sample were assembled by StringTie (v1.3.3b) (Mihaela Pertea.et al. 2015) in a reference-based approach. StringTie uses a novel network flow algorithm as well as an optional *de novo* assembly step to assemble and quantitate full length transcripts representing multiple splice variants for each gene locus.

The feature Counts v1.5.0-p3 was used to count the reads numbers mapped to each gene. And then FPKM of each gene was calculated based on the length of the gene and reads count mapped to this gene. FPKM, expected number of Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced, considers the effect of sequencing depth and gene length for the reads count at the same time, and is currently the most commonly used method for estimating gene expression levels.

Differential expression analysis of two groups (more than three biological replicates per group) was performed using the DESeq2 R package (1.20.0). DESeq2 provide statistical routines for determining differential expression in digital gene expression data using a model based on the negative binomial distribution. The resulting P-values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate. padj<=0.05 and |log2(foldchange)| >= 1 were set as the threshold for significantly differential expression. For the data downloaded from GEO database (GSE203346 and GSE154414), differential expression analysis of two groups (more than three biological replicates per group) was performed using the limma R package and selected with the same standard.

2.6 RNA extraction and qRT-PCR analysis

According to the manufacturer's instructions, total RNA was isolated from the placentas using Trizol reagent (Life Technologies). The Evo M-MLV reverse transcription kit (Accurate Biotechnology (Hunan) Co., Ltd.) was used for reverse transcription (RT-PCR). SYBR Green Pro Taq HS premixed qPCR kit from Accurate Biotechnology (Hunan) Co., Ltd. was used for the quantitative PCR. Reactions were conducted with 1μL RT-PCR cDNA, 0.5 μL forward and reverse primers (10 μmol/L), 8μL water and 10 μL SYBR Green. Each reaction was normalized by co-amplification of β-actin. The samples were run by the StepOne real-time PCR machine (ABI, USA). The primers used in this study were *ATP5ME* forward 5′- CGCGCTACAATTACCTAAAA -3′ and reverse 5′- ATATGCTGTCATCTTCTGCC -3′; *COX5B* forward 5′- TTGGGAAAAGCTGTCTGTTA -3′ and reverse 5′- GTCC CATTCATTGCATTACG -3′; *RPL35* forward 5′- AAGCTCTCT AAGATCCGAGTC -3′and reverse 5′- GCTTGTACTTCTTGCCC TTG -3′; *RPL37A* forward 5′- AAACGTACCAAGAAAGTCGG -3′ and reverse5′- CAGCTCGTCTCTTCATCTTG -3′ and β-actin forward 5′-GTCCACCTTCCAGCAGATGT-3' and reverse 5′- TCACCTTCACCGTTCCAGTT-3′.

2.7 GO and KEGG analysis of DEGs

Gene Ontology (GO) enrichment analysis of differentially expressed genes was implemented by the clusterProfiler R package (3.8.1), in which gene length bias was corrected. GO terms with corrected Pvalue less than 0.05 were considered significantly enriched by differential expressed genes. KEGG is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-through put experimental technologies (http://www.genome.jp/kegg/). We used clusterProfiler R package (3.8.1) to test the statistical enrichment of differential expression genes in KEGG pathways.

2.8 Seek hub genes and the PPI enrichment analysis of them

The STRING database is a search engine for interacting genes that seeks to build PPI networks of various genes based on known and projected PPIs and examine the proteins that interact with one

another (7). PPIs of DEGs of NM and DM were generated using the web tool STRING, and the confidence score (>0.4) was used as the screening criteria. Cytoscape software was then used to visualize the PPI network (version 3.7.2).CytoHubba was used to find Hub genes. Degree of CytoHubba plug-in was used to select the top 40 genes with the highest node connection closeness as the Hub genes (8).

The PPI enrichment analysis of Hub genes from two groups was constructed at STRING database (https://string-db.org/). MCODE plug-in (Node Score Cutoff: 0.2 Haircut: true Fluff: false K-Core: 2 Max. Depth from Seed: 100) was used to calculate accurate correlation level as well as identifying essential PPI network modules (9). Additionally, other Cytoscape add-ins namely, CytoHubba and CytoNCA were used to identify the network's highest linkage Hub genes (10).

2.9 Statistical analysis

The Kruskal Wallis test and *T*-test were used to calculate the statistical significance of the experimental data. Bonferroni-corrected P values to correct for account comparisons. The significance level was set as ** $p < 0.01$. Error bars denote standard deviations. The correlation between fetal weight and placenta weight was explored using the Spearman's rank correlation coefficient test.

3 Results

3.1 Hyperglycemia affected the weight of the placenta

The results of Kruskal Wallis test between three groups in seven variants, there are four significantly differences, which are pregestational BMI, Glucose, fetal Birth weight and placenta weight. The result of Kruskal Wallis test between two groups showed that significant differences in BMI occurred between the NN group and the DM group($P < 0.001$), significant differences in glucose content in blood occurred between the DM group and the other two group(both $P < 0.01$), significant differences in fetal birth weight occurred between the NN group and the other two groups (both $P < 0.001$), significant differences in placenta weight occurred between the NN group and the other two groups (both $P < 0.001$). Perhaps because the sample size was not large enough, there is no significant difference in BMI between NN group and NM group Compared with the NM group, the DM placenta weight rose by 6.3%, but the change is not significant ($p>0.05$).The weight of the fetus has a positive relationship with placental weight (Table 1). The results of the double-digit correlation analysis of placenta and fetal weight are displayed in Table 2. The Spearman's rho is 0.762, $p<0.01$.

3.2 Structure of placentas

The villi's size is uniform throughout the placenta tissue of the NN group (Figure 1A). Villi don't have any breaks or damage. The well-developed syncytial trophoblasts that make up the surface layer of the placental villi are dispersed in a flat, single-layer configuration, and the free surface has morphological rules, uniform distribution, and neatly aligned microvilli that are finger-shaped.

In the surface layer of the placenta villi of the NM placenta, the syncytial trophoblasts were arranged in a monolayer row (Figure 1B). In the placenta of extravillous and swelling villi have more concentrated deposits of fibrinoid. Syncytial trophoblasts are dispersed throughout the DM placenta's placental villi (Figure 1C). Villi come in various sizes. Red blood cells can be visible in the vascular lumen, the capillary lumen is intact, and some endothelial cells swell. The lumen of capillary endothelial cells has significantly shrunk and is clearly constricted.

3.3 Transcriptome assembly and annotation

RNA-Seq was used to compare the transcriptomic landscapes of NM verse NN, DM verse NN and DM verse NM placentas. All the samples sequenced on the Illumina HiSeq X platform produced about 43.4, 44.6 and 46.6 million raw reads for NN, NM and DM samples, respectively, covering 6.37, 6.21 and 6.69 GB of sequence data, respectively. The NN group received 42.45 million clean reads as a result of over 97% of the raw reads surviving quality and trimming. With almost 92% of the raw reads surviving quality checks and trimming, the NM and DM groups, respectively, produced 41.4 and 44.6 million clean reads. Supplementary Table 1 lists many characteristics, including average read size, Q30 percentage, and others. The genome was mapped using clean reads for the ensuing analysis.

