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Endocrine and metabolic effects on maternal-fetal and neonatal outcomes, volume II

Edited by
Huixia Yang, Moshe Hod, Cuilin Zhang, Jie Yan and Chen Wang

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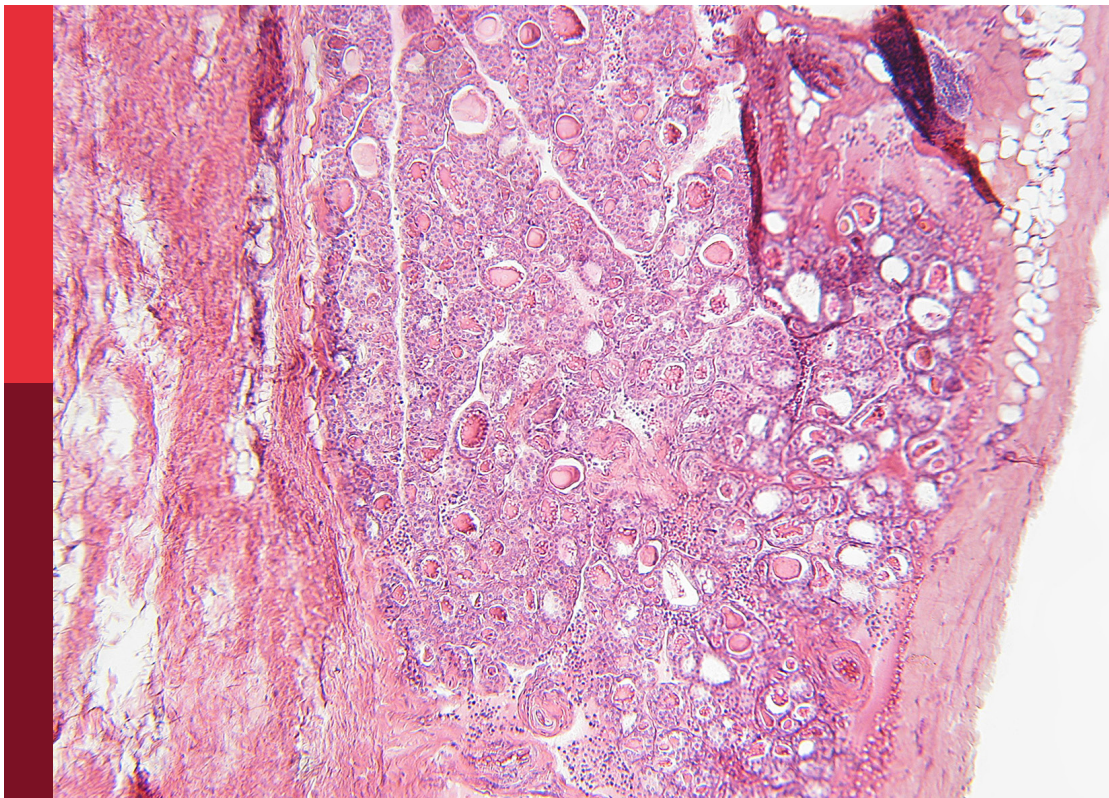

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Endocrine and metabolic effects on maternal-fetal and neonatal outcomes, volume II

Topic editors

Huixia Yang — Peking University, China
Moshe Hod — Mor Center, Israel
Cuilin Zhang — National University of Singapore, Singapore
Jie Yan — Peking University, China
Chen Wang — Peking University, China

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EDITED BY
Huixia Yang,
Peking University, China

REVIEWED BY
Anja Lisbeth Frederiksen,
Aalborg University Hospital, Denmark
Joao D.T.S. Anselmo,
Hospital do Divino Espírito Santo, Portugal

*CORRESPONDENCE
Herodes Guzman
guzmanh@chop.edu

†These authors have contributed equally to this work and share senior authorship

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Case report: Two unexpected cases of DGUOK-related mitochondrial DNA depletion syndrome presenting with hyperinsulinemic hypoglycemia

Herodes Guzman[1,2*], Sahr Yazdani[3], Jennifer L. Harmon[4], Kimberly A. Chapman[4], Bernadette Vitola[5,6], Louise Pyle[4], Heather McKnight[1], Winnie Sigal[1,7], Katherine Lord[1,7], Diva D. De Leon[1,7], Nadia Merchant[4,8†] and Rebecca Ganetzky[2,7†]

[1]Division of Endocrinology and Diabetes, Children's Hospital of Philadelphia, Philadelphia, PA, United States, [2]Division of Genetics, Children's Hospital of Philadelphia, Philadelphia, PA, United States, [3]Division of General Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA, United States, [4]Rare Disease Institute, Children's National Hospital, Washington, DC, United States, [5]Division of Gastroenterology, Hepatology and Nutrition, Children's National Hospital, Washington, DC, United States, [6]Transplant Institute, MedStar Georgetown University Hospital, Washington, DC, United States, [7]Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, United States, [8]Division of Endocrinology and Diabetes, Children's National Hospital, Washington, DC, United States

Timely diagnosis of persistent neonatal hypoglycemia is critical to prevent neurological sequelae, but diagnosis is complicated by the heterogenicity of the causes. We discuss two cases at separate institutions in which clinical management was fundamentally altered by the results of molecular genetic testing. In both patients, critical samples demonstrated hypoketotic hypoglycemia and a partial glycemic response to glucagon stimulation, thereby suggesting hyperinsulinism (HI). However, due to rapid genetic testing, both patients were found to have deoxyguanosine kinase (DGUOK)-related mitochondrial DNA depletion syndrome, an unexpected diagnosis. Patients with this disease typically present with either hepatocerebral disease in the neonatal period or isolated hepatic failure in infancy. The characteristic features involved in the hepatocerebral form of the disease include lactic acidosis, hypoglycemia, cholestasis, progressive liver failure, and increasing neurologic dysfunction. Those with isolated liver involvement experience hepatomegaly, cholestasis, and liver failure. Although liver transplantation is considered, research has demonstrated that for patients with DGUOK-related mitochondrial DNA depletion syndrome and neurologic symptoms, early demise occurs. Our report advocates for the prompt initiation of genetic testing in patients presenting with persistent neonatal hypoglycemia and for the incorporation of mitochondrial DNA depletion syndromes in the differential diagnosis of HI.

KEYWORDS

hypoglycemia, exome, mitochondria, DGUOK, hyperinsulinism

1 Introduction

Timely diagnosis of persistent neonatal hypoglycemia is critical to prevent neurological sequelae, but diagnosis is complicated by the heterogenicity of the causes. Deoxyguanosine kinase (DGUOK)-related mitochondrial DNA depletion syndrome (OMIM# 251880) or DGUOK deficiency can present with hypoglycemia but has additional phenotypic manifestations. This autosomal recessive mitochondrial disorder results from biallelic pathogenic variants in the *DGUOK* nuclear gene, which encodes the deoxyguanosine kinase involved in mitochondrial DNA (mtDNA) maintenance. Deficiency in DGUOK leads to impaired mitochondrial deoxynucleotide triphosphate production, resulting in mtDNA depletion and respiratory chain dysfunction (1, 2).

Patients with DGUOK deficiency typically present in two ways: multi-systemic disease (hepatocerebral type) in the neonatal period or isolated hepatic failure in infancy. In the multi-systemic form, psychomotor delay, rotary nystagmus, and hypotonia can be observed in addition to severe and progressive liver dysfunction. Those with isolated liver involvement suffer from hepatomegaly, cholestasis, and liver failure (2).

Diagnosis is facilitated by molecular genetic testing (3), which is usually done after finding biochemical derangements including conjugated hyperbilirubinemia, elevated gamma-glutamyltransferase, hepatitis with associated tyrosinemia, hypoglycemia, and lactic acidosis. Coagulopathies may also be observed secondary to liver failure. Despite the significance of presentation, head imaging is often normal. If tissue is collected, liver and muscle samples typically show a reduced mtDNA copy number, combined deficiency of liver respiratory chain complexes I, III, and IV, and a greater number of mitochondria with abnormal cristae on liver electron microscopy (2). Most patients with multi-systemic involvement pass away from liver failure at around 1-2 years of life (2, 4, 5).

In this report, we present two cases of persistent hypoketotic hypoglycemia initially undergoing work-up for hyperinsulinism (HI). Rapid genetic testing was critical in facilitating the unexpected diagnosis of DGUOK deficiency in each patient, fundamentally altering their clinical management. As HI has been only rarely reported in DGUOK deficiency (6, 7), our two patients establish a pattern of hyperinsulinemia in mtDNA depletion syndromes, warranting consideration of these disorders in the differential diagnosis of HI.

2 Case reports

Case 1: The first patient was a full-term male born at an outside hospital via vacuum-assisted delivery following an uncomplicated pregnancy with APGARS of 8 and 9 at 1 and 5 minutes of life, respectively, and a birth weight of 2,349 grams (1st percentile). This was the parents first child. On day of life (DOL) 0, he experienced hypoglycemia (plasma glucose [PG] 34 mg/dL; reference range: 70-99 mg/dL) and profound lactic acidosis (lactate 16.2 mmol/L; reference range: ≤1.5 mmol/L) with pH 7.29 and bicarbonate 10 mmol/L (reference range: 20-26 mmol/L). On physical examination, he was noted to have penoscrotal hypospadias and significant tachypnea. He was initiated on high-flow nasal cannula, ampicillin, gentamicin, and phototherapy given unconjugated hyperbilirubinemia at birth. He was then transferred to a quaternary care NICU for further management, including treatment of developing coagulopathy.

Further evaluation demonstrated persistently elevated plasma lactate, elevated plasma alanine, proline, and tyrosine, and low serum beta-hydroxybutyrate. Although the newborn screen was positive for an abnormally elevated tyrosine, succinylacetone was not detected in urine organic acids. The infant's normal acylcarnitine profile was not suggestive of a fatty acid oxidation disorder. A metabolic hypoglycemia genetic panel and trio whole exome sequencing were sent on DOL 4 and DOL 6, respectively. The family history was negative for acute liver failure, hypoglycemia, or sudden infant death. The parents were of Mexican ancestry and nonconsanguineous, though their families are from the same province in Mexico. To further characterize the patient's hypoglycemia, he underwent a diagnostic fast that demonstrated hypoketotic hypoglycemia and a partial glycemic response to glucagon stimulation, which was suggestive of HI although his persistent lactatemia was inconsistent with this diagnosis.

The infant was transferred to the endocrinology service on DOL 22 for continued hypoglycemia and received dextrose-containing fluids at a glucose infusion rate (GIR) of 8-10.5 mg/kg/min. On DOL 25, his metabolic hypoglycemia genetic panel resulted with a biparentally-inherited homozygous likely pathogenic variant in the *DGUOK* gene (c.749T>C, p.Leu250Ser), consistent with DGUOK deficiency. Notably, whole exome sequencing was not expected to produce a result for 2 more weeks. In light of this diagnosis, mitochondrial medicine, transplant hepatology, and palliative care were consulted. The patient was started on a mitochondrial cocktail of allopurinol and inosine, and liver transplantation was discussed. For his persistent hypoglycemia, a gastrostomy tube was placed for continuous enteral dextrose fluid infusion. With confirmation of this diagnosis and negative workup for congenital adrenal insufficiency, further management of his under-virilized genitalia was deferred. He was discharged home on DOL 49 with close outpatient follow-up.

At home, the family reported intermittent hypoglycemia requiring up-titration of the infant's enteral dextrose. By 4 months of age, he developed nystagmus, rendering a liver transplant inadmissible. Thereafter, he was admitted multiple times for worsening abdominal distension, ascites, and coagulopathy, eventually succumbing to his disease at 5 months of age.

Case 2: The second patient is a full-term male born at an outside hospital via precipitous spontaneous vaginal delivery to a mother with gestational diabetes with APGARS of 8 and 9 at 1 and 5 minutes of life, respectively, and birth weight of 2,680 grams (5th percentile). To our knowledge, the mother had insulin-dependent gestational diabetes that arose at 34 weeks gestation. Since the family had arrived in the United States at 32 weeks gestation, the mother's glycemic control was not known. After admission, the patient's plasma glucose levels progressively fell from 73 mg/dL to

50 mg/dL (reference range: 70-99 mg/dL). At 19 hours of life, he became hypothermic, tachypneic, and increasingly lethargic with multiple poor feeding attempts. He was transferred to the NICU where his plasma glucose returned undetectable. He was subsequently started on dextrose-containing fluids as well as IV ampicillin and gentamicin for early-onset sepsis.

On DOL 2, the patient was noted to have significant lactic acidosis (lactate 20 mmol/L; reference range: ≤1.5 mmol/L) with pH 7.13 and bicarbonate <15 mmol/L (reference range: 20-26 mmol/L). Additionally, he had multiple increased laboratory markers of liver dysfunction. Due to persistent hypoglycemia, GIR in his total parenteral nutrition (TPN) was increased to 5.5 mg/kg/min. On DOL 3, feeding initiation was stopped to limit protein intake given a developing concern for a metabolic disorder with ongoing lactic acidosis and hyperammonemia. Shortly after, he became coagulopathic and was transferred to a quaternary care NICU on DOL 4 for further management.

On DOL 5, metabolic testing in the setting of hypoglycemia revealed persistently elevated lactate, elevated plasma alanine, glycine, proline, and tyrosine, and low serum beta-hydroxybutyrate. Given the patient's history of a normal newborn metabolic screen and the non-diagnostic pattern of these amino acid elevations in the setting of liver dysfunction, these lab abnormalities were determined to signal liver failure rather than evidence of a metabolic disorder. Feeds, including protein, were slowly reintroduced without a significant increase in ammonia. Due to ongoing lactic acidosis, liver failure with synthetic dysfunction, and persistent hypoglycemia, genetic testing with trio whole exome sequencing was sent. There was no reported family history of acute liver failure, hypoglycemia, or sudden infant death. The parents were of Sudanese ethnicity and fifth cousins. Their only other child is a healthy 8-year-old girl.

Hypoglycemia was noted again with an attempted transition from parenteral to enteral nutrition, so a critical sample was obtained on DOL 18 that revealed hypoketotic hypoglycemia with partial response to glucagon stimulation, suggesting possible HI. This patient was started on diazoxide and chlorothiazide to wean off GIR through the TPN and facilitate the transition to full enteral feeds. However, he demonstrated only a partial response to diazoxide with a continued need for dextrose support.

Whole exome sequencing was reported on DOL 51 with biparentally-inherited homozygous likely pathogenic variants in the *DGUOK* gene (c.757_759del), revealing DGUOK deficiency. Following this diagnosis, diazoxide and chlorothiazide were discontinued. A gastrostomy tube was placed to allow for background continuous formula feeds to prevent hypoglycemia with the addition of PO ad libitum feeds on top. Given the patient's multisystem disease, hepatology determined him ineligible for liver transplantation.

After a multidisciplinary discussion, the family decided they wanted to spend time with their infant at home, so palliative care was consulted to facilitate the transition to home care. He was discharged home on DOL 79. At the time of his outpatient follow-up at 3 months of age, he was stable on his discharge regimen with no new symptoms. The family subsequently moved with the patient to be closer to extended family members, and his care was transitioned to local pediatric subspecialists. A timeline of the clinical course for each patient is presented in Figure 1.

3 Discussion

Rapid genetic testing fundamentally altered the management of both patients diagnosed with a rare mitochondrial disorder. While biochemical studies suggested a diagnosis of congenital HI, molecular genetic testing confirmed mtDNA depletion syndrome from DGUOK deficiency. Of the six other documented patients with the same homozygous variant as our first patient, four of them passed away from liver failure at a couple of months to 2 years of age (8, 9). Notably, one of the patients alive at the time of publication received a liver transplant despite neurologic symptoms and was reported to continue having severe neurologic dysfunction and developmental delay afterward (10). It is unclear whether any of these patients also suffered from hyperinsulinemic hypoglycemia. Similarly, lactic acidosis with rapidly progressive liver failure and neurologic dysfunction were observed in two patients with a heterozygous c.749T>C *DGUOK* missense variant (9, 11). The only other reported heterozygous patient was alive at the time of publication with isolated liver disease (11). Based on this prior variant evidence, phenotypic overlap in our patient, functional data showing significantly reduced enzyme activity and the lack of homozygotes present in the Genome Aggregation Database (gnomAD) with a minor allele frequency of 0.0032% (8/251,466 alleles); this variant was classified as likely pathogenic (8).

In contrast, the c.757_759del variant identified in our second patient has only been reported in one other individual in ClinVar as a variant of uncertain significance. No citations or clinical data are provided for this case. Moreover, functional data on this variant are not available in the literature. No homozygotes are present in gnomAD, and the variant has a minor allele frequency of 0.00040% (1/251,456 alleles). Although specific clinical data were not available from other patients with this variant, this patient's clinical presentation and disease course were consistent with other cases of DGUOK deficiency.

In both cases, liver transplantation was considered to treat their fulminant liver failure. As of 2020, a total of 20 patients with DGUOK deficiency have undergone liver transplantation and half have died from transplant-related complications (12). Those without neurologic features prior to transplant may still go on to develop progressive neurologic dysfunction, complicating the decision to transplant in any case of DGUOK deficiency (2, 11, 13, 14). In the first patient, his neurologic symptoms precluded this option. Due to a multisystem disease in the second patient, he was also determined to be a poor liver transplant candidate.

Various disorders lead to altered glucose homeostasis, including HI, panhypopituitarism, glycogen storage disorders, fatty acid oxidation defects, ketogenesis or ketolysis defects, disorders of gluconeogenesis, galactosemia, hereditary fructose intolerance, congenital disorders of glycosylation, mitochondrial disease, syndromic hypoglycemia, and select aminoacidopathies (15). Diagnosis is often delayed by the overlapping features seen in these disorders. Biochemical analysis is necessary for phenotyping

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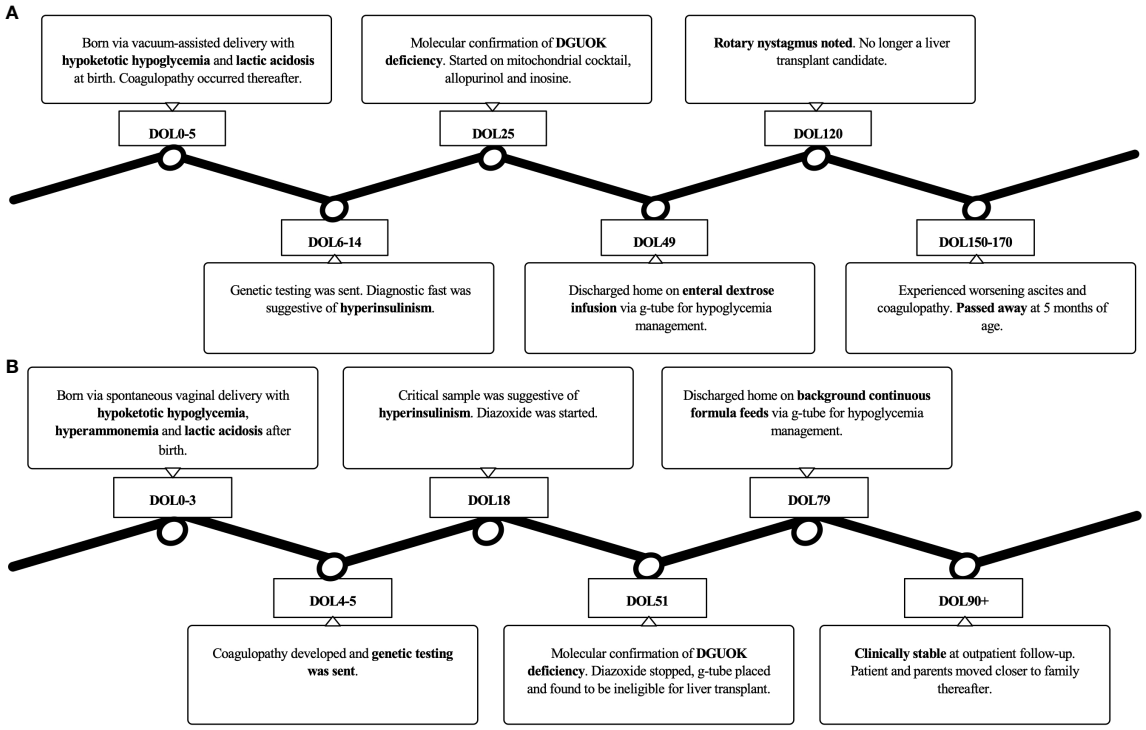

FIGURE 1
A timeline of the clinical course for **(A)** Patient 1 and **(B)** Patient 2.

and stratifying these patients (i.e., ketotic versus non-ketotic hypoglycemia), while molecular genetic testing is often required to confirm a diagnosis (15). Our presented equivocal cases help illustrate situations in which prompt initiation of genetic testing is essential to establishing an effective management plan.