TABLE 1 Clinical and analytical characteristics of the cohort.

	NN(n=30)	NM(n=15)	DM(n=15)	P value+
Maternal age (years)	32.29 ± 1.55	31.35 ± 3.29	34.14 ± 4.99	0.126
Pregestational BMI(kg/m^2)	38.81 ± 6.49	42.19 ± 7.25	44.3 ± 4.78**	0.001
Gestational weight gain (kg)	16.71 ± 6.97	16.13 ± 7.79	12.56 ± 4.24	0.106
Glucose(mg/dL)	4.54 ± 0.29	4.55 ± 0.82	6.17 ± 1.32**	0.000
Gestational delivery (weeks)	38.65 ± 0.19	38.74 ± 0.94	38.39 ± 1.09	0.503
Fetal birth weight (g)	3337.79 ± 313.75	4150 ± 159.86**	4221.42 ± 253.97**	0.000
Placental weight (g)	580.8 ± 93.89	734.31 ± 130.072**	780.02 ± 164.05**	0.000

**P-value<0.01vs control.

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TABLE 2 Correlation analysis.

	Fetal_weight	Placenta_weight
Fetal_weight	1	
Placenta_weight	.762**	1

**Correlation is significant at the 0.01 level (2-tailed).

3.4 Identification of differently expressed genes

We conducted a clustering analysis between the NN and NM, DM group based on the levels of gene expression. For comparative and enrichment analysis of DEGs, we defined genes with |log2fold| changes>1 and padj<0.05 as significantly differently expressed genes. The volcano plot analysis also showed significant DEGs NM versus NN (Figure 2A), DM versus NN (Figure 2B) and DM versus NM (Figure 2C). The up-regulated DEGs are represented by red dots, while the down-regulated DEGs are represented by green dots. In the NM versus NN group, a total of 614 genes showed differential expression, with 285 up-regulated and 329 down-regulated DEGs (Supplementary Table 2). In the DM versus NN group, 3207 genes were differentially expressed, with 1325 up-regulated and 1882 down-regulated DEGs (Supplementary Table 2). In the DM versus NM group, 394 genes were differentially expressed, with 73 up-regulated and 321 down-regulated DEGs. Additionally, the heatmap showed the placenta genes that were up-regulated in red and down-regulated in green in NM versus NN (Figure 2D), DM versus NN (Figure 2E), and DM versus NM (Figure 2F).

3.5 mRNA expression patterns were verified via qRT-PCR

Following confirmation using the gene-specific primers described in the procedure and qRT-PCR of the NM and DM group (n = 15) vs control group (n = 15), we discovered that the change direction of these four genes is consistent with the RNA-seq data. The results showed that the four randomly selected genes reduced in abnormal groups significantly. In comparison to the NN group, the expression of the genes for ATP5ME, COX5B, RPL35, and RPL37A was considerably lower in NM and DM ($p< 0.01$) (Figure 3). Their expression in the DM group was also lower than in the NM group, which were statistically significant ($p< 0.01$)

3.6 GO and KEGG analysis of DEGs

GO analysis using terminology related to biological process, cellular component, and molecular function was used to define the function of DEGs. As seen in Figure 4, the biological processes that were primarily impacted by the down-regulated DEGs of NM (Figure 4A) were protein targeting, nucleoside monophosphate metabolism, and oxidative phosphorylation. The ribosome, the mitochondrial inner membrane, and the respiratory chain were the three major areas where the DEGs of NM involved in the cellular component were down-regulated. The NM DEGs that were down-regulated primarily included ribosome structural components, proton transmembrane transporter activity, and oxidoreductase activity in molecular functions. The GO analysis of up-regulated DEGs in NM had poor enrichment ($p>0.05$) (Figure 4B). The biological processes that were primarily impacted by the down-regulated DEGs of DM versus NN (Figure 4C) involved protein targeting, RNA catabolism, and ribonucleotide metabolism. The DM DEGs that were down-regulated primarily implicated the cytosol, inner membrane of the mitochondria, and ribosomes in the cellular component. The down-regulated DEGs of DM vs NN involved in molecular functions were primarily linked to cadherin binding, electron transfer activity, and ribosome structural components. The biological processes that were engaged in the up-regulated DEGs of DM vs NN (Figure 4D) were primarily connected to cilium organization, blood circulation, and epithelial cell proliferation. The ciliary portion and the extracellular matrix structural constituent was the primary area where the up-regulated DEGs of DM versus NN engaged in the cellular component were most closely associated. The mitochondrial inner membrane, ribosome, and mitochondrial matrix were the primary components of the down-regulated DEGs of DM versus NM (Figure 4E) engaged in the cellular component. The molecular

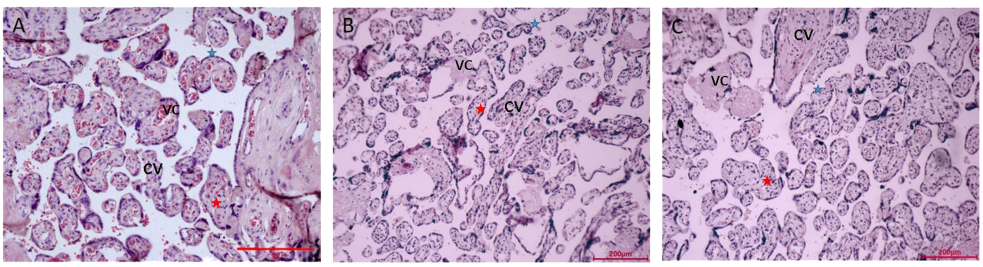

FIGURE 1
Microscopy of the placentas. **(A)** Low power view with the chorionic plate of normal pregnancy (38+ 6 weeks gestation). **(B)** Low power view with the chorionic plate of normal pregnancy with macrosomia (39+ 1 weeks gestation). **(C)** Low power view with the chorionic plate of hyperglycemia with macrosomia (38+ 3weeks gestation). Bar=200μm. CV, chorionic villi; VC, vascular congestion; blue star is syncytiotrophoblasts, red star is cytotrophoblastic cell.

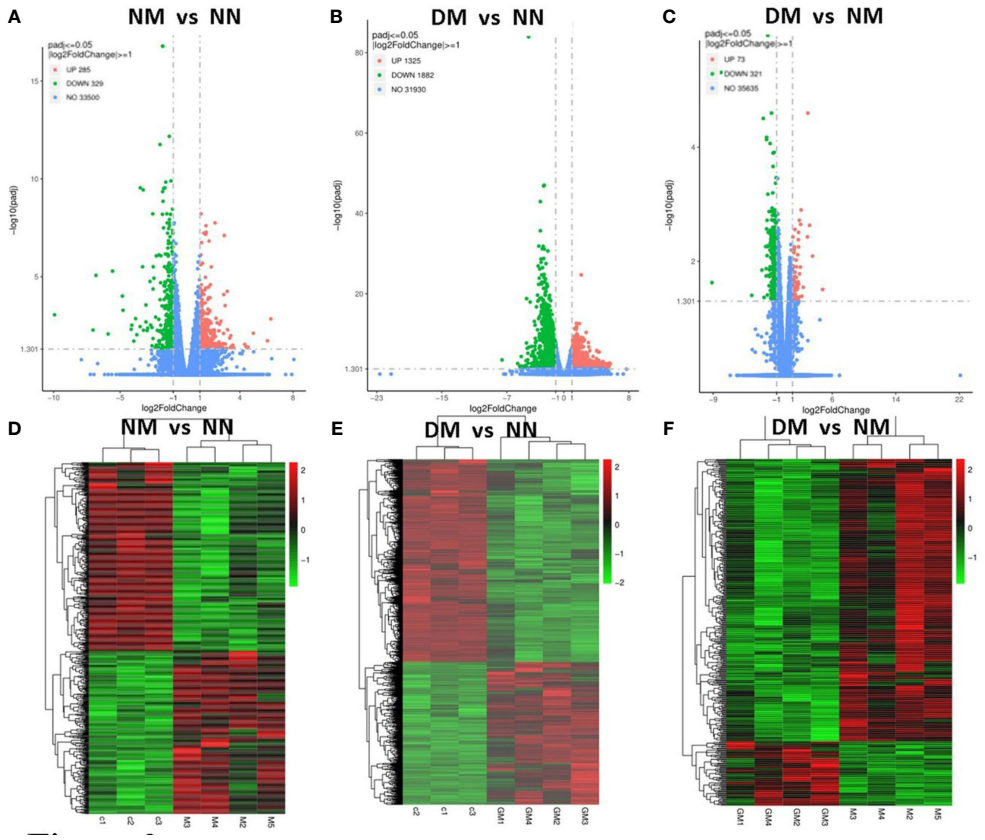

FIGURE 2
Identification of differential expressed genes in the placenta from macrosomia group and hyperglycemia with macrosomia. Volcano plots were used to display differential expressed RNAs of NM versus NN **(A)**, DM versus NN **(B)** and DM versus NM in term placenta**(C)**. **(D)** Heatmaps were used to display expressed RNAs of NM versus NN **(D)**, DM versus NN **(E)** and DM versus NM in term placenta **(F)**. NN, control group; NM, macrosomia; DM, hyperglycemia with macrosomia.

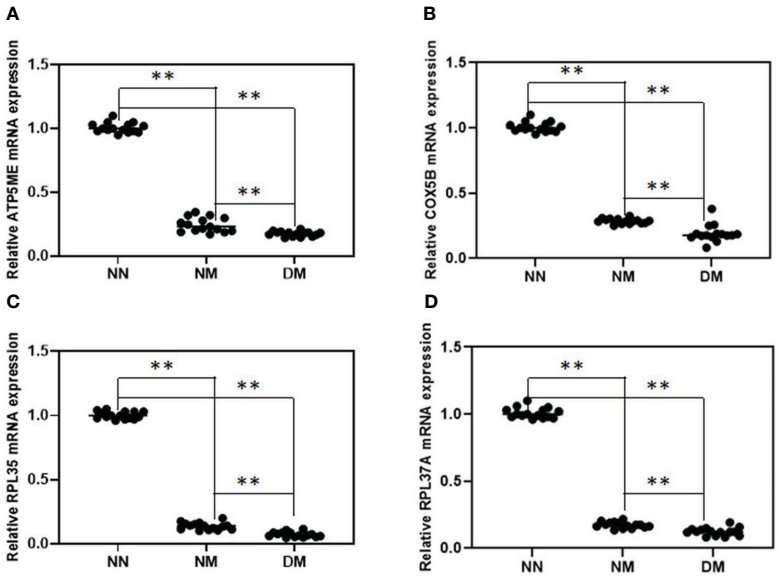

FIGURE 3
Relative expression analysis of four selected DEGs between NN and NM and DM group. qRT-PCR was used to analyze of the mRNA expression of ATP5ME **(A)**, COX5B **(B)**, RPL35 **(C)** and RPL37A **(D)** in term placenta between NN and NM and DM (n=15) respectively β-actin was used as the internal reference. **P-value<0.01.