For both of our patients, the diagnosis of HI was considered based on increased glucose requirements, the pattern of hypoketotic hypoglycemia, and the partial glycemic response to glucagon even though the elevated lactate was inconsistent with this disorder. HI is the most common cause of persistent hypoglycemia. It can be secondary to perinatal factors such as maternal diabetes or perinatal stress, or it can be genetic. Several genes are implicated in the development of HI, most commonly pathogenic variants in *ABCC8* and *KCNJ11* (15). Depending on the affected gene and inheritance pattern, responsiveness to medical treatment can vary with some cases requiring pancreatectomy (15, 16). Given these treatment considerations, genetic testing is considered the standard of care in suspected persistent HI. A two-tiered approach expedites molecular diagnosis: diazoxide-unresponsive patients who are more likely to have focal disease undergo Tier 1 testing that investigates for *ABCC8*, *KCNJ11*, and *GCK* mutations with a turnaround time of 5–7 days while diazoxide-responsive patients undergo more comprehensive genetic testing that results in ~4 weeks (16, 17). As nearly 50% of these patients with HI have non-diagnostic genetic testing results, expedited comprehensive genetic testing such as whole exome or genome sequencing may be warranted. This is especially critical in inconclusive cases like our own, in which the genetic diagnosis of DGUOK deficiency drastically altered clinical management and family decision making.

The characteristic features observed in multi-systemic DGUOK deficiency include lactic acidosis, hypoglycemia, cholestasis, progressive liver failure, and increasing neurologic dysfunction (2). However, diagnosis is complicated by phenotypic variability. Depending on the primary metabolic derangements observed, patients with DGUOK deficiency have been misclassified as neonatal hemochromatosis (hyperferritinemia and elevated alpha-fetoprotein) and tyrosinemia type 1 among other disorders (18). Most notably, an emerging feature of DGUOK deficiency is hyperinsulinemic hypoglycemia. In two deceased infants found to have DGUOK deficiency, autopsy tissue specimens revealed pancreatic islet cell hyperplasia suggestive of HI as the cause of their hypoketotic hypoglycemia. When alive, both autopsied patients had inappropriately high insulin levels and lower free fatty acids in the setting of hypoglycemia with a positive glycemic response to glucagon stimulation (6). A recent report of a third infant with DGUOK deficiency and hyperinsulinemic hypoglycemia reveals partial responsiveness to diazoxide therapy at 8 mg/kg/day dosing, which is similar to our second case (7). Our two cases now support a pattern of hyperinsulinemic hypoglycemia in DGUOK deficiency, thus warranting consideration of this mitochondrial disease in the workup of HI. Table 1 provides a summary of all reported cases of DGUOK deficiency with hyperinsulinemic hypoglycemia to date. It is important to note that we cannot rule out the possibility of perinatal stress-induced HI

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TABLE 1 All reported cases of deoxyguanosine kinase (DGUOK) deficiency with hyperinsulinemic hypoglycemia to date.

Reference	*DGUOK* variant	Age of presentation	Sex (M/F)	Clinical course	Critical sample[+]	Management
Patient 1	c.749T>C, p.Leu250Ser (homozygous)	At birth	M	Hypoketotic hypoglycemia with lactic acidosis and progressive liver failure resulting in death at 5 months	PG 37 mg/dL, BOHB <0.3 mmol/L, FFA 0.77 mmol/L, insulin 13.7 µIU/mL, c-peptide <0.1 ng/mL, lactate 4.2 mmol/L, delta glucose +22 in response to glucagon	Enteral dextrose infusion
Patient 2	c.757_759del (homozygous)	At birth	M	Hypoketotic hypoglycemia with lactic acidosis and transient hyperammonemia. Progressive liver failure, currently living	PG 38 mg/dL, BOHB <0.1 mmol/L, insulin 13.1 µIU/mL, delta glucose +13 in response to glucagon	Diazoxide, continuous formula feeds
Pronicka **et al.** (6)	c.766_767insGATT/ c.?, p.Phe256X/p.? (heterozygous)	At birth	F	Hypoketotic hypoglycemia with lactic acidosis and progressive liver failure resulting in death at 18 months	PG 22.8 mg/dL, BOHB 0.21 mmol/L, FFA 0.72 mmol/L, insulin 4.3 µIU/mL, delta glucose +77 in response to glucagon	Enteral dextrose infusion
	c.3G>A/ c.813_814insTTT, p.Met1Ile/ (compound heterozygous)	At birth	F	Hypoketotic hypoglycemia with lactic acidosis and progressive liver failure resulting in death at 6.5 months	PG 21.4 mg/dL, BOHB 0.15 mmol/L, FFA 0.1 mmol/L, insulin 5.7 µIU/mL, delta glucose +108 in response to glucagon	Enteral dextrose infusion
Arya **et al.** (7)	c.763_766dupGATT, p.Phe256* (homozygous)	At birth	M	Hypoketotic hypoglycemia with lactic acidosis and progressive liver failure resulting in death at 8 months	PG 36 mg/dL, BOHB <0.01 mmol/L, insulin 21.9 µIU/mL, lactate 4.1 mmol/L	Diazoxide, continuous formula feeds

*M, male; F, female; PG, plasma glucose; BOHB, beta-hydroxybutyrate; FFA, non-esterified free fatty acids.
[+]Referenc ranges: PG (70-99 mg/dL), BOHB (≥2.0-5.0 mmol/L), FFA (≥1.5 mmol/L), insulin (≤2 µIU/mL), c-peptide (0.0-3.3 ng/mL), lactate (≤1.5 mmol/L).

in the pathophysiology of hypoglycemia. Regardless, if there is evidence of HI, treatment with diazoxide may be of benefit because recurrent hypoketotic hypoglycemia may be contributing to the progressive neurologic dysfunction observed in these patients. Diazoxide-responsiveness has already been demonstrated in patients with tyrosinemia type 1 and HI, further supporting this treatment strategy for DGUOK deficiency (19).

Advances in next-generation sequencing have facilitated similar diagnoses in recent years, particularly in critically ill infants and children (20, 21). For example, among 354 infants receiving either early (15 days after study enrollment) or delayed (60 days after enrollment) whole genome sequencing results, twice as many participants with early genetic diagnosis had a change in clinical management, including subspecialty referrals, condition-specific medications, and surgical interventions (22). The overall diagnostic yield of genetic screening in this patient population is 37% with management changes reported in 20-100% of diagnosed patients (23). Not only does genetic testing affect clinical outcomes, but recent analyses also highlight the economic benefits of timely genetic diagnosis (20, 21, 24). By reducing the length of hospital stays, genetic testing can save up to two million US dollars per 100 patients tested (20). Moreover, one study demonstrated that both the public and affected families have a vested interest in rapid genetic testing for critically ill children despite the cost (25).

In conclusion, our experiences reinforce the importance of rapid genetic testing for patients with persistent neonatal hypoglycemia, particularly when biochemical testing is inconclusive. With these two new cases, there are now five patients with DGUOK deficiency and hyperinsulinemic hypoglycemia, extending the differential diagnosis of HI to include mtDNA depletion syndromes and giving further credence to the utility of expedited comprehensive genetic testing in these scenarios. Most significantly, timely molecular confirmation of DGUOK deficiency can shorten the diagnostic odyssey for these patients and allow for the cessation of futile interventions, thus maximizing the time a family has with their affected child.

4 Patient perspective

Not provided by the parents of Patient 1. We were unable to reach the parents of Patient 2.

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 minors' legal guardian/next of kin for the publication of this case report.

Author contributions

HG: Writing – original draft, Writing – review & editing. SY: Writing – original draft, Writing – review & editing. JH: Writing – original draft, Writing – review & editing. KC: Writing – review & editing. BV: Writing – review & editing. LP: Writing – review & editing. HM: Writing – review & editing. WS: Writing – review & editing. KL: Writing – review & editing. DL: Writing – review & editing. NM: Writing – original draft, Writing – review & editing. RG: Writing – review & editing.

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Conflict of interest

NM is currently on the advisory board of BioMarin and Pfizer; there is no conflict of interest in relation to this current paper or topic. KC is on the advisor board of Travere Therapeutics and PI for the HERO trial by HemoShear Therapeutics and her institution is reimbursed for her time; there is no conflict of interest in relation to this current paper or topic. BV is a consultant for Mirum Pharmaceuticals; there is no conflict of interest in relation to this current paper or topic. DL has received consulting fees from Eiger Biopharmaceuticals, Crinetics Pharmaceuticals, Hanmi Pharmaceuticals, and Zealand Pharma. She has also received research funding/contracts from Zealand Pharma, Hanmi Pharmaceuticals, Twist Pharma, Crinetics Pharmaceuticals, Rezolute, and Ultragenyx. There is no conflict of interest in relation to this current paper or topic.

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1268135/full#supplementary-material

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EDITED BY
Wei Ge,
University of Macau, China

REVIEWED BY
Stan Hileman,
West Virginia University, United States
Takeshi Onuma,
Osaka University, Japan

*CORRESPONDENCE
Ziliang Wang
wangziliang1986@126.com
Gengsheng He
gshe@shmu.edu.cn

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Associations between maternal urinary kisspeptin in late pregnancy and decreased fetal growth: a pregnancy-birth cohort study

Jiaxian Chen[1], Lan Yang[1], Yafei Chen[1], Wei Yuan[2], Yao Chen[2], Hong Liang[2], Maohua Miao[2], Gengsheng He[3]* and Ziliang Wang[2]*

[1]Shanghai-MOST Key Laboratory of Health and Disease Genomics, NHC Key Lab of Reproduction Regulation, Shanghai Institute for Biomedical and Pharmaceutical Technologies, School of Public Health, Fudan University, Shanghai, China, [2]Shanghai-MOST Key Laboratory of Health and Disease Genomics, NHC Key Lab of Reproduction Regulation, Shanghai Institute for Biomedical and Pharmaceutical Technologies, Shanghai, China, [3]Department of Nutrition and Food Hygiene, School of Public Health, Key Laboratory of Public Health Safety, Ministry of Education, Fudan University, Shanghai, China

Background: Kisspeptin has been indicated to be a biomarker of fetal growth. Although some evidence suggested that maternal kisspeptin concentrations in early pregnancy were associated with increased fetal growth, studies are still limited and the effect of kisspeptin in late pregnancy remains unknown. This study aimed to investigate the associations between maternal kisspeptin in late pregnancy and fetal growth.

Methods: Based on the Shanghai-Minhang Birth Cohort study, 724 mother-neonate pairs were included in this study. We measured maternal kisspeptin concentrations in the urine samples collected in late pregnancy and neonatal anthropometric indices at birth. The associations between maternal kisspeptin and neonatal anthropometry were investigated using multiple linear regression models.

Results: Higher maternal urinary kisspeptin concentrations were associated with lower neonatal birth weight, head circumference, upper arm circumference, abdominal skinfold thickness, triceps skinfold thickness, and back skinfold thickness. The inverse associations were more pronounced for the highest kisspeptin levels versus the lowest. These patterns were consistent in analyses stratified by neonatal sex, with notably stable associations between maternal kisspeptin concentrations and skinfold thickness.

Conclusion: The present study suggested that maternal kisspeptin concentrations in late pregnancy might be inversely associated with fetal growth. The physiological mechanisms of maternal kisspeptin might differ from those in early pregnancy. Further studies are required to assess associations

between maternal kisspeptin and energy homeostasis and explore the physiological roles of kisspeptin in late pregnancy.

KEYWORDS

kisspeptin, neonatal anthropometry, fetal growth, late pregnancy, skinfold thickness

1 Introduction

Kisspeptin is a family of natural neuropeptides consisting of kisspeptin-54, 14, 13, and 10, encoded by the gene *KiSS-1* (1, 2). It has been proved that kisspeptin is a key regulator of reproductive development and functions, as it stimulates the secretion of Gonadotrophin-Releasing Hormone (GnRH) and activates the Hypothalamic-Pituitary-Gonadal (HPG) axis by binding to its natural ligand G Protein-Coupled Receptor 54 (GPR54), which is encoded by the gene *KiSS-1R* (2).

Recent studies have suggested additional roles of kisspeptin in regulating placentation and later pregnancy. *KiSS-1* and *KiSS-1R* are found highly expressed by trophoblast cells in the placenta during pregnancy (3). With rapid proliferation and differentiation of trophoblast cells, maternal circulating kisspeptin levels continuously increase during pregnancy and peak at delivery (4). Compared with the nonpregnant, circulating kisspeptin levels of pregnant women increase 900-fold in the first trimester and 7000-fold in the third trimester (5). During the initial stages of gestation, kisspeptin plays a key role in inhibiting angiogenesis, restraining trophoblast invasion and migration, and regulating implantation and subsequent placental development (3). Several epidemiological studies have associated decreased maternal kisspeptin levels in early pregnancy with unfavorable pregnancy outcomes, such as spontaneous miscarriage (6), preeclampsia (7), and preterm birth (8). Further, kisspeptin in early pregnancy may also act as a biomarker to predict fetal growth. A few case-control studies have associated maternal kisspeptin levels in the first trimester with increased neonatal birth weight (9–11).

However, growing evidence supports that besides kisspeptin's important role in regulating early pregnancy, it is also involved in the later fine-tuning of many other key procedures related to fetal growth (11), including maternal energy homeostasis (12) and programming of fetal endocrine functions (13). A study has reported inverse associations between kisspeptin concentrations in late pregnancy and neonatal birth weight, which is inconsistent with the findings for early pregnancy (14). However, another study did not find any associations (15). In general, studies on associations between maternal kisspeptin and fetal growth are still scarce and inconsistent, especially in the lack of evidence of kisspeptin in late pregnancy. Therefore, this study aimed to investigate associations between maternal kisspeptin levels in late pregnancy and fetal growth reflected by a range of neonatal anthropometric indices.

2 Materials and methods

2.1 Study participants and design

We used data from the Shanghai-Minhang Birth Cohort Study (S-MBCS), which was an ongoing prospective study designed to examine the effects of environmental exposures on both mothers' and their children's health. Between April and December 2012, pregnant women were recruited when they visited Minhang Maternal and Child Health Hospital in Shanghai, China for their first prenatal care visit (12-16 weeks of gestation). Detailed inclusion and exclusion criteria have been described elsewhere (16). In total, 1,292 pregnant women were recruited in the study, and 1,233 delivered live infants in the study hospital (28 delivered in another hospital, 31 abortions or stillbirths). We further excluded 8 twin pregnancies, leaving 1,225 live singletons. Single-spot urine samples of pregnant women were collected at 31.6 weeks of gestation on average for kisspeptin measurement. Neonatal anthropometry collection was conducted 1 (with an interquartile range of 1 to 2) day after birth. Due to limited funding, we selected 724 women for urinary kisspeptin measurements, based on criteria such as sufficient urine volume and availability of follow-up visit information (17). Thus, a total of 724 mother-neonate pairs with both maternal kisspeptin concentrations and neonatal anthropometry records were included in this study (Figure 1).

All women provided informed consent for themselves and their children at recruitment and each follow-up visit. The study protocol was approved by the ethical committee of the Shanghai Institute for Biomedical and Pharmaceutical Technologies (formerly the Shanghai Institute of Planned Parenthood Research).

2.2 Measurement of maternal urinary kisspeptin

Urine samples were collected at 31.6 weeks of gestation on average and frozen at -80°C before being transferred to the laboratory for measurement. Total kisspeptin concentrations (kisspeptin-54, kisspeptin-14, kisspeptin-13, and kisspeptin-10)

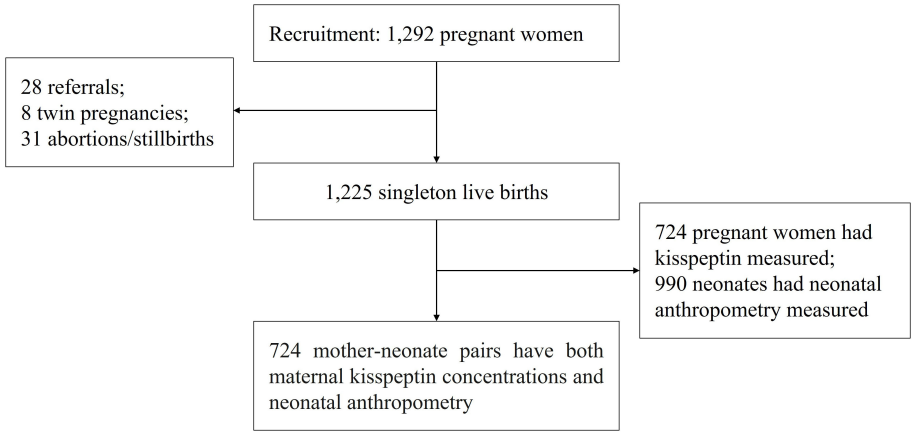

FIGURE 1
Participant recruitment and flow in the Shanghai-Minhang Birth Cohort Study.

were measured using the Human Kisspeptin 1(KiSS1) Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Blue Gene, Shanghai, China) according to the manufacturer's protocol, without any dilution (18). The assay had high sensitivity and excellent specificity according to the introduction of the kit, with inter-assay and intra-assay coefficients of variation<10% and recovery rates ranging from 94% to 103%. More details of kisspeptin measurement have been previously described (19).

2.3 Measurement of neonatal anthropometry

We utilized a range of neonatal anthropometric indices to evaluate fetal growth, including birth weight, upper arm circumference, abdominal circumference, head circumference, triceps skinfold thickness, back skinfold thickness, and abdominal skinfold thickness. Each index reflects the growth of distinct body parts. Birth weight, as a comprehensive parameter, is employed to monitor neonatal growth and nutritional status. Head circumference is used as a proxy for brain growth. Upper arm circumference is considered a parameter to reflect the combined muscle and fat. Abdominal circumference is employed to assess the size of the abdominal viscera. Skinfold thickness is utilized to estimate body fat, with upper arm skinfold thickness mainly reflecting peripheral subcutaneous fat and abdominal skinfold thickness mainly reflecting central subcutaneous fat (20, 21).

Neonatal anthropometry collection was conducted 1 (with an interquartile range of 1 to 2) day after birth. The neonate's birth weight (scaled with fine 1g graduation) was retrieved from the medical birth records (22). Head, arm, and abdominal circumference (scaled with fine 0.1 cm graduation) were measured by tape. Head circumference was measured with the tape placed from above the eyebrows to the maximum protrusion of the occiput. Abdominal circumference was measured with the tape placed around the abdomen just above the umbilicus and perpendicular to the long mid-axis of the trunk. Upper arm circumference was measured with the tape placed through the midpoint between the acromion and tip of the olecranon on the right arm (23). Skinfold thickness (scaled with fine 0.1 mm graduation) was measured using a skinfold caliper. While the neonate was lying in a prone position, physicians stood on the right side of the body and placed the skinfold caliper respectively over the right triceps, midway between the posterior border of the tip of the acromion and the olecranon (triceps skinfold thickness), below the lower angle of the right scapula (back skinfold thickness), and at the intersection point on the midline of the clavicle and parallel to the navel (abdominal skinfold thickness) (24). Each neonate was measured twice by the two proficient physicians who were blinded to the mothers' kisspeptin levels at the time of measurement, and the averages of their measurements were used for analysis.

2.4 Covariates collection

At enrollment, we used a structured questionnaire to collect information about demographic characteristics, reproductive history, health conditions, and lifestyle factors. Maternal pre-pregnancy body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Additionally, maternal urinary creatinine concentration was also measured to control for urine dilution.

2.5 Statistical analysis

The included and excluded mother-neonate pairs' demographic characteristics, pregnancy-related information, and neonatal anthropometric indices were tabulated. We also described the distributions of creatinine-adjusted maternal urinary kisspeptin concentrations among populations with different characteristics. Those data in different groups were compared using student's t-tests and chi-square tests.

Kisspeptin concentrations were first included as continuous variables in multiple linear regression models to examine the

general pattern of associations between maternal kisspeptin concentrations and neonatal anthropometric indices. We further investigated the effects of kisspeptin at different levels by tertiles (the first tertile group as the reference group) in model 2, since generalized additive models (GAM) suggested the existence of nonlinear associations between kisspeptin concentrations and some specific neonatal anthropometric indices. As previous studies had reported sex-specific effects of kisspeptin (12), we further stratified all our analyses by neonatal sex. Covariates were included based on the evidence of a potential confounder from previous literature (22). Additional covariates that changed the estimates by more than 10% were also included. Finally, the following variables were included in multiple linear regression models: maternal age (<25, 25-30 or ≥30 years), maternal education (middle school or below, high school, or college or above), family income per capita (<4000, 4000-8000, or ≥8000 Chinese Yuan (CNY)/month), maternal pre-pregnancy BMI (<18.5, 18.5-24, or ≥24.0 kg/m^2), paternal drinking before conception (yes or no), maternal weight gain during pregnancy (kg), parity (nulliparous or multiparous), gestational weeks (<37, 37-42, or ≥42 weeks), maternal disease status (yes for mothers with chronic diseases diagnosed before or during pregnancy, such as diabetes mellitus, hypertension, and hypothyroidism, or no) and neonatal sex (boys or girls). Urinary creatinine concentrations were $\log_{10}$-transformed and included as a covariate to control for urine dilution (25).

We further conducted several sensitivity analyses to test the robustness of the results. We removed pregnant women with chronic diseases such as diabetes mellitus, hypertension, and hypothyroidism to repeat main analyses among the remaining 499 mother-neonate pairs, considering that these diseases may have effect modifications on the associations between maternal kisspeptin and neonatal anthropometry (9–11). Similarly, we restricted the main analyses to 514 pregnant women whose pre-pregnancy BMI was normal (18.5-24 kg/m^2) but still included BMI as a continuous variable in linear regression models (10, 26).

All analyses were conducted with SAS version 9.4 (SAS Institute Inc., Cary, NC). A *p*-value less than 0.05 from two-tailed tests was considered statistically significant.

3 Results

3.1 General characteristics of the population

The characteristics of the participants are presented in Table 1. Among the 724 participating mothers, approximately half were between 25-30 years old at parturition (55.11%), reported a monthly family income per capita between 4000-8000 CNY/month (41.76%), and were exposed to passive smoking before conception (40.36%). The majority of participants were well-educated (77.32% graduated from college or above), had a normal weight before pregnancy (72.39% had a BMI between 18.5 and 24 kg/m^2), did not suffer from chronic diseases before or during pregnancy (68.92%), and reported no alcohol consumption before conception for their partners (68.10%). Compared with the excluded group, pregnant women included were more likely to be nulliparous (86.91% vs. 81.12%), gain more weight during pregnancy (16.56 kg vs. 15.79 kg), and have a longer gestational age (39.61 weeks vs. 39.33 weeks). About 57% of the included neonates were boys, higher than the proportion of excluded male neonates (50.2%). The mean (± SD) birth weight, head circumference, upper arm circumference, abdominal circumference, abdominal skinfold thickness, triceps skinfold thickness, and back skinfold thickness of the neonates included were 3442.72 (± 432.89) g, 35.16 (± 1.18) cm, 11.08 (± 1.00) cm, 33.63 (± 1.80) cm, 2.63 (± 0.76) mm, 4.04 (± 1.10) mm, and 3.98 (± 1.08) mm, respectively. All anthropometric indices were normally distributed. No significant differences in anthropometric indices were observed between the included and the excluded neonates.

3.2 Distributions of maternal urinary kisspeptin concentrations

The distributions of maternal urinary kisspeptin concentrations are shown in Supplementary Table 1. The mean concentration of creatinine-adjusted urinary kisspeptin was 1335.14 ng/g creatinine. Women with higher family incomes had higher kisspeptin concentrations. Additionally, pre-pregnancy BMI was inversely associated with kisspeptin concentrations.

3.3 Associations between maternal urinary kisspeptin and neonatal anthropometry

In general, inverse associations between maternal urinary kisspeptin concentrations in late pregnancy and neonatal anthropometric indices were observed. In model 1, we used kisspeptin as a continuous variable and found that higher maternal kisspeptin concentrations were consistently associated with lower neonatal anthropometric indices, with statistical significance reaching for all indices except abdominal circumference. When the analyses were stratified by neonatal sex, we found a consistent pattern among both male and female neonates (Figure 2; Supplementary Table 2).

Further, when kisspeptin was included as a categorical variable in model 2, the inverse associations were mainly found for the highest levels of maternal kisspeptin, compared with the lowest (Table 2). Compared with neonates with the first tertile of maternal kisspeptin, those with the highest maternal kisspeptin levels had lower birthweight (β=-88.81, 95% confidence interval (CI): -173.39, -4.24), upper arm circumference (β=-0.21, 95%CI: -0.41, -0.01), abdominal skinfold thickness (β=-0.27, 95%CI: -0.42, -0.11), triceps skinfold thickness (β=-0.25, 95%CI: -0.47, -0.02) and back skinfold thickness (β=-0.40, 95%CI: -0.62, -0.18). Similar patterns were observed in analyses stratified by neonatal sex, with the significance only reached for skinfold thickness. The highest levels of maternal kisspeptin were associated with lower abdominal skinfold thickness (β=-0.23, 95%CI: -0.42, -0.03) and back

TABLE 1 Characteristics of mother-neonate pairs included and not included.

Characteristics	Included (n=724)	Excluded (n=501)	p‡
Maternal age at parturition (years)			0.0837
<25	75 (10.36)	70 (14.62)	
25-30	399 (55.11)	249 (51.98)	
≥30	250 (34.53)	160 (33.40)	
Maternal education			0.3479
Middle school	64 (8.85)	55 (11.00)	
High school	100 (13.83)	75 (15.00)	
College or above	559 (77.32)	370 (74.00)	
Family income per capita (CNY/month)			0.0736
<4000	134 (18.72)	119 (24.14)	
4000-8000	299 (41.76)	190 (38.54)	
≥8000	283 (39.52)	184 (37.32)	
Maternal passive smoking before conception			0.9332
Yes	291 (40.36)	203 (40.60)	
No	430 (59.64)	297 (59.40)	
Paternal drinking before conception			0.7603
Yes	230 (31.90)	163 (32.73)	
No	491 (68.10)	335 (67.27)	
Pre-pregnancy BMI (kg/m^2)			0.6895
<18.5	138 (19.44)	106 (21.46)	
18.5-24	514 (72.39)	348 (70.44)	
≥24.0	58 (8.17)	40 (8.10)	
Parity			0.0061
Nulliparous	624 (86.91)	404 (81.12)	
Multiparous	94 (13.09)	94 (18.88)	
Gestational age at parturition (weeks)			<0.0001
<37	12 (1.66)	33 (6.60)	
37-42	701 (96.82)	457 (91.40)	
≥42	11 (1.52)	10 (2.00)	
Maternal disease			0.1378
Yes	225 (31.08)	136 (27.15)	
No	499 (68.92)	365 (72.85)	
Total weight gain during pregnancy (kg)	16.56 ± 4.47	15.79 ± 5.11	0.0109
Gestational age at maternal urine collection (weeks)	31.60 ± 1.60	31.77 ± 1.79	0.2941
Neonatal sex			0.0107
Male	417 (57.60)	250 (50.20)	
Female	307 (42.40)	248 (49.80)	
Birth weight (g)	3442.72 ± 432.89	3391.02 ± 546.08	0.1179
Head circumference (cm)	35.16 ± 1.18	35.10 ± 1.18	0.3395

(Continued)

TABLE 1 Continued

Characteristics	Included (n=724)	Excluded (n=501)	p‡
Upper arm circumference (cm)	11.08 ± 1.00	11.17 ± 1.11	0.4789
Abdominal circumference (cm)	33.63 ± 1.80	33.67 ± 1.79	0.7725
Abdominal skinfold thickness (mm)	2.63 ± 0.76	2.62 ± 0.77	0.6157
Triceps skinfold thickness (mm)	4.04 ± 1.10	4.03 ± 1.07	0.9231
Back skinfold thickness (mm)	3.98 ± 1.08	3.88 ± 0.97	0.3075

‡p-value for differences between included and not included mother-neonate pairs. BMI, body mass index; CNY, Chinese Yuan.

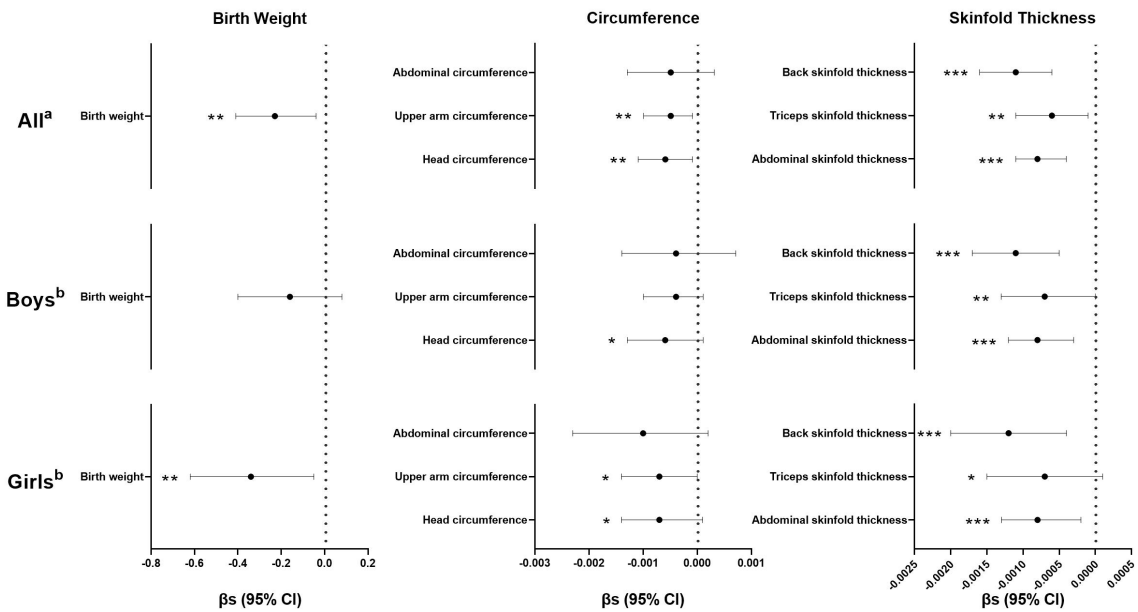

FIGURE 2
Regression coefficients (βs) and 95% confidence intervals (CIs) for the associations between maternal kisspeptin levels (continuous) in late pregnancy and neonatal anthropometry using multiple linear regression models. [a] Adjusted for log_{10}-transformed maternal creatinine concentrations, family income, maternal education, paternal drinking before conception, maternal age, maternal pre-pregnancy body mass index, parity, gestational age, total weight gain, maternal disease, and neonatal sex. [b] Adjusted for all the variables in Note a except for neonatal sex. Numeric data are available in Supplementary Table-2. *p< 0.10, **p< 0.05, ***p< 0.01, n(All)=724, n(Boys)=417, n(Girls)=307.

TABLE 2 Regression coefficients (βs) and 95% confidence intervals (CIs) for the associations between maternal kisspeptin levels (categorical) in late pregnancy and neonatal anthropometry using multiple linear regression models[a].

Neonatal anthropometric indices	Kisspeptin levels	All (n=724)[b]	Boys (n=417)[c]	Girls (n=307)[c]
Birth weight (g)				
	1st tertile	Ref.	Ref.	Ref.
	2nd tertile	4.98 (-70.43, 80.39)	-18.04 (-116.99, 80.92)	14.85 (-102.84, 132.55)
	3rd tertile	-88.81 (-173.39, -4.24)**	-72.70 (-182.71, 37.31)	-104.77 (-238.55, 29.02)
Head circumference (cm)				
	1st tertile	Ref.	Ref.	Ref.
	2nd tertile	0.03 (-0.18, 0.23)	0.01 (-0.27, 0.29)	0.00 (-0.31, 0.32)

(Continued)

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TABLE 2 Continued

Neonatal anthropometric indices	Kisspeptin levels	All (n=724)[b]	Boys (n=417)[c]	Girls (n=307)[c]
	3rd tertile	-0.23 (-0.46, 0.00)*	-0.24 (-0.55, 0.07)	-0.30 (-0.65, 0.06)
Upper arm circumference (cm)				
	1st tertile	Ref.	Ref.	Ref.
	2nd tertile	0.01 (-0.17, 0.19)	0.01 (-0.22, 0.23)	0.04 (-0.25, 0.34)
	3rd tertile	-0.21 (-0.41, -0.01)**	-0.16 (-0.41, 0.09)	-0.24 (-0.57, 0.10)
Abdominal circumference (cm)				
	1st tertile	Ref.	Ref.	Ref.
	2nd tertile	0.12 (-0.21, 0.44)	-0.08 (-0.51, 0.34)	0.31 (-0.20, 0.82)
	3rd tertile	-0.22 (-0.58, 0.15)	-0.29 (-0.76, 0.18)	-0.15 (-0.72, 0.43)
Abdominal skinfold thickness (mm)				
	1st tertile	Ref.	Ref.	Ref.
	2nd tertile	-0.04 (-0.18, 0.10)	-0.05 (-0.23, 0.13)	-0.01 (-0.24, 0.22)
	3rd tertile	-0.27 (-0.42, -0.11)***	-0.23 (-0.42, -0.03)**	-0.27 (-0.53, -0.01)**
Triceps skinfold thickness (mm)				
	1st tertile	Ref.	Ref.	Ref.
	2nd tertile	-0.06 (-0.26, 0.14)	-0.03 (-0.30, 0.23)	-0.01 (-0.33, 0.31)
	3rd tertile	-0.25 (-0.47, -0.02)**	-0.14 (-0.44, 0.15)	-0.29 (-0.65, 0.08)
Back skinfold thickness (mm)				
	1st tertile	Ref.	Ref.	Ref.
	2nd tertile	-0.07 (-0.27, 0.12)	-0.02 (-0.27, 0.22)	-0.08 (-0.40, 0.24)
	3rd tertile	-0.40 (-0.62, -0.18)***	-0.34 (-0.62, -0.07)**	-0.38 (-0.74, -0.01)**

[a]Kisspeptin concentrations were included as a categorical variable by tertiles.
[b]Adjusted for $\log_{10}$-transformed maternal creatinine concentrations, family income, maternal education, paternal drinking before conception, maternal age, maternal pre-pregnancy body mass index, parity, gestational age, total weight gain, maternal disease, and neonatal sex.
[c]Adjusted for all the variables in Note b except for neonatal sex.
*$p < 0.10$ vs. 1st tertile, **$p < 0.05$ vs. 1st tertile, ***$p < 0.01$ vs. 1st tertile.

skinfold thickness (β=-0.34, 95%CI: -0.62, -0.07) among male neonates, and lower abdominal skinfold thickness (β=-0.27, 95% CI: -0.53, -0.01) and back skinfold thickness (β=-0.38, 95%CI: -0.74, -0.01) among female neonates, respectively.

Exclusion of women with chronic diseases or restricting the analyses to women with normal pre-pregnancy BMI did not essentially change the patterns, while several estimates lost statistical significance due to the reduced sample size. Nevertheless, the significant associations between maternal kisspeptin levels in late pregnancy and decreased neonatal fat mass (abdominal skinfold thickness and back skinfold thickness) remained stable (Supplementary Tables 3, 4).

4 Discussion

To our knowledge, this is the first study to investigate the potential role of maternal kisspeptin levels in late pregnancy in fetal growth reflected by a range of neonatal anthropometric indices, including birth weight, circumference, and skinfold thickness. We observed a consistent pattern of the associations between maternal urinary kisspeptin concentrations in late pregnancy and decreased neonatal anthropometry among both sexes, particularly for the highest kisspeptin levels compared with the lowest. Notably, the significant associations between maternal kisspeptin levels and neonatal fat mass were relatively stable.

Consistent with our results, a case-control study reported the inverse associations between maternal kisspeptin concentrations in late pregnancy and neonatal birth weight among 40 healthy mother-neonate pairs (14). Similarly, inverse associations were also observed in studies on fetal growth and placental expressions of *KISS-1* which are highly correlated with circulating kisspeptin concentrations in late pregnancy since the placenta is the main source of maternal circulating kisspeptin during pregnancy (4, 27). Nevertheless, another case-control study reported no associations between maternal kisspeptin concentrations in any trimester and fetal growth among healthy mother-neonate pairs (15), whose ability to detect statistical significance might be limited by the relatively small sample size (around 25 participants). Notably, the inverse associations of kisspeptin in late pregnancy with neonatal

anthropometry in our study were different from those in early pregnancy (9–11, 28).

The different associations between maternal kisspeptin concentrations and neonatal anthropometry could potentially be explained by varying physiological roles and regulatory mechanisms of kisspeptin in different stages of pregnancy. In early pregnancy, placental kisspeptin has been reported to be associated with invasive capacity, with evidence showing that the peak of placental expressions of kisspeptin coincides with implantation and placentation (29). Decreased maternal kisspeptin concentrations in early pregnancy have been linked with impaired fetal growth due to dysfunction of implantation and placentation while administering kisspeptin in early gestation could alleviate the adverse consequences and further improve fetal growth (5, 29–31). Intriguingly, despite the fact that placental expressions of *KISS-1* peak in early pregnancy, circulating kisspeptin concentrations continuously rise until parturition when implantation and placentation have already completed, suggesting that maternal kisspeptin may influence fetal growth through other regulatory mechanisms in later stages of pregnancy (5, 32). Kisspeptin has been suggested to be related to energy homeostasis, with several animal studies showing positive associations between kisspeptin concentrations and energy-regulatory hormones like leptin and oxytocin which can reduce appetite and food intake and increase energy expenditure, especially among pregnant rats (32–35). Recently, kisspeptin has been found to exert direct effects on energy homeostasis (12), supported by the anatomical associations and functional feedback between kisspeptin and key appetite-regulating neurons found in rodent models (36, 37). Both animal and epidemiological studies have reported that lack of kisspeptin leads to increased appetite, body weight, and fat mass (10, 12, 26), which is also in line with the inverse associations between maternal kisspeptin concentrations and pre-pregnancy BMI observed in our study.

Although the physiological mechanisms underlying the interactions between kisspeptin and energy homeostasis are still unclear, the correlations between kisspeptin and energy-regulatory hormones, food intake, body weight, and fat mass may offer some potential explanations for our findings, considering that maternal energy homeostasis is closely linked with fetal growth (34). Moreover, since the placenta has also been supposed to be the main source of neonatal kisspeptin (38), maternal kisspeptin concentrations in late pregnancy may represent the concentrations of neonates. Given the fact that lack of kisspeptin leads to increased body fat mass (39), the stable inverse associations between maternal kisspeptin concentrations and neonatal fat mass observed in our study seemed explicable. In addition, maternal kisspeptin may be also positively associated with fetal leptin levels (13), which provides a further explanation for the inverse associations described above due to leptin's critical role in reducing body fat (34).