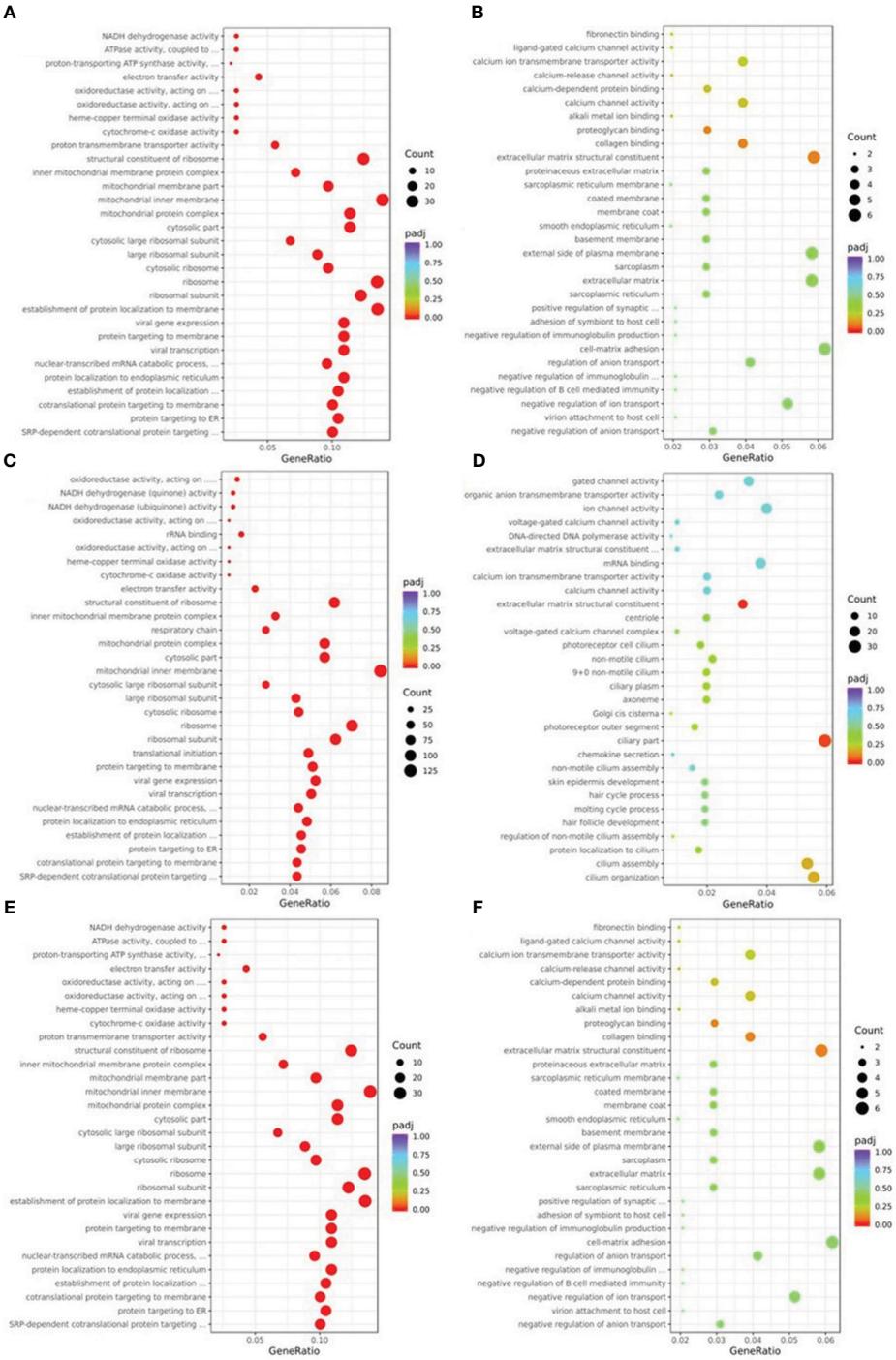

FIGURE 4
Gene ontology (GO) classification of differentially expressed genes (DEGs). **(A)** GO annotation showed that down-regulated DEGs of NM versus NN were associated with different biological processes, cell component and molecular functions. **(B)** GO annotation showed that up-regulated DEGs of NM versus NN were associated with different biological processes, cell component and molecular functions. **(C)** GO annotation showed that down-regulated DEGs of DM versus NN were associated with different biological processes, cell component and molecular functions. **(D)** GO annotation showed that up-regulated DEGs of DM versus NN were associated with different biological processes, cell component and molecular functions. **(E)** GO annotation showed that down-regulated DEGs of DM versus NM were associated with different biological processes, cell component and molecular functions. **(F)** GO annotation showed that up-regulated DEGs of DM versus NM were associated with different biological processes, cell component and molecular functions.

functions that the down-regulated DEGs of DM versus NM were involved in were primarily connected to ribosome structural components, electron transfer activity, and protein kinase inhibitor activity. The biological process-related up-regulated DEGs of DM versus NM were primarily connected to the response to xenobiotic stimuli. The GO analysis of the up-regulated DEGs of DM versus NM (Figure 4F) had poor enrichment.

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The pathways of DEGs were predicted using KEGG pathway analysis. As shown in Figure 5, the down-regulated DEGs in NM versus NN (Figure 5A) were primarily focused on ribosome (about 26 DEGs, including RPL37A and RPL35), Alzheimer's disease (about 20 DEGs, including SNCA and COX8A), and retrograde endocannabinoid signaling (about 6 DEGs, such as NDUFA1 and GNG5). The ECM-receptor interaction (about 5 DEGs, including FN1 and TNC) the primary areas of up-regulated DEGs in NM

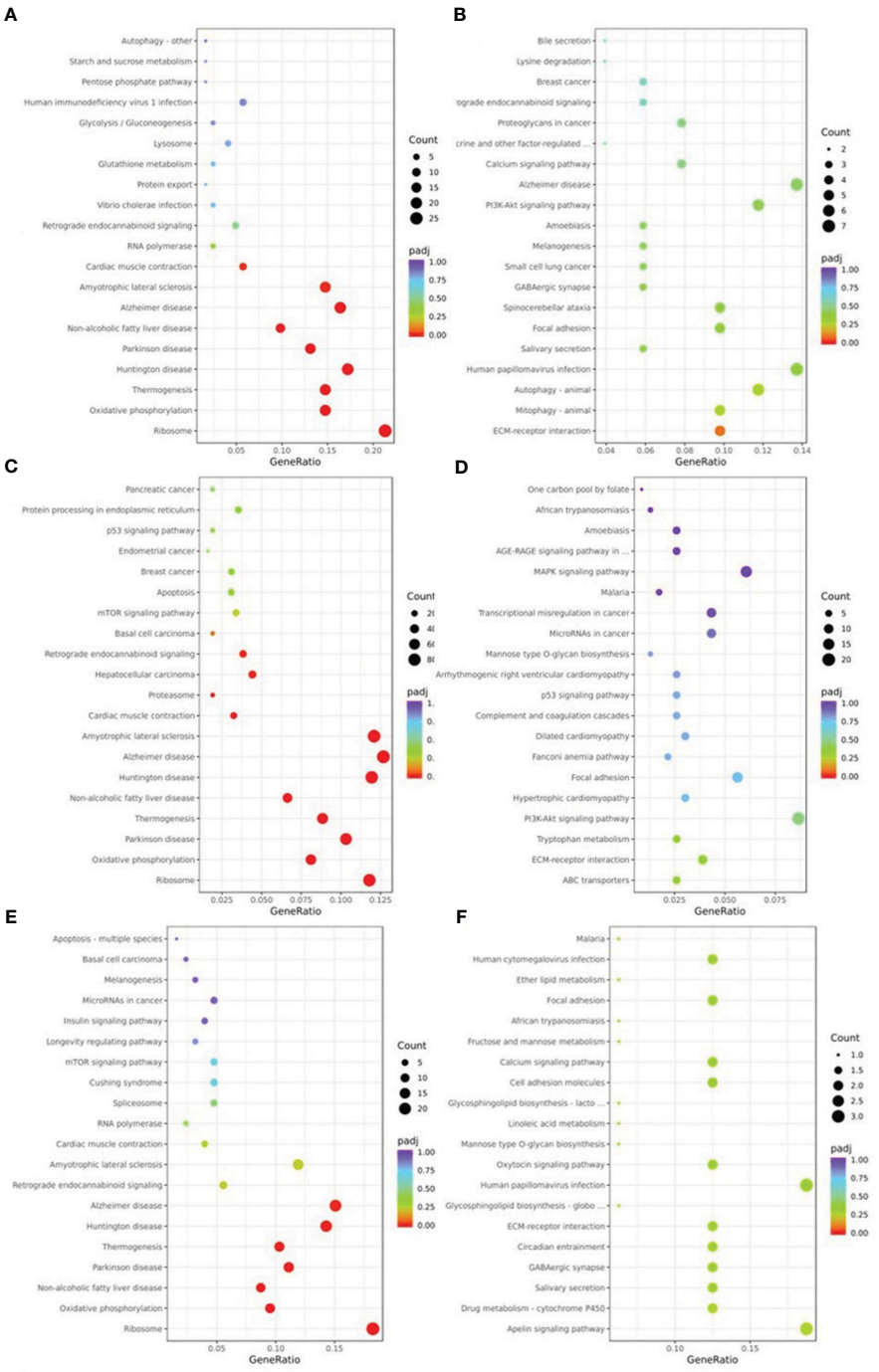

FIGURE 5
KEGG pathway classification of differentially expressed genes (DEGs) KEGG pathway analysis showed that DEGs were involved in different signaling pathways. **(A)** KEGG pathway analysis of the down-regulated DEGs of NM versus NN. **(B)** KEGG pathway analysis of the up-regulated DEGs of NM versus NN were associated with different biological processes, cell component and molecular functions. **(C)** KEGG pathway analysis of the down-regulated DEGs of DM versus NN were associated with different biological processes, cell component and molecular functions. **(D)** KEGG pathway analysis of the up-regulated DEGs of DM versus NN were associated with different biological processes, cell component and molecular functions. **(E)** KEGG pathway analysis of the down-regulated DEGs of DM versus NM were associated with different biological processes, cell component and molecular functions. **(F)** KEGG pathway analysis of the up-regulated DEGs of DM versus NM were associated with different biological processes, cell component and molecular functions.