Our study has several strengths. This is the first large-scale prospective study to examine the associations between maternal kisspeptin concentrations in late pregnancy and a range of anthropometric indices, suggesting the roles of kisspeptin in regulating fetal growth in late pregnancy, and thereby offering novel insights. Additionally, we collected a broad range of data on maternal and children's characteristics, allowing for adjustment for potential covariates. Last but not least, we considered the potential modification effects by neonatal sex, maternal diseases, and maternal pre-pregnancy BMI, and observed similar patterns, indicating the relative robustness of the associations between maternal kisspeptin concentrations and fetal growth.

Despite the strengths, we also have some limitations to acknowledge. Firstly, due to limited funding, our study selected about 60% of mother-neonate pairs for kisspeptin measurement from the cohort. The included and excluded participants had similar characteristics except for the slight differences in gestational age, parity, and maternal weight gain during pregnancy. In addition, no significant differences were found in neonatal characteristics between the included and excluded infants. Thus, our results were less likely to be attributed to selection bias. Secondly, we collected a single-spot urine sample, which may not accurately reflect the kisspeptin concentrations due to the short biological half-life of this biomarker (40). This may lead to non-differential misclassification, resulting in the attenuation of the associations (41). Finally, we did not investigate the interactions between maternal kisspeptin and energy-regulatory hormones in this study. It would be valuable to examine hormones like leptin to explore the mechanisms underlying the associations between maternal kisspeptin and fetal growth in future studies.

In conclusion, this study found that maternal urinary kisspeptin concentrations in late pregnancy were inversely associated with neonatal anthropometry and these associations were mainly found for the highest kisspeptin levels, compared with the lowest. Our results suggest that maternal kisspeptin in late pregnancy may be associated with decreased fetal growth and the related physiological mechanisms may differ from those in early pregnancy. Considering the new findings, further studies are required to corroborate our results. In addition, studies on the potential role of kisspeptin in regulating energy homeostasis are encouraged to explore the physiological mechanisms of the associations we observed.

Data availability statement

The datasets presented in this article are not readily available because some or all datasets generated may refer to individual privacy. Requests to access the datasets should be directed to ZW, ziliangwang1986@126.com.

Ethics statement

The studies involving humans were approved by the ethical committee of the Shanghai Institute for Biomedical and Pharmaceutical Technologies (formerly the Shanghai Institute of Planned Parenthood Research). 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

JC: Formal Analysis, Writing – original draft, Writing – review & editing. LY: Formal Analysis, Methodology, Writing – review & editing. YFC: Formal Analysis, Methodology, Writing – review & editing. WY: Writing – review & editing, Funding acquisition, Supervision. YC: Formal Analysis, Methodology, Writing – review & editing. HL: Funding acquisition, Methodology, Resources, Writing – review & editing. MM: Funding acquisition, Supervision, Writing – review & editing. GH: Conceptualization, Supervision, Writing – review & editing. ZW: Conceptualization, Funding acquisition, 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1257248/full#supplementary-material

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EDITED BY
Wei Ge,
University of Macau, China

REVIEWED BY
Xuebin Fu,
Ann & Robert H. Lurie Children's Hospital of Chicago, United States
Antonietta Franco,
Washington University in St. Louis, United States

*CORRESPONDENCE
Gabriela Pavlinkova
gpavlinkova@ibt.cas.cz

†These authors share first authorship

‡These authors share senior authorship

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Reprogramming of the developing heart by Hif1a-deficient sympathetic system and maternal diabetes exposure

Hana Kolesova[1,2†], Petra Hrabalova[3,4†], Romana Bohuslavova[3], Pavel Abaffy[5], Valeria Fabriciova[3], David Sedmera[1,2‡] and Gabriela Pavlinkova[3*‡]

[1]Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, Czechia, [2]Department of Developmental Cardiology, Institute of Physiology Czech Academy of Sciences (CAS), Prague, Czechia, [3]Laboratory of Molecular Pathogenetics, Institute of Biotechnology Czech Academy of Sciences (CAS), BIOCEV, Vestec, Czechia, [4]Faculty of Science, Charles University, Prague, Czechia, [5]Laboratory of Gene Expression, Institute of Biotechnology Czech Academy of Sciences (CAS), BIOCEV, Vestec, Czechia

Introduction: Maternal diabetes is a recognized risk factor for both short-term and long-term complications in offspring. Beyond the direct teratogenicity of maternal diabetes, the intrauterine environment can influence the offspring's cardiovascular health. Abnormalities in the cardiac sympathetic system are implicated in conditions such as sudden infant death syndrome, cardiac arrhythmic death, heart failure, and certain congenital heart defects in children from diabetic pregnancies. However, the mechanisms by which maternal diabetes affects the development of the cardiac sympathetic system and, consequently, heightens health risks and predisposes to cardiovascular disease remain poorly understood.

Methods and results: In the mouse model, we performed a comprehensive analysis of the combined impact of a *Hif1a*-deficient sympathetic system and the maternal diabetes environment on both heart development and the formation of the cardiac sympathetic system. The synergic negative effect of exposure to maternal diabetes and *Hif1a* deficiency resulted in the most pronounced deficit in cardiac sympathetic innervation and the development of the adrenal medulla. Abnormalities in the cardiac sympathetic system were accompanied by a smaller heart, reduced ventricular wall thickness, and dilated subepicardial veins and coronary arteries in the myocardium, along with anomalies in the branching and connections of the main coronary arteries. Transcriptional profiling by RNA sequencing (RNA-seq) revealed significant transcriptome changes in *Hif1a*-deficient sympathetic neurons, primarily associated with cell cycle regulation, proliferation, and mitosis, explaining the shrinkage of the sympathetic neuron population.

Discussion: Our data demonstrate that a failure to adequately activate the HIF-1α regulatory pathway, particularly in the context of maternal diabetes, may contribute to abnormalities in the cardiac sympathetic system. In conclusion, our findings indicate that the interplay between deficiencies in the cardiac sympathetic system and subtle structural alternations in the vasculature,

microvasculature, and myocardium during heart development not only increases the risk of cardiovascular disease but also diminishes the adaptability to the stress associated with the transition to extrauterine life, thus increasing the risk of neonatal death.

KEYWORDS

mouse model, maternal diabetes, coronary arteries, sympathetic neurons, cardiac sympathetic system

1 Introduction

The cardiac sympathetic system is a part of the autonomic nervous system that controls heart performance. This regulation is dependent on accurate connections between postganglionic sympathetic neurons and the heart, which is established during embryonic and postnatal development. Abolishing sympathetic system function affects survival due to cardiac failure (1–3). Most sympathetic postganglionic neurons innervating the heart are located in the stellate ganglion, with a smaller number present in the middle cervical and upper thoracic sympathetic ganglia of the sympathetic chains (4). Postganglionic neurons in these ganglia receive terminals from cardiac sympathetic preganglionic neurons in the upper thoracic spinal segments. Sympathetic innervation density is variable between regions both across and within the heart muscle layers and between the chambers of the heart (5). The highest density of sympathetic innervation is in the subepicardium and central conduction system, and it gradually decreases from the atria to the ventricles and from the base to the apex of the heart (5, 6). The cardiac sympathetic nervous system uses norepinephrine as a neurotransmitter.

Sympathetic neurons originate from neural crest cells that migrate near the dorsal aorta and form the primary sympathetic chain ganglia around embryonic day (E) 10.5 in mice (7, 8). These cells undergo neuronal and catecholaminergic differentiation, marked by the initiation of the expression of enzymes involved in norepinephrine biosynthesis, tyrosine hydroxylase (TH), and dopamine β-hydroxylase (7, 9). In the subsequent migration phase, sympathetic progenitors move away from the dorsal aorta to form secondary sympathetic ganglia at E13.5. Within these ganglia, sympathetic neuroblasts complete proliferation with a peak exit from the cell cycle at E14.5 and undergo final differentiation into sympathetic neurons (8). Proliferating sympathetic neurons begin to extend first axons as early as E12.5 (10, 11). Axons extend along the arteries attracted by vascular-derived guidance cues, including neurotrophin 3 (12), artemin (13), and endothelins (14). Nerve growth factor (NGF) produced by the heart controls the final stages of cardiac sympathetic innervation (15). Cardiomyocyte-derived Sema3a, a neural chemorepellent, is necessary for sympathetic innervation patterning by inhibiting axonal growth (16). The first axonal projections in the heart are detected in the dorsal subepicardium of the ventricles around E14 (17), and large coronary veins serve as an intermediate template for distal sympathetic axon extension (6). Subsequently, in the myocardial layer, sympathetic axons are guided by arteries toward the final target cells in the myocardium.

Target cell-derived factors control axon growth, branching, synaptic and electrophysiological properties, and release of neurotransmitters during development and in the establishment of mature properties of the sympathetic system (10, 18). Disrupting sympathetic innervation reciprocally affects heart development. For instance, neonatal chemical sympathectomy disrupts the cardiomyocyte cell cycle, resulting in a reduced heart size (19). The treatment by β-adrenergic receptor (AR) antagonists, which are used clinically to treat conditions associated with excessive effects of norepinephrine, promoted the progression of cytokinesis in neonatal mice, which reduced adverse remodeling after myocardial infarction in adults (20). Similarly, infusions of β-AR blockers induce significant neurite outgrowth in the *in vitro* assay system of neonatal sympathetic neurons and myocardial sympathetic axon density in the rat heart with elevated ventricular contractility (21). Sympathetic neurons have a significant role in the regulation of cardiomyocyte maturation, as shown by *in vitro* co-cultures of human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes with sympathetic neurons (22). Furthermore, sympathetic defects have been associated with sudden infant death syndrome, cardiac arrhythmic death, and certain congenital heart defects in children (23, 24).

Changes in cardiac sympathetic innervation and sympathetic system activity are implicated in many pathologies in adults, including sudden cardiac death, myocardial ischemia, cardiac death, hypertension, diabetic heart disease, and heart failure (25, 26). Emerging evidence suggests that many of the sympathetic dysfunctions in various pathological heart conditions have a developmental origin (19, 20, 27, 28). Using conditional deletion of oxygen-sensitive subunit HIF-1α, we previously revealed a key role for the transcription factor hypoxia-inducible factor 1 (HIF-1) in the development of sympathetic neurons and sympathetic innervation of the developing heart and its negative effects on heart function in adults (29). HIF-1 coordinately regulates responses to hypoxia and ischemia and plays multifactorial roles in pathophysiological responses in myocardial ischemia, infarction,

metabolic and structural remodeling, and heart failure (30–33). Additionally, HIF-1-regulated pathways direct cardiac development (34–36). A number of studies highlight the combinatorial effects of HIF-1 deregulation and environment on heart development, including fetal hypoxia or maternal diabetes, influencing the cardiovascular and metabolic health of offspring (37–40). Despite significant progress in understanding this phenomenon known as fetal or developmental programming [reviewed in (41–43)], numerous questions pertaining to its underlying penetrance and disease predisposition remain unresolved.

In this study, we present a comprehensive analysis of the combined impact of the *Hif1a*-deficient cardiac sympathetic system and the adverse maternal diabetes environment on embryonal development. Our analysis of *Hif1aCKO* embryos revealed a negative synergistic effect of *Hif1a* deletion and the diabetic environment on the development of cardiac innervation and chromaffin cells of the adrenal medulla of the sympathetic system, affecting heart development. Thus, exposure to maternal diabetes and *Hif1a*-deficient cardiac sympathetic system heighten the risk of cardiovascular disease in the offspring.

2 Materials and methods

2.1 Experimental animal models

This study was approved by the local Animal Care and Use Committee of the Institute of Molecular Genetics CAS and the Institute of Anatomy, First Faculty of Medicine, Charles University. All experiments were performed with embryo littermates (females and males). Animals were housed in a controlled environment with 12-h light/dark cycles and free access to water and food.

The previously described experimental model of the conditional deletion of *Hif1a* (*Hif1aCKO*) genotype $Isl1^{tm1(cre)Sev/+}$;$Hif1a^{loxP/loxP}$ was used (29). Briefly, floxed $Hif1^{atm3Rsjo}$ with exon 2 of the *Hif1a* gene flanked by loxP sites (44) on a mixed C57BL/6J;C57BL/6N genetic background were obtained from Jackson Laboratories (Bar Harbor, ME, USA; #Strain 007561). $Isl1^{Cre/+}$ mice were on the FVB background. $Hif1a^{loxP/+}$ or $Hif1a^{loxP/loxP}$ mice without the *Isl1-Cre* allele individuals were used as the control. For the breeding scheme, female mice $Hif1a^{loxP/loxP}$ were crossed with $Hif1a^{loxP/+;}$ $Isl1^{Cre/+}$ males, in which the *Isl1-Cre* knock-in allele was inherited paternally to minimize the potential influence of maternal genotype on the developing embryos.

To visualize sympathetic neurons, we used *tdTomatoAi14* reporter mice with *Rosa-CAG-LSL-tdTomato* allele (Ai14, B6.Cg-$Gt(ROSA)26Sor^{tm14(CAG-tdTomato)Hze}$, Stock No. 7914 Jackson Laboratories) to generate the reporter *Hif1aCKO-Ai14* (genotype: $Hif1a^{loxP/loxP}$;$Isl1^{Cre}$;*tdTomatoAi14*) and control-*Ai14* mice (genotype: $Hif1a^{loxP/+}$;$Isl1^{Cre}$;*TdtomatoAi14*). To examine the formation of the secondary sympathetic chain, we used *peripherin-enhanced green fluorescent protein* (*Prph-eGFP*) genomic reporter transgenic mice (45, 46) and crossed them with the *Hif1a* mutant line.

To visualize the pattern of the developing coronary arteries, $Hif1a^{loxP/loxP}$ mice were crossed *to Cx40:eGFP* strain with eGFP signal in the coronary arteries, the atria, the atrioventricular node, and the His–Purkinje system (47). Double homozygote females ($Cx40:^{eGFP/+}$;$Hif1a^{loxP/loxP}$) with background CD1/129SvJxSwiss were then crossed with $Hif1a^{loxP/+}$;$Isl1^{Cre}$ males.

The noon of the day on which the vaginal plug was found was designated E0.5. Animals were euthanized by cervical dislocation in our study. Embryos were collected for analysis at different ages (Figure 1A). All comparisons were made between animals with the same genetic background. Phenotyping and data analysis were performed blind to the genotype of the mice. Genotyping was performed by PCR on tail DNA (Supplementary Table 1).

2.2 Diabetes induction

Diabetes was induced in 6-week-old females ($Hif1a^{loxP/loxP}$) by two intraperitoneal injections of 100 mg/kg body weight of streptozotocin (STZ, S0130, Sigma-Aldrich, Dorset, UK) within a 1-week interval, as described (37, 48). The level of blood glucose was checked in a drop of blood from the tail vessel using a glucometer (CONTOUR plus ONE, Ascensia Diabetes Care, Basel, Switzerland). Mice with a level of glucose maintained above 13.9 mmol/l in blood were classified as diabetic. Diabetic females were mated with males, and the next morning, the vaginal plug was checked. Maternal blood glucose levels at the time of embryo collection are shown in Supplementary Figure 1. Embryos collected from diabetic pregnancies were labeled as "diabetic" or "DIA", and from non-diabetic pregnancies were labeled as "non-diabetic" or "non-DIA".

2.3 Immunohistochemistry and morphological evaluations

Whole-mount immunohistochemical staining of embryonic hearts (E16.5 and E18.5) was performed on cleared tissue, as described (49). Embryos were perfused with 0.05% heparin in phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde (PFA) for 90 minutes. The heart was cleared using CUBIC reagent at 37°C with gentle shaking for 1 week (50). Used primary and secondary antibodies are described in Supplementary Tables 2 and 3. The nuclei were stained with Hoechst. The fluorescent signals were detected using LSM 880 NLO (AxioObserver Z1, Carl Zeiss, Oberkochen, Germany) and AxioZoomV16 (Carl Zeiss, Germany).

Positive areas (TH and TUJ1) were quantified using the Threshold tool in NIH ImageJ software and expressed as a percentage of total areas (heart and adrenal gland). The total number of positive cells (NeuN) in the whole stellate ganglion (STG) was quantified using a cell counter in NIH ImageJ software. Microvasculature density was assessed using

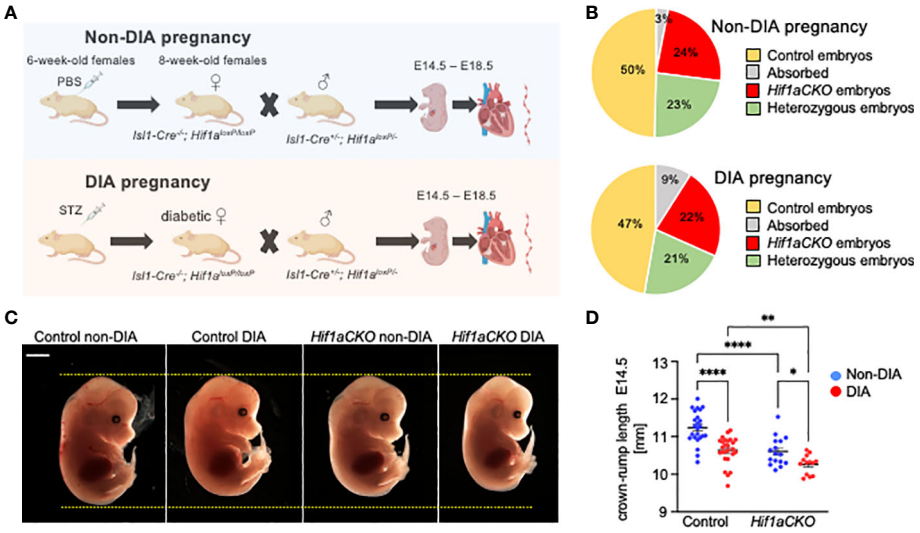

FIGURE 1
Maternal diabetes affects the distribution of genotypes and size of embryos. **(A)** Experimental design. Diabetes was induced in 6-week-old females by intraperitoneal injections of streptozotocin (STZ). Diabetic females were mated with males, and embryos were collected for analysis from E14.5 to E18.5 from diabetic and non-diabetic pregnancies. Created with BioRender.com. **(B)** The distribution of embryo genotypes collected at E14.5 was influenced by maternal diabetes. χ^2 test **p = 0.0012, non-diabetic pregnancies: n = 33 absorbed, n = 260 *Hif1aCKO*, n = 253 heterozygous *Hif1aCKO*, and n = 538 control embryos from 124 litters; diabetic pregnancies: n = 18 absorbed, n = 45 *Hif1aCKO*, n = 42 heterozygous *Hif1aCKO*, and n = 94 control from 22 litters. **(C)** Representative images of E14.5 embryos. Scale bar, 2 mm. **(D)** The evaluation of the crown–rump length of control and mutant embryos from non-diabetic and diabetic pregnancies at E14.5. Data are presented as mean ± SEM, n = 24 non-DIA Control, n = 26 DIA Control, n = 16 non-DIA *Hif1aCKO*, and n = 13 DIA *Hif1aCKO*. Two-way ANOVA followed by *post hoc* Fisher's multiple comparisons test; *p < 0.05, **p < 0.01, ****p < 0.0001.

immunohistochemically stained 8-µm paraffin sections labeled with anti-PECAM-1 (an endothelial marker) and wheat germ agglutinin (WGA). Sections were imaged on an Olympus confocal microscope, and positive signals of PECAM-1 and WGA were combined and thresholded in NIH ImageJ. Then, the positive area was measured in the region of interest (compact layer) in the right ventricle. Subsequently, the measured microvasculature-positive area was normalized to the background signal (all tissue autofluorescence) and expressed as a percentage, thus leading to the density of microvasculature in the right ventricular wall.