versus NN (Figure 5B). The down-regulated DEGs in DM versus NN (Figure 5C) were primarily focused on ribosome (approximately 80 DEGs, including RPL37 and RPS11), Alzheimer's disease (about 86 DEGs), and cardiac muscle contraction (about 22 DEGs, such as UQCRQ and TNNC1). The KEGG analysis of up-regulated DEGs in DM versus NN (Figure 5D) had poor enrichment. As shown in Figure 5E, the DEGs that were down-regulated in DM compared to NM were primarily related to the ribosome (about 23 DEGs, including RPL37A and RPS11), Alzheimer's disease (about 19 DEGs, including APC2 and COX8A), and non-alcoholic fatty liver disease (about 11 DEGs, such as SNCA and COX8A). In the KEGG analysis (Figure 5F), the up-regulated DEGs in DM versus NM had poor enrichment.

3.7 PPI hub genes identification

To mine the Hub genes of NM and DM, the DEGs were loaded into string and MC>0.4 was cutoffs. A network consisted of 354 nodes and 1164 edges with p< 1.0e-16 were obtained in NM versus NN. The Hub gene was chosen from the PPI network using the CytoHubba plug-in and Degree method, as illustrated in Figure 6A and Table 3. All 26 genes were downregulated. The score for the last Hub gene is 58. Because String can only analyze the limit of 2000 proteins, we further analyzed the named DEGs in DM versus NN with |log2fold| changes>1.12 with String, which got a network with 1676 nodes, 9131 edges and PPI enrichment p-value <1.0e-16. The Hub gene was illustrated in Figure 6B. All of the Hub genes of DM group are down-regulated. The score of the last Hub gene is 162. In the DM group, these 72 genes showed reduced expression. The Hub genes of the NM group were included in the Hub gene of the DM group except TIMM10, UQCRH, SEC61B and ATP5I.

3.8 Exploration the relationship between macrosomia and hyperglycemia by DEGs

We scanned the DEGs of NM versus NN, DM versus NN, and DM versus NM and studied the overlapped DEGs between two and three comparisons (Figure 7A). There were 517 DEGs overlapped in NM and DM groups, which changed in the same direction, containing 299 down-regulated DEGs and 218 up-regulated DEGs. That is to say, more than 84% of DEGs in the NM group changed in the same direction in the DM group. These genes involved in the biological process were mainly related to metabolic process, cellular process, signaling and so on (Figure 7B). There are 79 genes overlapped in NM versus NN and DM versus NM including 10 Hub genes of NM group. The 74 overlapped DEGs of three groups involved in the biological process were mainly related to metabolic process, cellular process and positive regulation of biological process (Figure 7C). These 74 DEGs includes RPS29, RPL35, RPS11, RPS2, NDUFB7, RPL37A, FAU, RPL36, RPL8 and RPL18A, which are Hub genes in macrosomia. From this, we infer that hyperglycemia changes the expressions of these Hub genes and promotes the incidence of macrosomia.

3.9 Exploration the relationship between hyperglycemia and macrosomia by hub genes

We acquired a network consisted of 303 nodes and 724 edges with p< 1.0e-16 in DM versus NM. The cytoHubba plug-in and the Degree algorithm were used to select the Hub gene from the PPI network as shown in Figure 8A. All of these 21 genes were down-regulated. Furthermore, RPS29, RPL35, RPS11, RPS2, NDUFB7, RPL37A, FAU, RPL36, RPL8 and RPL18A are Hub genes of NM versus NN. Except MRPL12 and CCDC124, the other Hub genes were included in the Hub gene of the DM group. We then analyzed the relationship between the other Hub gene in the DM versus NM group and the Hub gene in the NM versus NN group (Figure 8B). This result shows that in addition to the overlapped Hub gene, there is a strong link between the Hub genes in the DM versus NM group.

4 Discussion

Hyperglycemia represents the most common form of altered glucose in pregnant women, and may cause macrosomia, hypertension and cardiovascular disease (11). Pregnant women

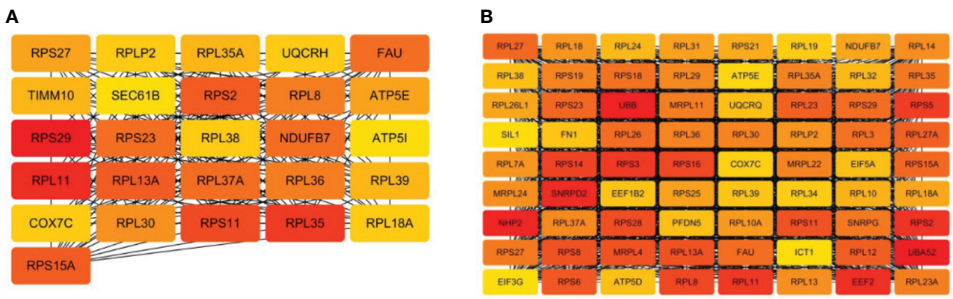

FIGURE 6
Hub genes of macrosomia and hyperglycemia with macrosomia. **(A)** the Hub genes of macrosomia, **(B)** the Hub genes of hyperglycemia with macrosomia. The color of the key gene represents its degree. The higher the degree of gene, the more redd her color.

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TABLE 3 Changes of hub genes in two groups.

Group	Gene ID	Degree	Change
NM	RPS29	82	down
NM	RPL11	80	down
NM	RPL35	78	down
NM	RPS11	76	down
NM	RPS2	74	down
NM	RPS15A	74	down
NM	RPL13A	74	down
NM	NDUFB7	72	down
NM	RPL37A	72	down
NM	RPS23	72	down
NM	FAU	72	down
NM	RPL36	70	down
NM	RPL8	70	down
NM	RPL30	66	down
NM	ATP5ME	64	down
NM	RPS27	64	down
NM	RPL39	62	down
NM	RPL35A	62	down
NM	TIMM10	62	down
NM	UQCRH	60	down
NM	COX7C	60	down
NM	RPLP2	60	down
NM	RPL18A	60	down
NM	RPL38	60	down
NM	SEC61B	58	down
NM	ATP5I	58	down
DM	UBA52	326	down
DM	UBB	280	down
DM	NHP2	254	down
DM	SNRPD2	248	down
DM	EEF2	244	down
DM	RPS2	238	down
DM	RPS3	236	down
DM	RPL11	236	down
DM	RPS5	230	down
DM	RPS14	226	down
DM	RPL8	224	down
DM	RPS16	224	down
DM	RPS28	222	down
DM	MRPL4	220	down
DM	RPS11	220	down
DM	RPL13A	220	down
DM	RPS8	220	down
DM	RPS6	216	down
DM	RPL12	214	down
DM	RPL23	210	down
DM	RPL27A	210	down
DM	RPS23	208	down
DM	RPS15A	208	down
DM	RPL27	208	down
DM	RPS29	206	down
DM	FAU	206	down
DM	RPL26	206	down
DM	RPL3	204	down
DM	RPS18	204	down
DM	RPL35	202	down
DM	RPS27	202	down
DM	SNRPG	202	down
DM	RPL23A	200	down
DM	MRPL11	200	down
DM	RPL36	198	down
DM	RPS19	198	down
DM	RPL30	198	down
DM	MRPL22	198	down
DM	RPL35A	198	down
DM	RPL29	196	down
DM	RPL37A	196	down
DM	RPL13	192	down
DM	RPL14	192	down
DM	MRPL24	192	down
DM	RPL7A	190	down
DM	RPLP2	190	down
DM	RPL18	190	down
DM	RPL10A	188	down
DM	RPS25	186	down
DM	RPL31	186	down
DM	RPL18A	184	down
DM	NDUFB7	184	down