To visualize the pattern of the developing coronary arteries together with TH^+ innervation, the hearts were dissected and immunolabeled with anti-TH and Cy5-conjugated secondary antibodies. The atria were then carefully cut off to expose the origin of the coronary arteries at the base of the heart, cleared in CUBIC for 48 h (51), and visualized with 4× and 10× dry objectives on an Olympus FluoView 1000 confocal system. For quantification of TH^+ innervation, maximum intensity projections (MIPs) of confocal series taken with a 4× objective with 25-µm z-step were used. The image from the far-red (Cy5) channel containing the signal from the anti-TH antibody was oriented with the base on the top and the apex at the bottom (Valentine projection). Two semicircular segmented lines at 25% and (basal) and 75% (apical) apex–base distance were drawn in ImageJ, and line profiles were then generated (Supplementary Figure 2). The peaks in fluorescence intensity, corresponding to individual nerve bundles, were then easily counted in an unbiased manner.

2.4 Light-sheet fluorescence microscopy and analysis of images

Embryonic hearts were microdissected from non-diabetic and diabetic control and *Hif1aCKO* embryos (E16.5 and E18.5). An advanced CUBIC protocol (49) with some modifications (50) was used for tissue clearing to enable efficient imaging by light-sheet microscopy. Whole-mount immunohistochemical staining of embryonic hearts was performed, and samples were stored before imaging in Cubic 2 at room temperature. The secondary sympathetic chain was microdissected from non-diabetic and diabetic control-*Ai14* and *Hif1aCKO-Ai14* embryos (E14.5) and whole-mount stained with NeuN. Zeiss Lightsheet Z.1 microscope with illumination objective Lightsheet Z.1 5×/0.1 and detection objective Dry objective Lightsheet Z.1 5×/0.16 was used for imaging at the Light Microscopy Core Facility of the Institute of Molecular Genetics of the Czech Academy of Sciences. IMARIS software v8.1.1 (Bitplane AG, CA, San Francisco, USA) was used for image processing.

2.5 RNA sequencing of fluorescence-activated cell-sorted sympathetic ganglion neurons

Sympathetic ganglia were dissected from E14.5 embryos *Hif1aCKO-Ai14;Prph-eGFP* (n = 3) and *Control-Ai14;Prph-eGFP* (n = 2). Sympathetic chains were homogenized, and neuronal cells were dissociated using 0.1% collagenase (C9263, Sigma-Aldrich, UK) and 0.05% trypsin in Dulbecco's PBS for 7 minutes (T4799,

Sigma-Aldrich, UK). Enzymatic activity was stopped by adding fluorescence-activated cell sorting (FACS) buffer [2% fetal bovine serum (FBS) in Dulbecco's PBS and 10 mM EGTA]. FACS of eGFP and tdTomato-positive cells was performed at the Imaging Methods Core Facility at BIOCEV on a BD FACS Aria Fusion flow cytometer operated using BD FACSDiva™ Software. A total of 100 $eGFP^+$ and $tdTomato^+$ cells per biological sample were collected into individual wells of a 96-well plate containing 5 µL of lysis buffer of NEBNext single-cell low input RNA library prep kit for Illumina (#E6420, New England Biolabs, Ipswich, MA, USA). Plates were frozen immediately on dry ice and stored at −80°C. The total time from euthanasia to cell collection was ∼3 h.

The RNA library preparation, RNA sequencing (RNA-seq), and data processing were performed as described previously (27). Briefly, the NEBNext single-cell low-input RNA library prep kit for Illumina (#E6420, New England Biolabs) was used for library generation at the Gene Core Facility (Institute of Biotechnology CAS, Czechia), and the libraries were sequenced on an Illumina NextSeq 500 next-generation sequencer with NextSeq 500/550 High Output kit 75 cycles (Illumina #200024906) at the Genomics and Bioinformatics Core Facility (Institute of Molecular Genetics CAS, Czechia). RNA-seq reads in FASTQ files were mapped to the mouse genome GRCm38 primary assembly release M8 using STAR [version 2.7.0c (52)]. Using cutadapt v1.18 (53), the number of reads (minimum, 32 million; maximum, 73 million) was trimmed by Illumina sequencing adaptor and bases with reading quality lower than 20; subsequently, reads shorter than 20 bp were filtered out. TrimmomaticPE version 0.36 (54). Ribosomal RNA and reads mapping to UniVec database were filtered out using bowtie v1.2.2. with parameters -S -n 1 and SortMeRNA (55). A count table was generated using the Rsubread v2.0.1 package with default parameters without counting multi-mapping reads. The raw RNA-seq data were deposited at NIH GEO under accession number GSE250606 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE250606).

DESeq2 [v1.26.0 (56)] default parameters were used to normalize data and compare the different groups. Differentially expressed genes between *Hif1aCKO* and control sympathetic neurons were identified based on an adjusted p-value $p_{adj} < 0.05$, log2 fold change (log2FC >0.3, <−0.3), and a base mean ≥50. The functional annotation of the differentially expressed genes was performed using g: Profiler (Raudvere et al., 2019). Complete query details are available in Query info Tables in Dataset S1. The resulting GEM and combined GMT files using term size <1,800 were loaded into Cytoscape (57) plugin "EnrichmentMap" (58) using 0.01 false discovery rate (FDR) q-value cutoff to generate a network. The edge cutoff was set to 0.35, and nodes were set to 0.007 Q value.

2.6 Quantitative real-time PCR

RNA was isolated from the microdissected sympathetic chains of individual embryos from diabetic and non-diabetic litters using TRIzol (Invitrogen, Carlsbad, CA, USA). The concentration and purity were quantified using NanoDrop (ND-2000 Spectrophotometers, Thermo Fisher Scientific, Waltham, MA, USA). cDNA samples were prepared using Maxima H Minus First Strand cDNA Synthesis Kit with dsDNA (#K1682, Thermo Scientific, USA) from 300 ng isolated RNA/sample. Quantitative real-time PCR (qRT-PCR) was performed using 10× diluted cDNA samples. cDNA at a volume of 4 µL was added to 5 µL of SybrGreen (GrandMaster Mix, TATAA Biocenter, Gothenburg, Sweden) with 0.2 µM reverse and forward primers. Primers were designed using Primer3 software, and sequences are shown in Supplementary Table S2. Validation of RNA-seq targets was performed using Bio-Rad C1000 Thermal Cycler (CFX384 Real-Time System, Bio-Rad Laboratories, Hercules, CA, USA), and activation was performed using AmpliTaq at 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 s for denaturation and 60°C for 60 s for extension. The relative expression levels of mRNA of target genes were normalized to the reference gene *Hprt1*. All reactions were conducted in duplicates, and the data were calculated using the ΔΔCp method, as previously described (37, 59). Primer sequences are presented in Supplementary Table 4.

2.7 Statistical analysis

Statistical analyses were performed using two-way ANOVA (GraphPad Prism 10), testing differences among experimental groups based on the genotype and experimental condition (diabetic or non-diabetic pregnancy) followed by multiple Fisher's comparisons; results are expressed as mean ± SD or mean ± SEM, with significance level $p < 0.05$. A chi-square (χ^2) test was used to compare the distribution of genotypes between the non-diabetic and diabetic groups (GraphPad Prism 10). Sample sizes and individual statistical results for all analyses are provided in the figure legends and tables.

3 Results

3.1 Maternal diabetes affects genotype distribution and the size of embryos

We analyzed the combined impact of the *Hif1a*-deficient cardiac sympathetic system and the adverse maternal diabetes environment on heart development (schematics of experimental study design in Figure 1A). The observed distribution of embryo genotypes collected at E14.5 was influenced by maternal diabetes (**p = 0.0012, χ^2 test; Figure 1B). We did not observe any developmental delay or structural abnormalities among embryos from diabetic and non-diabetic pregnancies. However, we identified a significant decrease in the average crown–rump length of both diabetic control and *Hif1aCKO* embryos compared to non-diabetic controls at E14.5 (Figures 1C, D). Furthermore, the length of diabetic *Hif1aCKO* embryos was significantly smaller than that of diabetic controls or non-

diabetic *Hif1aCKO*, indicating a synergistic effect of *Hif1a* deletion and the diabetic environment.

3.2 Cardiac sympathetic innervation is attenuated by *Hif1a* mutation and diabetic exposure

To assess the extent of cardiac innervation, we employed double immunolabeling of TH, a marker of sympathetic neurons and sympathetic innervation, and class III β-tubulin (TUJ1), a neuronal marker expressed in all cardiac fibers, representing sympathetic, parasympathetic, and sensory innervation. While parasympathetic cardiac innervation precedes sympathetic innervation, sympathetic fibers move alongside established vagal nerve tracts to innervate the heart (60). In the developing mouse heart, autonomic innervation precedes sensory innervation, with sensory axons becoming detectable at E18.5 (6, 61). In line with our previous study (29), the conditional deletion of *Hif1a* in sympathoadrenal progenitor lineage led to a profound deficit in cardiac sympathetic innervation (Figures 2A–D; Supplementary Video S1-S4). At E16.5, our immunohistochemical staining revealed that the majority of TH^+ axons in the posterior part of the ventricles of diabetic *Hif1aCKO* were lost, with no cardiac fibers observed in the apex (Figures 2B, D). Maternal diabetes and *Hif1a* mutation significantly reduced TH^+ and $TUJ1^+$ cardiac innervation when compared to hearts from non-diabetic pregnancies and control hearts, respectively (Figures 2D, E). Notably, a negative synergistic effect of *Hif1a* deletion and the diabetic environment on cardiac innervation was observed when comparing *Hif1aCKO* and control hearts from diabetic pregnancies.

Next, we evaluated the sympathetic innervation of the posterior and anterior parts of the ventricular wall in detail. Sympathetic innervation, as marked by anti-TH, was notably reduced in the *Hif1aCKO* ventricles, with a loss of both proximal and distal branches compared to the control hearts at E17.5, mirroring the observation at E16.5 (Figure 3A). The difference at the base of the heart, where the thick bundles dominated, was less affected between genotypes; however, a clear decrease in the number of (usually thinner) bundles was

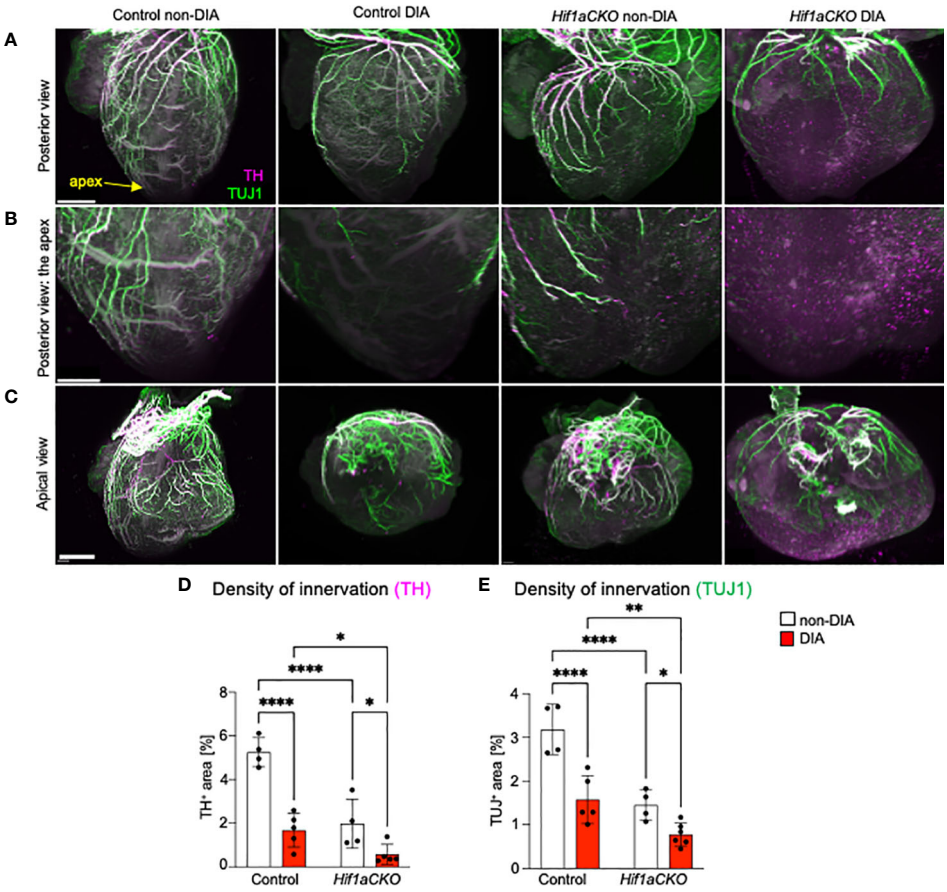

FIGURE 2
Reduced innervation in the diabetic *Hif1aCKO* heart at E16.5. **(A)** Representative images of immunolabeling of sympathetic innervation using anti-tyrosine hydroxylase (TH) and anti-class III β-tubulin (TUJ1) in the heart in the posterior view (scale bars, 500 μm). See also Supplementary Videos S1-S4. **(B)** The heart apex in detail in the posterior view (scale bar, 300 μm) and **(C)** the apical view (scale bar, 600 μm). **(D)** TH^+ and **(E)** $TUJ1^+$ fibers were quantified using the threshold tool in ImageJ and expressed as a percentage of the measured heart area. Cardiac innervation is reduced in *Hif1aCKO*, and the effect is further potentiated by diabetes. Data are presented as the mean ± SD (n = 4–5 samples). Two-way ANOVA followed by *post hoc* Fisher's multiple comparisons test; *p < 0.05, **p < 0.01, ****p < 0.0001.

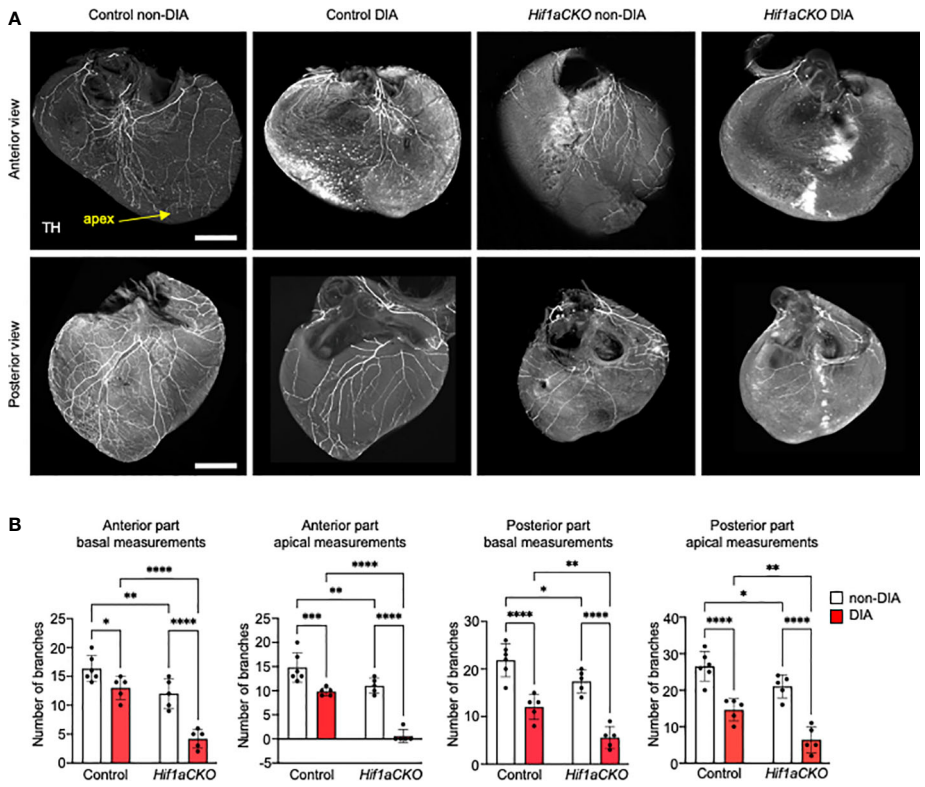

FIGURE 3
Sympathetic innervation of the anterior and posterior parts of the ventricles reduced by *Hif1a* deletion and maternal diabetes at E17.5. **(A)** Representative images of immunohistochemical staining of sympathetic innervation in the anterior and posterior parts of the ventricles using tyrosine hydroxylase (TH) (scale bar, 500 μm). Posterior part of heart ventricles contains more branches, and their plexus is more complex compared to the anterior part of heart ventricles. The greatest reduction of sympathetic innervation is noticeable in diabetic *Hif1aCKO* with a few main branches innervating the anterior part of the ventricular wall, but all distal branches of the ventricles are lost. Similarly, the posterior part of diabetic *Hif1aCKO* ventricles has a significant loss of innervation with only few remaining nerves. **(B)** The fluorescence intensity corresponding to individual nerve proximal and distal branches in the ventricular wall was quantified using ImageJ at 25% (basal) and 75% (apical) apex–base distance (Supplementary Figure S2). Data are presented as the mean ± SD (n = 5–6 samples). Two-way ANOVA followed by *post hoc* Fisher's multiple comparisons test; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

evident at the apex, attesting to a deficient innervation. The maternal diabetes exposure resulted in a significant attenuation of sympathetic innervation in the control hearts (Figures 3A, B). However, the synergistic detrimental effects of *Hif1a* deletion and diabetic pregnancy were most pronounced in the diabetic *Hif1aCKO* hearts. Only a few main branches innervated the basal area of the myocardial wall in the anterior view, and all distal ventricular branches were missing. While the posterior part of the heart contained a higher number of branches with a more complex plexus, the combination of *Hif1a* deletion and maternal diabetes led to a nearly complete loss of innervation, with only a few remaining nerves (Figures 3A, B).

At E18.5, the most pronounced deficit in cardiac sympathetic innervation was detected in *Hif1aCKO* embryos exposed to maternal diabetes, indicating a long-lasting synergistic effect of *Hif1a* deletion and the diabetic environment (Figures 4A–D). Conversely, TUJ1$^+$ axons were equally reduced in both non-diabetic and diabetic *Hif1aCKO* hearts, highlighting the impact of *Hif1a* mutation rather than maternal diabetes (Figure 4E). Similarly, exposure to maternal diabetes did not significantly affect TUJ1 innervation in the control heart compared to control hearts from non-diabetic embryos. Both sympathetic and TUJ1$^+$ fibers were significantly reduced in non-diabetic *Hif1aCKO* hearts when compared to non-diabetic control hearts (Figures 4D, E), indicating abnormalities in the formation of cardiac innervation of *Hif1aCKO*. Consequently, these changes could have significant implications for heart function and postnatal survival of *Hif1aCKO* mutants.

3.3 Myocardial changes induced by maternal diabetes

Given the implication of the sympathetic nervous system in heart size regulation and cardiomyocyte proliferation (19, 20), we conducted an analysis of the wall thickness of both the left ventricle (LV) and right ventricle (RV) and the thickness of the interventricular septum, using the sections of the E17.5 heart (Figure 5A). First, the length of the heart (from apex to base) and width across the widest part of both ventricles were measured. Maternal diabetes and *Hif1a* mutation significantly reduced both measured parameters, although the ratio of length and width was not altered, indicating that the ventricular proportions were

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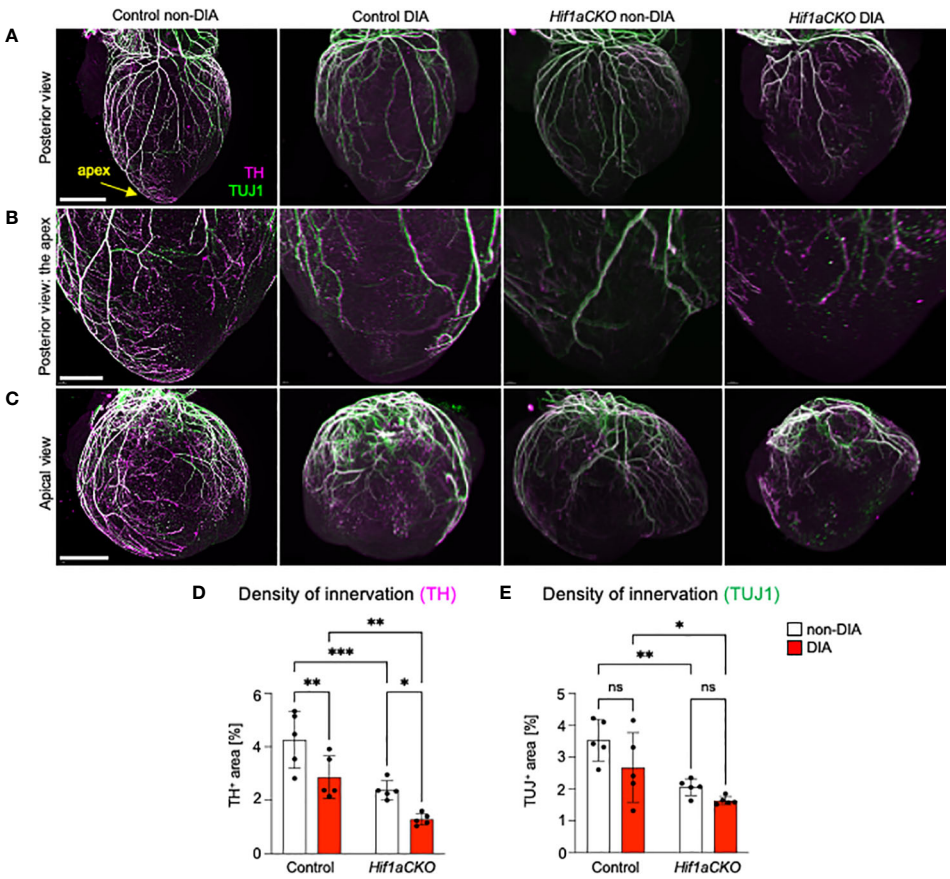

FIGURE 4
Reduced sympathetic innervation in the diabetic *Hif1aCKO* heart at E18.5. **(A)** Representative images of immunohistochemical staining of sympathetic innervation using tyrosine hydroxylase (TH) and class III β-tubulin (TUJ1) in the posterior view of the heart (scale bar, 1,000 µm), **(B)** in the apex in detail (scale bar, 500 µm), and **(C)** in detail the apical view of the heart (scale bar, 1,000 µm). **(D)** TH^+ and **(E)** $TUJ1^+$ innervations were quantified using the threshold tool in ImageJ and expressed as a percentage of the measured heart area. The combination of *Hif1a* deficiency and exposure to maternal diabetes led to the greatest reduction in sympathetic innervation. Data are presented as the mean ± SD (n = 5). Two-way ANOVA followed by *post hoc* Fisher's multiple comparisons test; *p < 0.05, **p < 0.01, ***p = 0.0002. ns, not significant.

similar among groups (Figures 5A, B). The compact myocardium of the LV and RV of the *Hif1aCKO* heart was significantly thinner compared to those of their control littermates from non-diabetic pregnancies (Figures 5A, C). Additionally, the control embryos from the diabetic pregnancy exhibited thinner LV and RV walls when compared to control non-diabetic embryos. Interestingly, the thickness of the interventricular septum increased in both diabetic and non-diabetic *Hif1aCKO* and diabetic control hearts compared to the control heart of embryos from non-diabetic pregnancies (Figures 5A, C). However, we did not detect an additive effect of the combination of *Hif1a* mutation and the diabetic environment on these parameters when compared to diabetic control embryos.

3.4 Diabetes and *Hif1a* deletion result in abnormalities in coronary vasculature

Next, we investigated the coronary vascular architecture, as the development of arterial and sympathetic nerve networks is coordinated in terms of both spatial distribution and molecular signaling. Furthermore, our previous study revealed a higher neonatal mortality rate among *Hif1aCKO* mice, which could be partially linked to major coronary artery anomalies (29). Building upon these findings, our current study expands our analyses of the formation of coronary artery architecture, using the *Cx40:eGFP* knock-in model with eGFP signal in the coronary arteries (47). The normal pattern of the coronary arteries together with examples of abnormal findings recorded from the mutant and diabetic hearts is summarized in Figure 6. Abnormalities included multiple smaller branches instead of a single large one, arterial "windows", and anomalous connections to the aorta (separate orifices of the circumflex and left anterior descending branches of the left coronary artery and separate orifice of the septal branch of the right coronary artery) instead of single opening of the left and right coronary arteries. These abnormalities alone cannot account for the approximately 40% perinatal mortality observed in the *Hif1aCKO* mice (29). Thus, the prenatal evaluations did not uncover any new, more severe malformations in the coronary artery architecture in the *Hif1aCKO* embryos, in comparison to

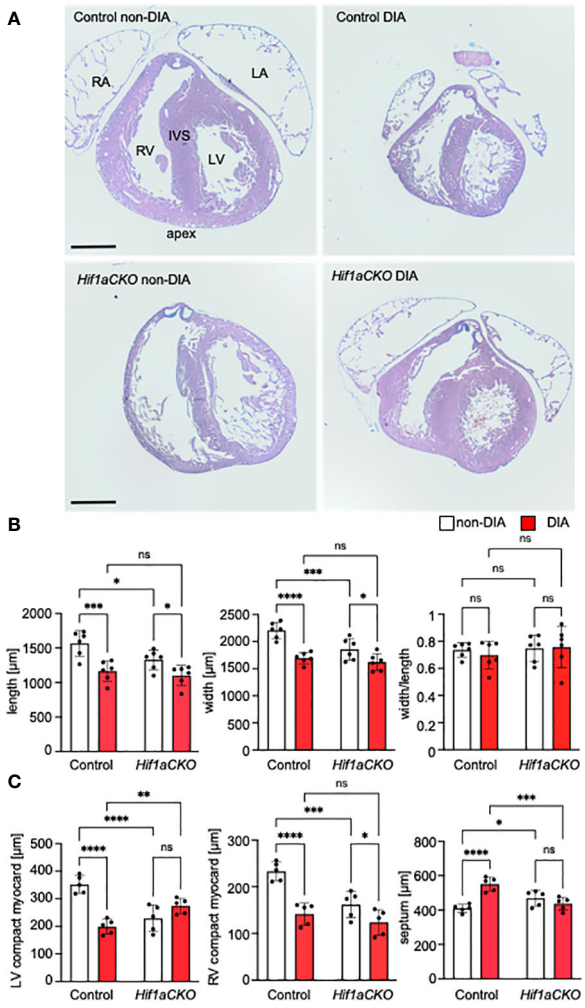

FIGURE 5
Morphological changes induced by the *Hif1a*-deficient sympathetic system and maternal diabetes exposure. **(A)** Representative images of histological staining with Alcian Blue/hematoxylin and eosin show transverse sections of E17.5 hearts; four-chamber view at the level of the pulmonary valve. RV, right ventricle; LV, left ventricle; IVS, interventricular septum; RA, right atrium; LA, left atrium. **(B)** The length and width of the mutant heart were significantly decreased; this effect was also induced by exposure to maternal diabetes. The heart shape, as assessed by the width/length ratio, remained similar among groups. **(C)** The ventricular thickness of the compact myocardium layer (µm) was reduced in the *Hif1aCKO* and diabetic hearts compared to controls. In contrast, the interventricular septum was thickened in the normoglycemic mutants versus controls and in the control group with diabetes. Data are presented as the mean ± SD (n = 5). Two-way ANOVA followed by *post hoc* Fisher's multiple comparisons test, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. ns, not significant. Scale bars, 500 µm.

the analyses of the adult *Hif1aCKO* heart reported previously (29).

We next visualized the microvasculature within the heart. We used a combination of anti-PECAM-1 antibody (a pan-endothelial marker) and WGA labeling. Subepicardial veins were noticeably dilated in both non-diabetic and diabetic *Hif1aCKO* in the RV (white arrows in Figures 7C, D) when compared to the control hearts from diabetic and non-diabetic embryos (Figures 7A, B). Interestingly, coronary arteries in the compact myocardium of the RV appeared dilated in embryos from diabetic pregnancies (yellow arrows in Figures 7B, D) but not in non-diabetic *Hif1aCKO* hearts. Although the relative vessel density, indicative of myocardial perfusion and oxygenation in the RV, did not show significant differences among the groups (Supplementary Figure 3), the thinner ventricular wall represents a reduction in the absolute amount of microvasculature compared to the controls. The microvasculature of the LV seemed to be less affected, although subepicardial veins were also dilated in diabetic and non-diabetic *Hif1aCKO* and in diabetes-exposed control embryos compared to the non-diabetic control group (Figures 7E–H).

3.5 HIF-1α deficiency alters the development of adrenal chromaffin cells in diabetic embryos

Sympathetic neurons and neuroendocrine chromaffin cells in the adrenal medulla originate from a common catecholaminergic sympathoadrenal progenitor (62). Therefore, we proceeded to examine the development of chromaffin cells in the adrenal medulla. Consistent with our earlier findings that HIF-1α deficiency adversely impacted the development of chromaffin cells in the adrenal medulla (29), we observed a significant reduction in TH expression in the adrenal medulla *Hif1aCKO* embryos as early as E14.5 (Figures 8A, B). However, the detrimental effect of maternal diabetes on TH-expressing chromaffin cells became evident at a later stage, specifically at E18.5. Maternal diabetes exposure led to a significant reduction in the size of the adrenal medulla of control embryos, but diabetic *Hif1aCKO* embryos exhibited the most substantial reduction when compared to the other groups.

3.6 *Hif1a* deletion and maternal diabetes impair postganglionic neural development

Considering the compromised cardiac sympathetic innervation resulting from HIF-1α deficiency and exposure to a maternal diabetic environment, we assessed the development of postganglionic neurons. The stellate ganglia and thoracic sympathetic chain were evaluated using light-sheet fluorescence microscopy and tdTomato and *Prph*-eGFP reporter expression at E14.5 (Figures 9A, B). The size of the sympathetic chain was reduced in response to maternal diabetes and *Hif1a* deletion when compared to non-diabetic control sympathetic chains (Figure 9D). Similarly, the number of neurons expressing NeuN, a marker of mature neurons, exhibited a decrease in both maternal diabetes and *Hif1a* mutation conditions, with no observed additive combinatorial effect of *Hif1a* mutation within the diabetic environment (Figures 9C, E).

To analyze the molecular changes resulting from the elimination of *Hif1a* in developing sympathetic neurons, we

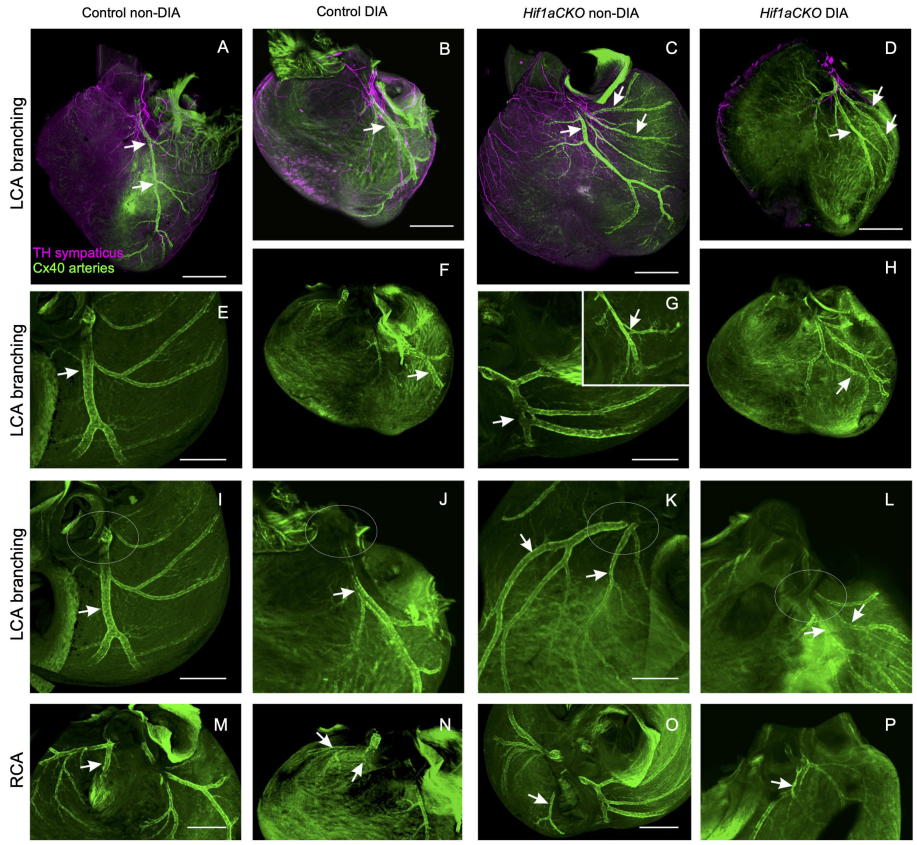

FIGURE 6
Anomalies of main coronary arteries. **(A)** Normal branching of the left coronary artery (LCA) in control mouse embryo at E17.5 with anterior interventricular branch [left anterior descending (LAD); arrows]. **(B)** Almost normal branching of LCA in embryo of diabetic mother with slightly twisted branches of the LCA. **(C)** Multiple branches of the LCA are radiating to the base of the left ventricle in *Hif1aCKO* embryo. **(D)** An example of abnormal branching of the LCA in diabetic *Hif1aCKO*. Note that the heart is also generally smaller. **(E, F)** More examples of normal shorter and convoluted branches of the LCA (arrow indicates LAD). **(G, H)** Examples of abnormal and doubled branches of the LCA. Arterial windows in *Hif1aCKO* embryos (arrow and inset in **G**). Arrow in panel H shows two parallel branches next to each other with a small anastomosis. **(I, J)** Normal single orifice of LCA from the aorta and two separate openings into the aorta of the anterior interventricular and the circumflex branch of the LCA. The area of the left aortocoronary orifice is outlined by the ovals. **(K, L)** Double opening of the LCA into the aorta. The ovals outline the aortocoronary openings, and the respective branches (LCA and the circumflex branch) are indicated by the arrows. **(M, N)** Normal connection of the septal branch (arrow) of the right coronary artery (RCA). **(O)** Abnormal RCA connection shown in the *Hif1aCKO* heart: the interventricular branch (arrow) is connected directly to the aorta instead of to the RCA. **(P)** Abnormally dilated segment of the RCA (arrow) just before its entry to the aorta. Scale bars, 500 μm **(A–D, F, H)** and 200 μm **(E, G, I–P)**.

performed a bulk-cell RNA sequencing (experimental design in Figure 10A). Each biological replicate for the bulk RNA-seq analysis contained 100 *Prph*-eGFP$^+$ and tdTomato$^+$ FACS-sorted single cells from the E14.5 stellate ganglia and thoracic sympathetic chain.

Differential expression analysis identified 36 downregulated and 32 upregulated transcripts of protein-coding genes in *Hif1aCKO* neurons (Supplementary Data 1). Functional enrichment analysis of the set of upregulated genes demonstrated a significant enrichment of Gene Ontology (GO) categories associated with *proliferation*, *cell cycle*, and *mitosis*, likely reflecting the main compensatory mechanisms for aberrant neuronal development (Figures 10B, C; Supplementary Data 1). For example, upregulated genes encoding proteins are involved in cell cycle and mitosis regulation including *Cdc20*, the coactivator of mitotic progression, which is also required for dendrite development (63); the transcription factor *E2f1*, which is important for cell cycle progression and apoptosis (64); cyclin B1 (*Ccnb1*) (65); *Kif23*, which is important for cell division and implicated in neuronal migration (66); *Cenpe*, *Cenpf*, and *Mki67*, which are important for cell cycle and mitosis (67); and *Mcc*, which is a regulator of cell cycle and Wnt/β-catenin signaling pathway (68). Additionally, we detected an elevated expression of glutamic acid decarboxylase 2 (*Gad2*), the enzyme that catalyzes the formation of γ-aminobutyric acid (GABA) from glutamic acid. GABAergic signaling, the main inhibitory neurotransmitter system, is important in sympathetic and cardiovascular regulation (69). *Slc38a11*, a member of the SLC38 family of amino acid transporters, was upregulated, although its function and substrate specificity are unknown (70). Moreover, elevated levels of *Gata2*, a key transcription factor expressed in developing sympathetic neurons (71), were found in *Hif1aCKO* neurons.

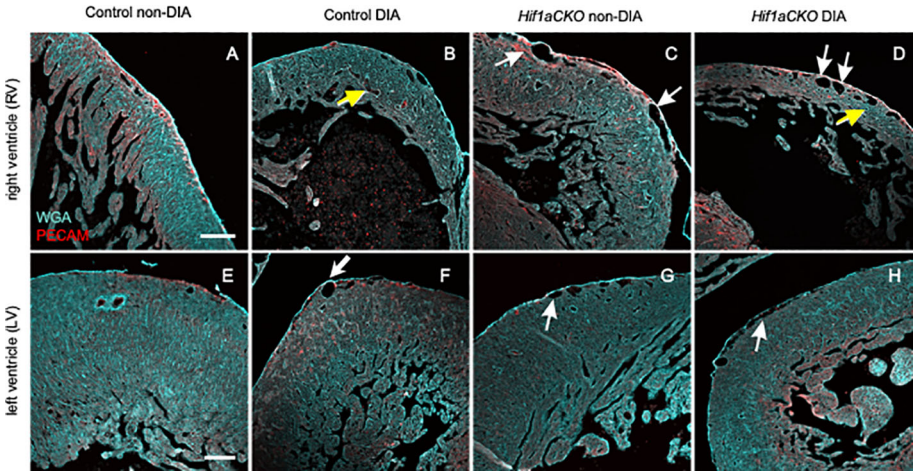

FIGURE 7
Changes in microvasculature in the embryonal heart induced by maternal diabetes and *Hif1a* deletion. **(A–H)** Representative images of visualized microvasculature using a combination of anti-PECAM-1 (a pan-endothelial marker), and wheat germ agglutinin (WGA) labeling depict changes in the E17.5 heart. White arrows indicate dilated subepicardial veins, and yellow arrows indicate round and dilated coronary arteries in the myocardium. Dilated subepicardial veins are observed in the right ventricle (RV) in non-diabetic and diabetic *Hif1aCKO* compared to the control groups. Round and dilated coronary arteries in the myocardium are in the RV of diabetic control and diabetic *Hif1aCKO*. Dilated subepicardial veins in left ventricles (LVs) are found in diabetic control, diabetic, and non-diabetic *Hif1aCKO* groups. Microvasculature in the LV is less affected compared to the RV, although the relative vessel density in the RV is unchanged among the evaluated groups (Supplementary Figure S3). Note the thinner compact myocardium of the RV specifically in the diabetic control and *Hif1aCKO* **(B, D)** compared to non-diabetic hearts **(A, C)**. Scale bars, 100 μm.

The GO analysis of downregulated genes revealed a notable enrichment of biological processes linked to *nervous system development*, *neuron death*, *secretion*, and *synapse function*, indicating significant alternations in neuronal development, survival, and function. The downregulated set included genes such as HIF-1, regulated and highly expressed in neurons glucose transporter (*Slc2a3*) (72), the neuronal calcium sensor neurocalcin delta (*Ncald*) (73), a synaptic organizer and adhesion molecule neurexin 1 (*Nrxn1*) (74), and neurotrophic receptor tyrosine kinase 1, *Ntrk1* (also known as *TrkA*), a receptor for nerve growth factor with a key role in the regulation of proliferation, differentiation, and survival of sympathetic neurons (75, 76).