(Continued)

(Continued)

TABLE 3 Continued

Group	Gene ID	Degree	Change
DM	RPL26L1	184	down
DM	RPS21	182	down
DM	RPL10	180	down
DM	RPL32	178	down
DM	RPL24	178	down
DM	ATP5D	176	down
DM	EIF5A	176	down
DM	UQCRQ	176	down
DM	PFDN5	176	down
DM	RPL38	176	down
DM	FN1	174	down
DM	RPL39	174	down
DM	EEF1B2	174	down
DM	RPL19	170	down
DM	RPL34	170	down
DM	COX7C	166	down
DM	ATP5E	164	down
DM	SIL1	162	down
DM	EIF3G	162	down
DM	ICT1	162	down

with hyperglycemia through the placenta will stimulate the production of a large amount of insulin secretion of the fetus to be able to make full use of blood sugar, and promote the synthesis of protein and fat, so that the fetus grows larger (12). This study aims to provide light on the gene expression level of the diabetic macrosomia mechanism. 614 DEGs were found using RNA-seq in the placenta of pregnant women who delivered macrosomia. 3207 DEGs were found in the placenta of pregnant women with macrosomia who also had high blood sugar levels throughout pregnancy. In comparison to NM, 394 DEGs were discovered in DM. Four of DEGs were verified by qRT-PCR.

In order to analyze the pathogenesis of macrosomia, hyperglycemic macrosomia and the impact of hyperglycemia on macrosomia, KEGG and GO were used to analyze the differential genes in the three groups of alignment. Through GO analysis, it was discovered that NM DEGs are involved in protein targeting, nucleoside monophosphate metabolism, oxidative phosphorylation, cell-matrix adhesion, cellular calcium ion homeostasis, and nutritional response. The results of the GO analysis showed that DM DEGs are involved in the targeting of proteins, RNA catabolism, ribonucleotide metabolism, cilium organization, blood circulation, and proliferation of epithelial cells. According to GO analysis, DM versus NM DEGs were found to be involved in protein targeting, the electron transport chain, oxidative phosphorylation, and the response to xenobiotic stimulation. The KEGG results and GO results were not entirely in agreement. In three comparisons, the down-regulated DEGs in the ribosome overlapped. The substantial downregulation of ribosomal genes in this study implies that ribosome anomalies play a significant role in the development of macrosomia. A diverse array of disorders' etiology is due to defects in ribosome biosynthesis and function (13). Nucleolar stress results from the ribosome genesis process being disturbed. Fat formation is a result of nucleolar stress (14).

In this work, the intersection of DEGs in three sets of different alignments was further studied. According to the results, there is a significant relationship between DM and NM since more than 84% of the DEGs in the NM group altered in the same direction as those in the DM group. In comparison to NM versus NN alignment, 74 DEGs in the DM versus NM alignment changed in the same way. This means they are linking genes between hyperglycemia and macrosomia. Three sets of distinct alignments contain 74 DEGs that have had their orientation reversed, indicating that they are significant in hyperglycemia and macrosomia.

Cytoscape was used to evaluate the Hub genes of the DM and NM groups in order to investigate the fundamental cause of increased macrosomia in hyperglycemia. Among the 26 Hub genes in the NM group, 22 genes were DM group Hub genes, and 10 genes were DM group versus NM group. These ten genes are located in the DEGs that overlap in three different sets of alignments. This leads us to hypothesize that these 10 genes are crucial linkers between hyperglycemia and macrosomia. They were all deregulated. Except NDUFB7, the other proteins are ribosome protein. Ribosome impairment is important in obesity (15).

RPS29 induced apoptosis, which means the downregulation of RPS29 is associated with cell proliferation (16). In GSE154414, it was likewise downregulated. RPL35 encodes a ribosomal protein that is a component of the 60S subunit. RPL35 was revealed as putative key drivers of stress granules (17). RPS11encodes a member of the S17P family of ribosomal proteins that is a component of the 40S subunit. RPS11 is also a stress response marker (18). RPS2 plays a critical role in the regulation of p53 signaling including the ribosomal stress response (19). RPL37A encodes a ribosomal protein that is a component of the 60S subunit. It related pathways including peptide chain elongation and rRNA processing in the nucleus and cytosol (20). FAU encodes a fusion protein consisting of the ubiquitin-like protein fubi at the N terminus and ribosomal protein S30 at the C terminus. Processing FAU is required for 40S maturation and depends on USP36 (21). RPL36 encodes a ribosomal protein that is a component of the 60S subunit (22). RPL8 overexpression enhances apoptosis brought on by FasL (23). RPL18A encodes a member of the L18AE family of ribosomal proteins that is a component of the 60S subunit (24). Confusion in the "production and processing" of ribosomal RNA can cause nucleolar stress (25).

This investigation also constructed PPI between DM versus NM and NM versus NN non-overlapping Hub genes and revealed that there is an unbreakable link between them, further illuminating the association between hyperglycemia and macrosomia. So, we shouldn't