Using our RNA-seq data, we selected specific genes for qRT-PCR analysis to examine their expression in the sympathetic chain ganglia of control and *Hif1aCKO* embryos from non-diabetic and diabetic pregnancies at E14.5 (experimental design in Figure 10A). Consistent with the RNA-seq results, we found significantly increased expression levels of several selected genes, including *Cdc20*, *Gad2*, *Gata2*, *Kif23*, *Mki67*, and *Scl38a11*, in *Hif1aCKO* compared to the control group (Figure 10D). Additionally, some of these genes (*Gad2*, *Kif23*, and *Mki67*) were also upregulated in the sympathetic chain ganglia of control embryos exposed to maternal diabetes. While there was no additive effect of maternal diabetes in conjunction with *Hif1a* deletion on their expression, these results suggest possible compensatory mechanisms associated with proliferation, cell cycle, and mitosis in response to the adverse diabetic environment or *Hif1a* deficiency in developing sympathetic neurons. Interestingly, genes associated with synapse function, such as *Nrxn1* and *Ncald*, exhibited reduced expression in both control and *Hif1aCKO* embryos from diabetic pregnancies. In contrast, *Plk1* encoding Polo-like kinase 1, a regulator of mitosis (77), was upregulated by maternal diabetes but not affected by *Hif1a* deletion. Overall, these results align with our analyses of cellular changes in sympathetic chains, with no observed additive combinatorial effect of *Hif1a* mutation in the diabetic environment.

4 Discussion

This study investigates the combined impact of the *Hif1a*-deficient sympathetic system and maternal diabetes on heart development, specifically focusing on cardiac innervation, coronary artery formation, and the development of postganglionic sympathetic neurons of the cardiac sympathetic system. It provides the first comprehensive analysis of how maternal diabetes exposure affects the development of the cardiac sympathetic system, highlighting the combined impact of the *Hif1a*-deficient sympathetic system and the maternal diabetes environment on the heart. Inadequate activation of the HIF-1α regulatory pathway, particularly in the context of maternal diabetes, may contribute to abnormalities in the cardiac sympathetic system.

Diabetic pregnancies are associated with an increased incidence of congenital anomalies (78, 79) and an increased risk of fetal and infant death (80). The risk of fetal death is over four times higher, and the risk of infant death is nearly doubled in diabetic pregnancies (80). Furthermore, both human and animal studies indicate that exposure to diabetes *in utero* increases cardiovascular risk factors in the offspring, with long-term consequences for cardiovascular and metabolic health (37, 79, 81–83). Given the known associations between

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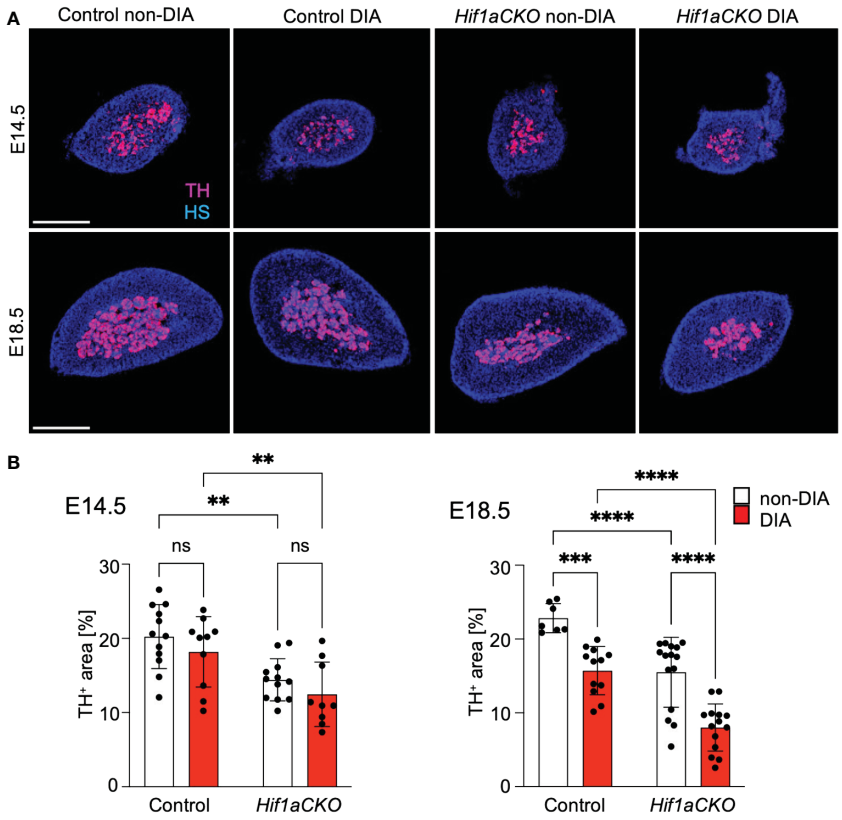

FIGURE 8
Reduced size of the adrenal medulla in diabetic *Hif1aCKO* mice. **(A)** Representative confocal images of cross-sections through the left adrenal glands of control and *Hif1aCKO*, and diabetic and non-diabetic embryos at E14.5 and E18.5, showing the size of the adrenal medulla by immunolabeled tyrosine hydroxylase (TH), a marker of sympathoadrenal cells. Hoechst-stained cell nuclei (HS). Scale bars, 300 µm. **(B)** TH^+ areas were quantified using the thresholding tool ImageJ and expressed as a percentage of the total adrenal gland area. The greatest reduction in the size of the adrenal medulla is observed in E18.5 embryos as the result of the combination of *Hif1a* deficiency and maternal diabetes exposure. Data are presented as the mean ± SD (E14.5 n = 12 non-DIA Control, n = 10 DIA Control, n = 12 non-DIA *Hif1aCKO*, and n = 9 DIA *Hif1aCKO*; E18.5 n = 7 non-DIA Control, n = 12 DIA Control, n = 15 non-DIA *Hif1aCKO*, and n = 14 DIA *Hif1aCKO*). Two-way ANOVA followed by *post hoc* Fisher's multiple comparisons test, **p < 0.01, ***p = 0.001, ****p < 0.0001. ns, not significant.

sympathetic defects and conditions like sudden infant death syndrome, cardiac arrhythmic death, and certain congenital heart defects in children (23, 24), we hypothesize that the *Hif1a* deficiency combined with maternal diabetes may further compromise the development of the cardiac sympathetic system. This compromise could potentially contribute to cardiac abnormalities and heightened health risks.

Consistent with our previous research (29), we observed a significant deficiency in cardiac sympathetic innervation and the development of neuroendocrine chromaffin cells in *Hif1aCKO* mice. Notably, the combination of the *Hif1a*-deficient sympathetic system and exposure to maternal diabetes accelerated the impairment of sympathetic innervation in the developing heart. Additionally, the negative impact on the size of the adrenal medulla in *Hif1aCKO* was further amplified by maternal diabetes, indicating an additive effect of *Hif1a* deletion and diabetes exposure. Chromaffin cells of the adrenal medulla are an important component of the sympathetic system and modulators of metabolic stress responses (84).

Previously, we reported a 40% increased neonatal mortality rate among *Hif1aCKO* mice (29). A similar reduced survival was also reported for mice with germline deletion of *Th*, although the cause of death in these mice was undetermined (1). In the current study, considering the coordinated development of peripheral sympathetic innervation and the coronary arterial network, we investigated the presence of anomalies in the coronary vasculature. We used genetically labeled coronary arteries in the *Cx40:eGFP* knock-in model (47) to examine coronary vascular development. While we observed abnormalities in the architecture of coronary arteries of *Hif1aCKO* embryos, they alone cannot account for the 40% neonatal mortality in *Hif1aCKO*.

Given the implication of the sympathetic nervous system in heart size regulation and cardiomyocyte proliferation (19, 20), we compared the size of the heart and the compact myocardial wall thickness. Exposure to maternal diabetes and *Hif1a* mutation had adverse effects on both heart size and compact myocardium of the LV and RV. We did not detect an additive effect of the combination of the *Hif1a*-deficient sympathetic system and the diabetic environment on these parameters compared to diabetic control embryos. The reduction in ventricular wall thickness observed in mutant and diabetes-exposed embryos, in comparison to non-diabetic controls, implies a corresponding decrease in the absolute

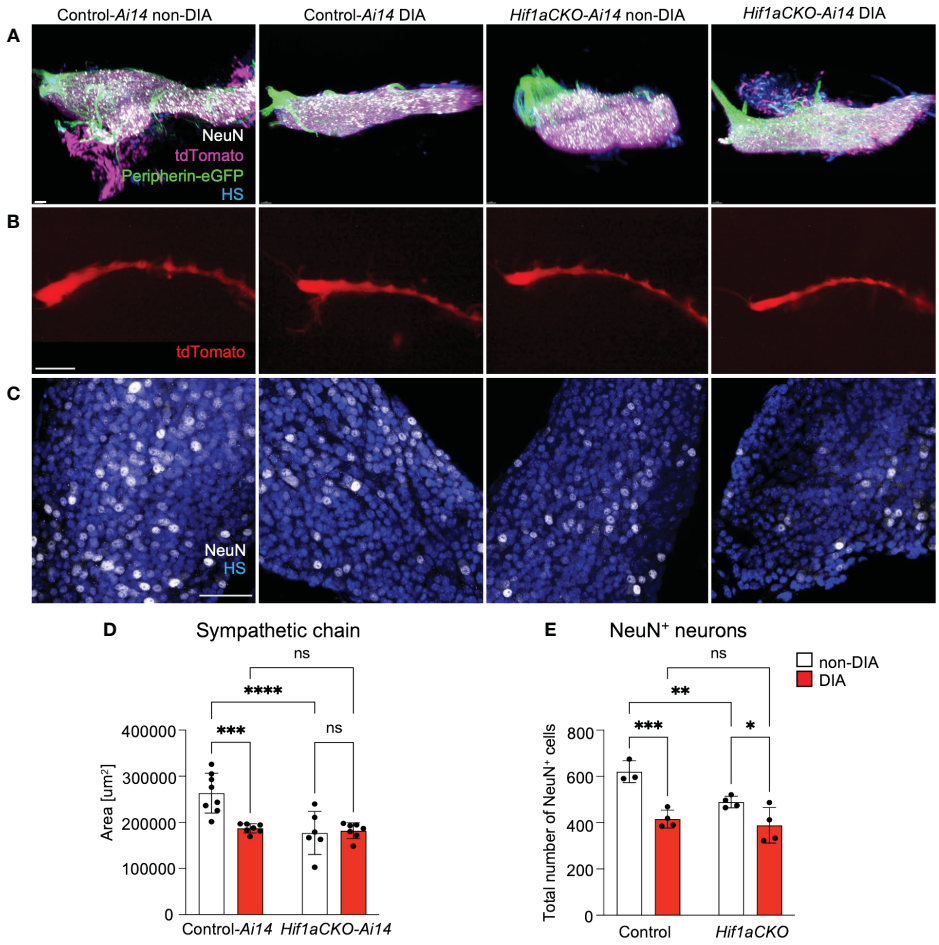

FIGURE 9
An altered development of sympathetic chain ganglia induced by maternal diabetes and *Hif1a* mutation. **(A)** Representative images of microdissected stellate ganglion (STG) of the secondary sympathetic chain of reporter control-*Ai14* and *Hif1aCKO-Ai14* embryos at E14.5. Samples were cleared (CUBIC protocol), imaged, and reconstructed using 3D light-sheet fluorescence microscopy showing tdTomato$^+$ and *Prph*-eGFP$^+$ neurons immunolabeled by anti-NeuN (a marker of differentiated neurons). Hoechst-stained cell nuclei (HS). Scale bar, 50 µm. **(B)** Representative images of the secondary sympathetic chain (SG) from STG to fourth thoracic ganglion at E14.5, reconstructed using 3D light-sheet fluorescence microscopy. Scale bar, 500 µm. **(C)** Confocal images of immunostaining for NeuN show neuronal density in the STG ganglion at E14.5. Hoechst-stained cell nuclei (HS). Scale bars, 50 µm. **(D)** Quantification of the area of the sympathetic chain (SG) from STG to fourth thoracic ganglion at E14.5. Data are presented as the mean ± SD (n = 7 non-DIA Control, n = 7 DIA Control, n = 6 non-DIA *Hif1aCKO*, and n = 7 DIA *Hif1aCKO*). **(E)** Density of NeuN$^+$ cells was quantified per area of the STG at E14.5. Data are expressed as mean ± SD (n = 3–4 samples per genotype, 3 areas per sample). Two-way ANOVA followed by *post hoc* Fisher's multiple comparisons test; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. ns, not significant.

amount of microvasculature. Consequently, this reduction is anticipated to impact myocardial perfusion and oxygenation. Moreover, dilated subepicardial veins and coronary arteries in the myocardium in both diabetic and non-diabetic *Hif1aCKO*, as well as diabetic control embryos, indicate compromised cardiac function. Increased thickness of the interventricular septum in these hearts suggests a compensatory response to failing hearts, given the smaller heart size and thinner ventricular walls. Whether this is a secondary effect or a direct result of the deficient adrenergic innervation remains unclear.

Our assessment of the cellular and molecular changes in developing postganglionic sympathetic neurons revealed significant alternations induced by *Hif1a* deletion or maternal diabetes at E14.5. However, we did not observe an additive combinatorial effect of *Hif1a* mutation in the diabetic environment. We selected the E14.5 developmental stage of sympathetic neurons for two main reasons. First, E14.5 represents a peak exit from the cell cycle, although ~25% of neurons are still cycling at E18.5 (8). Second, the first axons reach the heart ~E14 (17); therefore, we hypothesize that molecular changes associated with aberrant axonogenesis of sympathetic neurons might be detectable by RNA-seq at this stage. We found a significant enrichment of upregulated genes associated with proliferation, cell cycle, and mitosis, reflecting the main compensatory mechanisms for aberrant neuronal development of *Hif1aCKO*. In contrast, downregulated genes in *Hif1aCKO* were associated with nervous system development, synaptic function, and neuronal death, indicating altered neuronal development.

It is important to acknowledge the limitations of our study design. By focusing our molecular analysis (RNA-seq) on a single time point, we obtained only limited information about the temporal aspects of these molecular changes in *Hif1aCKO*

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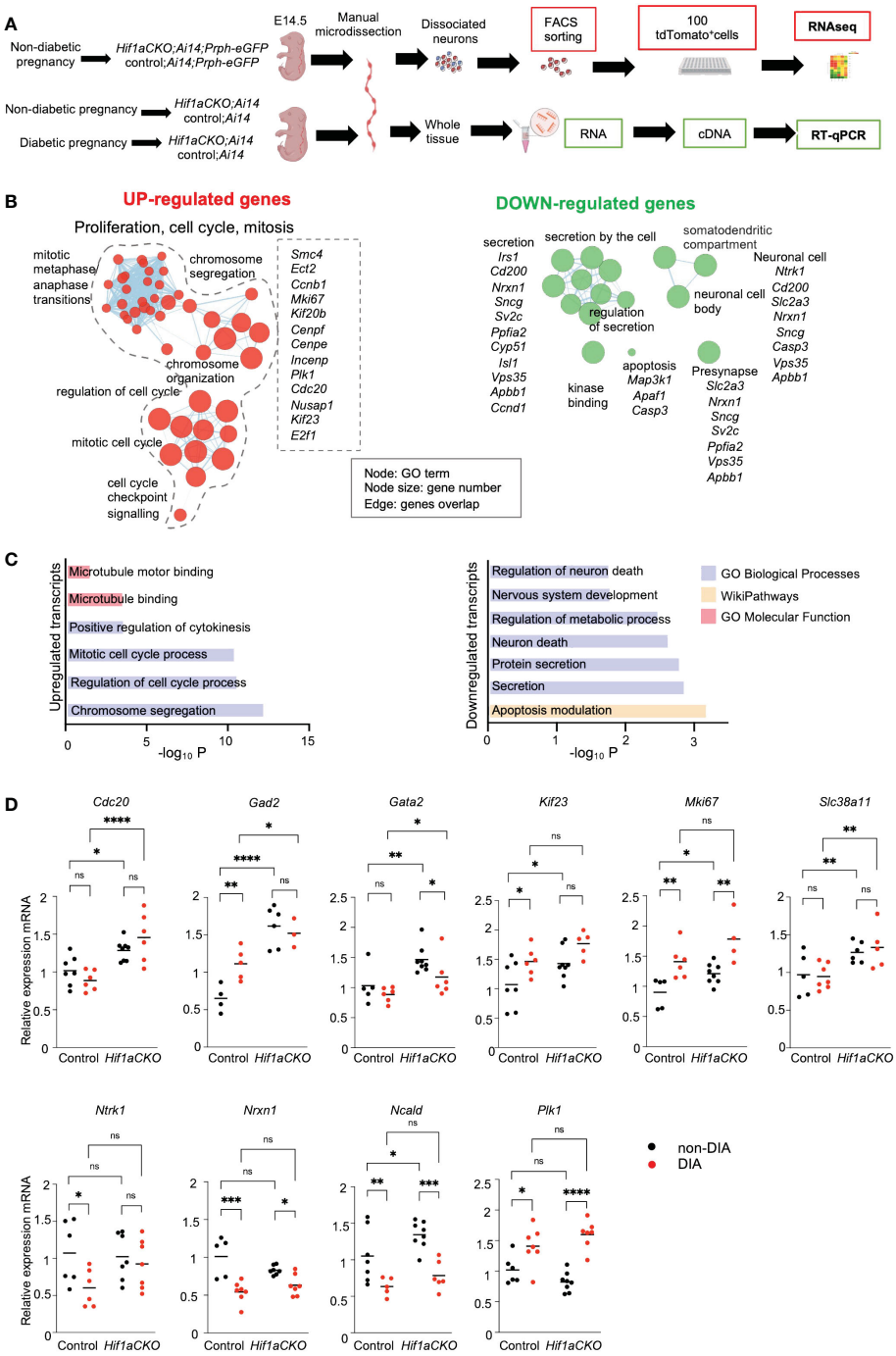

FIGURE 10
Hif1a-mediated transcription signature in sympathetic neurons. **(A)** Workflow depicts microdissection, dissociation, fluorescence-activated cell sorting (FACS) of single tdTomato$^+$ and *Prph*-eGFP$^+$ sympathetic neurons from control-*Ai14;Prph-eGFP* and *Hif1aCKO-Ai14;Prph-eGFP* non-diabetic embryos for a bulk of 100 cell RNA-seq analysis. RT-qPCR analyses were performed using RNA isolated from the microdissected sympathetic chain from E14.5 control and *Hif1aCKO* embryos from diabetic and non-diabetic pregnancies. Created with BioRender.com. **(B)** Gene set enrichment map of downregulated and upregulated differentially expressed genes visualized by the network. The complete list of identified down- and up-differentially expressed genes (adjusted p-value < 0.05, fold change >0.3, and <−0.3 cutoff values) is in Supplementary Data 1. Each node represents a Gene Ontology (GO) term; edges are drawn when there are shared genes between two GO terms. Each GO set cluster was assigned representative keywords; a full list of significant GO terms is available in Supplementary Data 1. **(C)** The bar graphs demonstrate a significant enrichment of GO terms, which reflect notable changes in sympathetic neurons. **(D)** The expression of RNA-seq-identified differentially expressed genes was analyzed by qRT-PCR. RNA mRNA was isolated from the microdissected secondary sympathetic chain of diabetic and non-diabetic control-*Ai14* and *Hif1aCKO-Ai14* embryos. Two-way ANOVA followed by *post hoc* Fisher's multiple comparisons test; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns, not significant. Abbreviations: *Cdc20*, cell division cycle 20; *Gad2*, glutamic acid decarboxylase 2; *Gata2*, GATA binding protein 2; *Kif23*, kinesin family member 23; *Mki67*, antigen identified by monoclonal antibody Ki 67; *Ncald*, neurocalcin delta; *Ntrk1*, neurotrophic tyrosine kinase receptor, type 1; *Nrxn1*, neurexin I; *Plk1*, polo like kinase 1; *Slc38a11*, solute carrier family 38, member 11.

sympathetic neurons. Another limitation of this study is that bulk-cell RNA sequencing approaches provide an average of expressional differences from multiple neurons, obscuring cell-specific differences. To address these limitations, future investigations using single-cell RNA-seq will be needed to fully establish molecular differences linked to specific cell states, cell-to-cell variability, to uncover the pathways of cell lineage differentiation affected in sympathetic neurons.