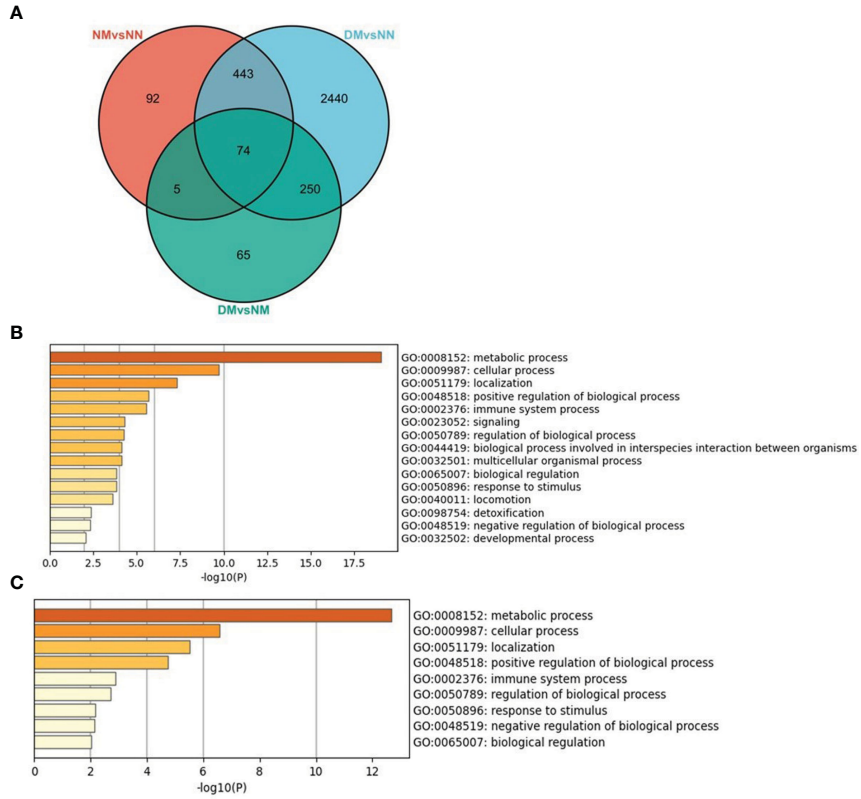

FIGURE 7
The overlapped DEGs of three sets of crossover genes with different alignments. **(A)** the overlapped DEGs of three sets of crossover genes with different alignments, **(B)** GO annotation of the overlapped DEGs of NM versus NN and DM versus NN. **(C)** GO annotation of the overlapped DEGs of three sets of crossover genes with different alignments. The color of the key gene represents its degree. The higher the degree, the more redder her color.

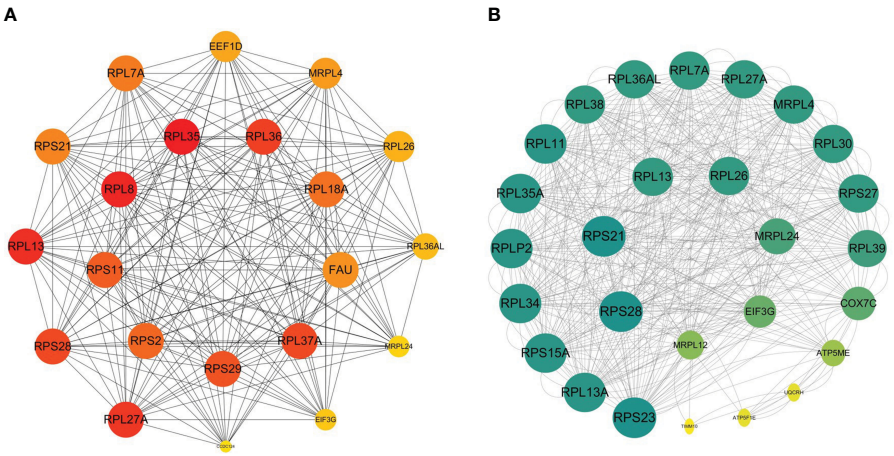

FIGURE 8
Relations between hyperglycemia with macrosomia and macrosomia. **(A)** The Hub genes of DM versus NM, **(B)** the PPI between the Hub genes of DM vs NM and NM vs NN except the overlapped Hub genes. The color of the key gene represents its degree. The higher the degree of the gene, the greener her color.

ignore their connection while exposing additional macrosomia in hyperglycemia. Ribosomal proteins including RPL18 maintain the identity of mouse embryonic stem cells (mESCs) and regulate the expression of 2C transcripts through a unique RP-RPL11-MDM2-P53-DUX cascade (26). RPL11 encodes a ribosomal protein that is a component of the 60S subunit. RPL11 promotes the active of p53, which can induce apoptosis (27). If these genes were verified in maternal blood in the future, they maybe biomarker in clinical practice. We can also consider increasing the expression of these genes to reduce the occurrence of macrosomia

5 Conclusions

In conclusion, we first detected the placenta's aberrant structure. After that, using RNA-seq analysis, the team investigated the molecular causes of aberrant placental morphological structures. The team then used GO and KEGG to analyze the internal mechanism of macrosomia and hyperglycemia. Then, the team analyzed the reasons for the high incidence of macrosomia in hyperglycemia from the perspectives of overlapping differential genes and Hub genes. The results showed 74 genes that served as bridges between hyperglycemia and macrosomia and the 10 Hub genes played a crucial part in this process. Also, it is the opinion of this work that non-overlapping Hubs should not be disregarded because of their close connections.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA853493.

Ethics statement

The studies involving humans were approved by medical research ethics review committee of the Dezhou Maternal and Child Health Hospital. 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.

Author contributions

QG: Conceptualization, Writing – original draft, Funding acquisition. GX: Writing – review & editing, Resources, Data curation. GW: Writing – review & editing, Resources, Investigation. WW: Writing – review & editing, Data curation. CZ: Writing – review & editing, Validation, Data curation. YS: Writing – review & editing, Investigation. CG: Writing – review & editing, Investigation. JC: Writing – review & editing, Resources, Investigation. HM: Writing – review & editing, Data curation. DS: Writing – review & editing, Funding acquisition. XM: Writing – review & editing, Supervision.

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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.2024.1330704/full#supplementary-material

References

1. Beta J, Khan N, Khalil A, Fiolna M, Ramadan G, Akolekar R. Maternal and neonatal complications of fetal macrosomia: systematic review and meta-analysis. *Ultrasound Obstet Gynecol.* (2019) 54:308–18. doi: 10.1002/uog.20279

2. Getahun D, Nath C, Ananth CV, Chavez MR, Smulian JC. Gestational diabetes in the United States: temporal trends 1989 through 2004. *Am J Obstet Gynecol.* (2008) 198:525.e521–5. doi: 10.1016/j.ajog.2007.11.017

3. Burton GJ, Fowden AL, Thornburg KL. Placental origins of chronic disease. *Physiol Rev.* (2016) 96:1509–65. doi: 10.1152/physrev.00029.2015

4. Sun M, Zhao B, He S, Weng R, Wang B, Ding Y, et al. The alteration of carnitine metabolism in second trimester in GDM and a nomogram for predicting macrosomia. *J Diabetes Res.* (2020) 2020:4085757. doi: 10.1155/2020/4085757

5. Balachandiran M, Bobby Z, Dorairajan G, Gladwin V, Vinayagam V, Packirisamy RM. Decreased maternal serum adiponectin and increased insulin-like growth factor-1 levels along with increased placental glucose transporter-1 expression in gestational diabetes mellitus: Possible role in fetal overgrowth. *Placenta.* (2021) 104:71–80. doi: 10.1016/j.placenta.2020.11.008

6. Zheng Y, Huang M, Lu X, Xu J, Han Y, Ji J, et al. Association of hyperglycaemia with the placenta of GDM-induced macrosomia with normal pre-pregnancy BMI and the proliferation of trophoblast cells. *J Obstet Gynaecol.* (2022) 42:1759–68. doi: 10.1080/01443615.2022.2036969

7. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.* (2015) 43:D447–452. doi: 10.1093/nar/gku1003

8. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst Biol*. (2014) 8 Suppl 4:S11. doi: 10.1186/1752-0509-8-S4-S11

9. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. (2003) 13:2498–504. doi: 10.1101/gr.1239303

10. Tang Y, Zhang Y, Hu X. Identification of potential hub genes related to diagnosis and prognosis of hepatitis B virus-related hepatocellular carcinoma via integrated bioinformatics analysis. *BioMed Res Int*. (2020) 2020:4251761. doi: 10.1155/2020/4251761

11. Oliveira AP, Calderon IM, Costa RA, Roscani MG, Magalhães CG, Borges VT. Assessment of structural cardiac abnormalities and diastolic function in women with gestational diabetes mellitus. *Diabetes Vasc Dis Res*. (2015) 12:175–80. doi: 10.1177/1479164114563302

12. Ornoy A, Becker M, Weinstein-Fudim L, Ergaz Z. Diabetes during pregnancy: A maternal disease complicating the course of pregnancy with long-term deleterious effects on the offspring. A clinical review. *Int J Mol Sci*. (2021) 22:1–36. doi: 10.3390/ijms22062965

13. Kang J, Brajanovski N, Chan KT, Xuan J, Pearson RB, Sanij E. Ribosomal proteins and human diseases: molecular mechanisms and targeted therapy. *Signal Transduct Target Ther*. (2021) 6:323. doi: 10.1038/s41392-021-00728-8

14. Wu J, Jiang X, Li Y, Zhu T, Zhang J, Zhang Z, et al. PHA-4/FoxA senses nucleolar stress to regulate lipid accumulation in Caenorhabditis elegans. *Nat Commun*. (2018) 9:1195. doi: 10.1038/s41467-018-03531-2

15. Snieckute G, Ryder L, Vind AC, Wu Z, Arendrup FS, Stoneley M, et al. ROS-induced ribosome impairment underlies ZAKα-mediated metabolic decline in obesity and aging. *Science*. (2023) 382:eadf3208. doi: 10.1126/science.adf3208

16. Khanna N, Sen S, Sharma H, Singh N. S29 ribosomal protein induces apoptosis in H520 cells and sensitizes them to chemotherapy. *Biochem Biophys Res Commun*. (2003) 304:26–35. doi: 10.1016/S0006-291X(03)00532-1

17. Raps S, Bahr L, Karkossa I, Rossol M, von Bergen M, Schubert K. Triclosan and its alternatives, especially chlorhexidine, modulate macrophage immune response with distinct modes of action. *Sci Total Environ*. (2024) 914:169650. doi: 10.1016/j.scitotenv.2023.169650

18. Yong WH, Shabihkhani M, Telesca D, Yang S, Tso JL, Menjivar JC, et al. Ribosomal proteins RPS11 and RPS20, two stress-response markers of glioblastoma stem cells, are novel predictors of poor prognosis in glioblastoma patients. *PloS One*. (2015) 10:e0141334. doi: 10.1371/journal.pone.0141334

19. Cho J, Park J, Shin SC, Kim JH, Kim EE, Song EJ. Ribosomal protein S2 interplays with MDM2 to induce p53. *Biochem Biophys Res Commun*. (2020) 523:542–7. doi: 10.1016/j.bbrc.2020.01.038

20. Carrara GFA, Evangelista AF, Scapulatempo-Neto C, Abrahão-MaChado LF, Morini MA, Kerr LM, et al. Analysis of RPL37A, MTSS1, and HTRA1 expression as potential markers for pathologic complete response and survival. *Breast Cancer*. (2021) 28:307–20. doi: 10.1007/s12282-020-01159-z

21. van den Heuvel J, Ashiono C, Gillet LC, Dörner K, Wyler E, Zemp I, et al. Processing of the ribosomal ubiquitin-like fusion protein FUBI-eS30/FAU is required for 40S maturation and depends on USP36. *Elife*. (2021) 10:1–36. doi: 10.7554/eLife.70560

22. Khatter H, Myasnikov AG, Natchiar SK, Klaholz BP. Structure of the human 80S ribosome. *Nature*. (2015) 520:640–5. doi: 10.1038/nature14427

23. Hao Y, Kong X, Ruan Y, Gan H, Chen H, Zhang C, et al. CDK11p46 and RPS8 associate with each other and suppress translation in a synergistic manner. *Biochem Biophys Res Commun*. (2011) 407:169–74. doi: 10.1016/j.bbrc.2011.02.132

24. Liang X, Zuo MQ, Zhang Y, Li N, Ma C, Dong MQ, et al. Structural snapshots of human pre-60S ribosomal particles before and after nuclear export. *Nat Commun*. (2020) 11:3542. doi: 10.1038/s41467-020-17237-x

25. Wu M, Xu G, Han C, Luan PF, Xing YH, Nan F, et al. lncRNA SLERT controls phase separation of FC/DFCs to facilitate Pol I transcription. *Science*. (2021) 373:547–55. doi: 10.1126/science.abf6582

26. Yi Y, Zeng Y, Sam TW, Hamashima K, Tan RJR, Warrier T, et al. Ribosomal proteins regulate 2-cell-stage transcriptome in mouse embryonic stem cells. *Stem Cell Rep*. (2023) 18:463–74. doi: 10.1016/j.stemcr.2022.12.007

27. Ko HM, Jee W, Lee D, Jang HJ, Jung JH. Ophiopogonin D increase apoptosis by activating p53 via ribosomal protein L5 and L11 and inhibiting the expression of c-Myc via CNOT2. *Front Pharmacol*. (2022) 13:974468. doi: 10.3389/fphar.2022.974468

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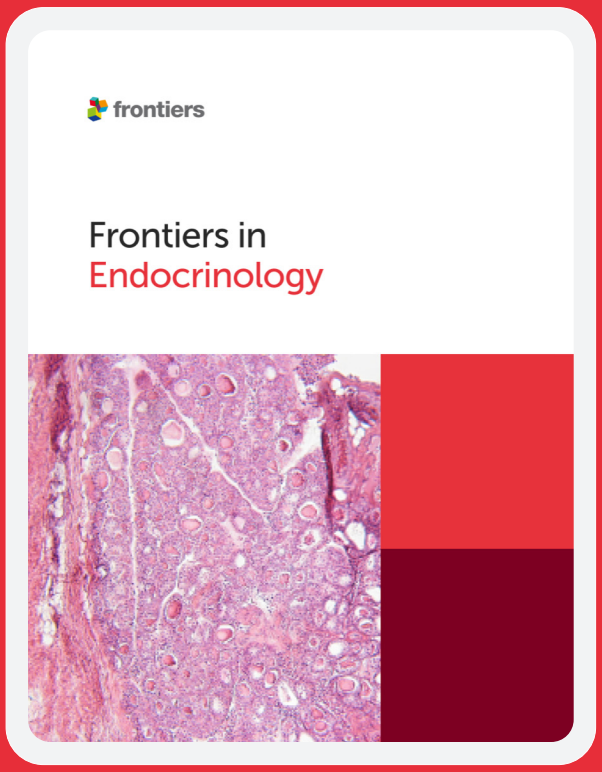

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