In summary, our results indicate that the interplay between deficiencies in the sympathetic system and subtle structural alternations in the vasculature, microvasculature, myocardium, and septum during heart development increases the risk of fetal demise for *Hif1aCKO*. Furthermore, even normal coronary vasculature, when experiencing deficient innervation, may not adequately react to the stress associated with birth and the subsequent rapid adaptation to oxygen breathing. Specifically, insufficient vasodilatation *via* beta-adrenergic receptors could lead to compromised cardiac function and neonatal death. This hypothesis is supported by considerable variability in the degree of sympathetic innervation in *Hif1aCKO*; it is likely that those with the most severe deficit are among the 40% of neonatal deaths. Considering the rapid clearance of dead pups by the mother, this hypothesis was difficult to validate. Deficient sympathetic innervation was reported from failing human hearts (85) or animal models of heart failure (86). Both deficient sympathetic innervation of the fetal heart along with the hypoplastic adrenal medulla affecting circulating catecholamine levels found in our study can contribute to this cascade of adverse peripartum events.

Data availability statement

The RNAseq data presented in the study are deposited in the GEO depository, accession number GSE250606.

Ethics statement

The animal study was approved by the Animal Care and Use Committee of the Institute of Molecular Genetics, CAS, and First Faculty of Medicine, CUNI. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

HK: Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. PH: Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft. RB: Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing – review & editing. PA: Investigation, Methodology, Writing – review & editing. VF: Investigation, Methodology, Visualization, Writing – review & editing. DS: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. GP: Conceptualization, Funding acquisition, Project administration, Supervision, 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/fendo.2024.1344074/full#supplementary-material

SUPPLEMENTARY VIDEO S1
Non-diabetic control embryonic heart at E16.5. Microdissected embryonic heart was cleared (CUBIC protocol), imaged, and reconstructed in 3D using light-sheet fluorescence microscopy (LFSM). Video shows the cardiac innervation using immunohistochemical staining of the sympathetic neuron marker, tyrosine hydroxylase (TH), and TUJ1 as a neuron marker.

SUPPLEMENTARY VIDEO S2
Diabetic control embryonic heart at E16.5. Microdissected embryonic heart was cleared (CUBIC protocol), imaged, and reconstructed in 3D using light-sheet fluorescence microscopy (LFSM). Video shows the cardiac innervation using immunohistochemical staining of the sympathetic neuron marker, tyrosine hydroxylase (TH), and TUJ1 as a neuron marker.

SUPPLEMENTARY VIDEO S3
Non-diabetic Hif1aCKO embryonic heart at E16.5. Microdissected embryonic heart was cleared (CUBIC protocol), imaged, and reconstructed in 3D using light-sheet fluorescence microscopy (LFSM). Video shows the cardiac innervation using immunohistochemical staining of the sympathetic neuron marker, tyrosine hydroxylase (TH), and TUJ1 as a neuron marker.

SUPPLEMENTARY VIDEO S4
Diabetic Hif1aCKO embryonic heart at E16.5. Microdissected embryonic heart was cleared (CUBIC protocol), imaged, and reconstructed in 3D using light-sheet fluorescence microscopy (LFSM). Video shows the cardiac innervation using immunohistochemical staining of the sympathetic neuron marker, tyrosine hydroxylase (TH), and TUJ1 as a neuron marker.

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EDITED BY
Richard Ivell,
University of Nottingham, United Kingdom

REVIEWED BY
Rui Zhao,
Shandong Provincial Qianfoshan Hospital, China
Yan Li,
Shandong University, China
Xiaolei Liang,
First Hospital of Lanzhou University, China

*CORRESPONDENCE
Yi Lin
yilinonline@126.com

†These authors share first authorship

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Effect of maternal serum albumin level on birthweight and gestational age: an analysis of 39200 singleton newborns

Jiayi Wu[1,2,3†], Xiaorui Liu[1,2,3†], Chuanmei Qin[1,2,3], Jinwen Zhang[1,2,3], Xueqing Liu[1,2,3], Jianing Hu[1,2,3], Fan Wu[1,2,3], Cailian Chen[4,5] and Yi Lin[1,2,3,6*]

[1]The International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China, [2]Shanghai Key Laboratory of Embryo Original Diseases, School of Medicine, Shanghai Jiao Tong University, Shanghai, China, [3]Institute of Birth Defects and Rare Diseases, School of Medicine, Shanghai Jiao Tong University, Shanghai, China, [4]Department of Automation, Shanghai Jiao Tong University, Shanghai, China, [5]Key Laboratory of System Control and Information Processing, Ministry of Education of China, Shanghai, China, [6]Reproductive Medicine Center, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China

Background: Serum albumin plays a pivotal role in regulating plasma oncotic pressure and modulating fluid distribution among various body compartments. Previous research examining the association between maternal serum albumin levels and fetal growth yielded limited and inconclusive findings. Therefore, the specific influence of serum albumin on fetal growth remains poorly understood and warrants further investigation.

Methods: A retrospective study involved 39200 women who had a singleton live birth at a tertiary-care academic medical center during the period from January 2017 to December 2020. Women were categorized into four groups according to the quartile of albumin concentration during early pregnancy: Q1 group, ≤41.0 g/L; Q2 group, 41.1-42.6 g/L; Q3 group, 42.7-44.3 g/L and Q4 group, >44.3 g/L. The main outcome measures were mid-term estimated fetal weight, birthweight and gestational age. Multivariate linear and logistic regression analysis were performed to detect the independent effect of maternal serum albumin level on fetal growth after adjusting for important confounding variables.

Results: In the crude analysis, a significant inverse correlation was found between early pregnancy maternal serum albumin levels and fetal growth status, including mid-term ultrasound measurements, mid-term estimated fetal weight, birthweight, and gestational age. After adjustment for a number of confounding factors, mid-term estimated fetal weight, birthweight, and birth height decreased significantly with increasing albumin levels. Compared to the Q2 group, the Q4 group had higher rates of preterm birth (aOR, 1.16; 95% CI, 1.01–1.34), small-for-gestational-age (aOR, 1.27; 95% CI, 1.11–1.45) and low birthweight (aOR, 1.41; 95% CI, 1.18–1.69), and lower rate of large-for-gestational-age (aOR, 0.85; 95% CI, 0.78–0.94). Moreover, to achieve the optimal neonatal outcome, women with higher early pregnancy albumin levels required a greater reduction in albumin levels in later pregnancy stages.

Conclusions: A higher maternal serum albumin level during early pregnancy was associated with poor fetal growth, with the detrimental effects becoming apparent as early as the mid-gestation period. These findings provided vital information for clinicians to predict fetal growth status and identify cases with a high risk of adverse neonatal outcomes early on.

KEYWORDS

serum albumin, fetal growth, birthweight, gestational age, mid-term fetal growth

Introduction

Optimal fetal growth and development are known to be the foundation for long-term human health, according to the well-known developmental origins of health and disease theory (1). Numerous studies have confirmed that intrauterine growth restriction or low birthweight can lead to diseases in children and adults, such as cognitive dysfunction and cardiovascular and metabolic diseases (2–4). More seriously, it is difficult to reverse the health status of an individual after birth, and some health defects may even cross generations (5). Although placental insufficiency, gestational hypertension, and preeclampsia have been reported to be risk factors for poor fetal growth (6, 7), its etiology is still unclear. A better understanding of modifiable factors associated with fetal growth would be vital to ensuring the maximum growth potential in early life.

Human serum albumin, an important indicator of nutritional status and hepatic function, is a widely used clinical marker. A lower serum albumin level may increase the risk of morbidity and mortality in both adults and children with various medical conditions, including stroke, renal disease, and malignancies (8). Notably, albumin, a major component of plasma proteins, plays a role in maintaining oncotic pressure and reflects plasma expansion (9). During pregnancy, inadequate plasma expansion is associated with the risk of low birthweight (LBW) (10), oligohydramnios (11), and preeclampsia (12). Growing evidence suggests that serum albumin levels could serve as an indicator of the risk and severity of preeclampsia (13, 14). Little is known, however, regarding the effect of maternal serum albumin on neonatal outcomes such as birthweight and birth length. Since albumin is a routine component of antenatal care, if proven to be an independent predictor for fetal growth, it could be a simple and low-cost method for early diagnosis of adverse neonatal outcomes.

To date, very few studies have examined the possible impact of maternal serum albumin on fetal growth (15–18), and their results are limited and contradictory. The main limitation to drawing robust and definitive conclusions is the absence of information on pregnancy complications, specifically gestational hypertension, preeclampsia, and gestational diabetes. Moreover, existing research were largely focused on women with term delivery, so the influence of maternal serum albumin levels on gestational age remains unknown. Therefore, there is clear need for a comprehensive investigation on the association between maternal serum albumin levels and fetal growth outcomes.

In the present study, we aimed to explore the impact of maternal serum albumin levels in early pregnancy on fetal growth by examining a large cohort of women with live-born singletons. Both neonatal outcomes and mid-term fetal growth were analyzed to predict the trajectory of fetal development throughout the duration of pregnancy, and the results offer crucial reference data for early interventions targeting inadequate fetal growth.

Methods

Study design and population

A retrospective study was conducted at the International Peace Maternity and Child Health Hospital (IPMCH) of Shanghai Jiao Tong University School of Medicine, a tertiary care hospital in China. The study protocol was approved by the Institute Medical Ethics Committee of IPMCH (reference number GKLW2021-17) and carried out according to the tenets of the Declaration of Helsinki. All women who had regular antenatal examination records and had a live birth (≥24 weeks of gestation) at IPMCH during January 2017 to December 2020 were included.

The exclusion criteria were: (1) multiple pregnancy, (2) *in vitro* fertilization, (3) maternal liver dysfunction (19), (4) maternal liver or renal disease, and (5) loss to follow-up or unavailability of main hepatic function records in the electronic database, including data on albumin, AST, and ALT levels.

Data collection

The following demographic characteristics were extracted from the medical record system: maternal age, pre-pregnancy body mass index (BMI), gravidity, parity, education level, cigarette or alcohol consumption before pregnancy, medical history, pregnancy complications, ultrasound measurements, delivery method,

gestational age, birthweight, birth length, and newborn sex. Gestational diabetes mellitus (GDM) was diagnosed based on a 2-h 75-g oral glucose tolerance test done at 24–28 weeks of gestation (20). Pregnancy-induced hypertension, including preeclampsia and gestational hypertension, was diagnosed based on diastolic blood pressure ≥90 mm Hg or systolic blood pressure ≥140 mm Hg measured twice after 20 weeks of gestation, with or without proteinuria. The records of liver biochemistry tests, including albumin, AST, and ALT levels, during the early pregnancy period (8–14 weeks of gestation) were measured by professional laboratory technicians, as previously described (19), and acquired from the hospital's laboratory database. The reference normal range for alanine transaminase [ALT] and aspartate aminotransferase [AST] in the Chinese population is considered to be not exceeding 40 U/L for both enzymes (21). The normal local laboratory serum albumin level range is 35–52 g/L (17). Records of the maternal serum albumin level during the final antenatal examination prior to delivery were also obtained, and the change in albumin level was calculated as the albumin value from the last assessment minus the albumin value from early pregnancy.

Ultrasound measurements, including biparietal diameter (BPD), humerus length (HL), femur length (FL), head circumference (HC), and abdominal circumference (AC), were performed by highly trained and experienced sonographers using standard protocols and identical instruments. These biometric measurements were recorded during 21–23 weeks of gestation. BPD was defined as the linear distance from the outer edge of the proximal parietal bone to the inner edge of the distal parietal bone on a cross-section of the fetal brain. FL and HL were measured as the linear distance along the long axis of the femur and humerus, respectively. With the ellipse function of the ultrasonic equipment, HC was measured at the same level as BPD, while AC was measured in a plane perpendicular to the level of the fetal umbilical plexus. To better assess fetal growth during the second trimester, estimated fetal weight (EFW) was calculated using HC, AC, BPD, and FL, according to the Hadlock formula (22).

The primary outcomes were mid-term EFW, singleton birthweight, and gestational age. The secondary endpoints included birth length, birthweight z-score, rates of preterm birth (PTB), small-for-gestational-age (SGA), large-for-gestational-age (LGA), LBW, and macrosomia. Birthweight z-scores were computed based on a set of general population reference values for Chinese singleton births to correct for the effect of newborn gender and gestational age on birthweight (23). Macrosomia and LBW were defined as birth weight >4000 g and <2500 g, respectively. Very-small-for-gestational-age (VSGA), SGA, LGA, and very-large-for-gestational-age (VLGA) were defined as birthweight <3rd, <10th, >90th, and >97th percentiles for gestational age, respectively. Very preterm birth (VPTB) and PTB were defined as delivery at <32 gestational weeks and <37 gestational weeks, respectively.

Statistical analysis

Continuous variables were described as mean values with standard deviations and compared by one-way analysis of variance. Categorical variables were presented as numbers with corresponding percentages and compared by Fisher's exact test or Pearson's chi-squared test, as appropriate. Following a methodology similar to previous literature (17), the women were further divided into four groups based on their albumin concentration: Q1, ≤41.0 g/L; Q2, 41.1–42.6 g/L; Q3, 42.7–44.3 g/L; and Q4, >44.3 g/L. The Q2 group was used as the reference for all comparisons. This grouping approach was implemented to facilitate a more comprehensive and detailed analysis of the influence of varying albumin levels on birth outcomes. Multivariable linear and logistic regression were performed to explore the association of maternal serum albumin level with fetal growth status after adjusting for several potential confounders, including maternal age, BMI, gravidity, parity, educational level, alcohol and cigarette consumption before pregnancy, ALT, AST, gestational age at sampling, pregnancy-induced hypertension, and GDM. The inclusion of potential confounders was determined based on the results of univariate and stepwise regression combined with variables related to serum albumin and neonatal outcomes indicated in previous studies. To further explore the dose-response association between serum albumin level and fetal growth, spline smoothing (24) on the basis of a generalized additive model were performed after adjustment for confounding factors. Given that serum albumin levels may vary as pregnancy progresses, to further minimize biases that may be caused by measurement of albumin levels at different gestational weeks, a sensitivity analysis was performed using women with a sampling time of 12 gestational weeks. Within this study cohort, the number of measurements conducted at 12 weeks was the highest, comprising 49% of the total population. All statistical analyses were performed using SAS software, version 9.4 (SAS Institute). Two-tailed P values <0.05 were considered to indicate significance.

Results

A total of 62299 women with a singleton live birth were selected from our electronic database, of which 39200 women fulfilling the inclusion criteria of the study were finally included. The details of the participant selection process are displayed in Supplementary Figure 1. A comparative analysis of the demographic characteristics was performed between the excluded and included participants (Supplementary Table 1).

The baseline demographic and clinical characteristics of the study population are presented in Table 1. In brief, the mean maternal age and BMI were 31.11 ± 3.88 years and 21.24 ± 2.82 kg/m^2, respectively. Of all the women, 26902 (68.6%) were primipara, and 22235 (56.7%) underwent vaginal delivery. The mean albumin level during early pregnancy was 42.68 ± 2.51 g/L, with the mean ALT level and AST level being 16.03 ± 14.10 U/L and 18.43 ± 7.62 U/L, respectively. Hepatic function measurements were taken at 11.96 ± 1.10 gestational weeks. The proportions of women diagnosed with pregnancy-induced hypertension and GDM were 4.8% and 14.3%, respectively.

With regard to the stratification of women into groups according to the quartiles of albumin concentration, 10269, 9741,

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TABLE 1 Basic characteristics of study population.

Characteristics	Participants, No. (%)
N	39200
Age (years)	31.11 ± 3.88
BMI (kg/m^2)	21.24 ± 2.82
Gravidity	
0	19037 (48.6)
≥1	20163 (51.4)
Parity	
0	26902 (68.6)
≥1	12298 (31.4)
Educational level	
Below college degree	10183 (26.0)
Bachelor's degree	20402 (52.0)
Master's or PHD degree	8615 (22.0)
Drinking before pregnancy (yes)	581 (1.5)
Smoking before pregnancy (yes)	228 (0.6)
Albumin (g/L)	42.68 ± 2.51
Alanine transaminase (U/L)	16.03 ± 14.10
Aspartate aminotransferase (U/L)	18.43 ± 7.62
Gestational age at sampling (weeks)	11.96 ± 1.10
Change in albumin level (g/L)	-6.67 ± 2.80
Delivery method	
Vaginal	22235 (56.7)
Cesarean	16965 (43.3)
Hypertension	
Pregnancy induced	1845 (4.8)
Preexisting	695 (1.8)
Diabetes	
Pregnancy induced	5620 (14.3)
Preexisting	94 (0.2)

Data are presented as mean ± SD for continuous variables and n (%) for dichotomous variables.

9707, and 9483 women were assigned to Q1, Q2, Q3, and Q4 groups, respectively. The baseline characteristics across the Q1-Q4 groups were shown in Supplementary Table 2. The associations between maternal serum albumin level and fetal growth are shown in Table 2. Women with higher levels of albumin had significantly lower BPD, HL, FL, HC, AC, and EFW in the second trimester ($P < 0.001$ for all). With regard to the neonatal outcomes, participants in the Q4 group (the highest quartile of albumin concentration) had significantly lower birthweight, birthweight z-scores, and birth length ($P < 0.001$). With increase in albumin concentrations, that is, from Q1 to Q4, the rates of PTB, LBW, SGA, and VSGA significantly increased ($P = 0.036$ for PTB; $P < 0.001$ for the rest), whereas the rates of macrosomia, LGA, and VLGA decreased ($P < 0.001$ for all). Stratified analyses of birthweight and birth length were further conducted based on the newborns' gender. The results demonstrated that, regardless of gender, birthweight, birthweight z-score, and birth length decreased with increasing serum albumin levels (Supplementary Table 3).

Multiple linear regression analyses were run to explore the relationship between albumin level and fetal growth (Table 3). According to the results of the fully adjusted analysis, compared with participants in the second quartile, those in the higher quartiles of albumin level had significantly lower EFW during the second trimester (Q3: $\beta = -4.18$, 95% CI = -5.97 to -2.39, $P < 0.001$; Q4: $\beta = -9.36$, 95% CI, -11.23 to -7.49; $P < 0.001$). Infants with higher maternal albumin levels had significantly lower birth weights than those in the second quartile, with the following values of adjusted regression coefficients: Q3: $\beta = -17.32$, 95% CI = -29.23 to -5.41 ($P = 0.004$); Q4: $\beta = -36.89$, 95% CI = -49.32 to -24.45 ($P < 0.001$). In addition, elevated albumin levels were associated with a decrease in gestational age-adjusted birthweight z-score and birth length after adjustment for confounding factors.

As shown in Table 4, after adjustment for several confounding variables, the highest quartile of albumin values was associated with higher risks of PTB (adjusted odds ratio (aOR) = 1.16; 95% CI = 1.01–1.34; $P = 0.038$), LBW (aOR = 1.41; 95% CI = 1.18–1.69; $P < 0.001$) and SGA (aOR = 1.27; 95% CI = 1.11–1.45; $P = 0.001$) than the second quartile. Furthermore, the fourth quartiles were associated with lower rates of LGA (aOR = 0.85, 95% CI = 0.78–0.94, $P = 0.001$) than the second quartile. Given the gradual decline of maternal albumin levels during pregnancy, we strategically selected women with a sampling time of 12 weeks for sensitivity analysis to minimize potential sampling time bias. A total of 19189 women were included in the sensitivity analysis, and the findings remained consistent with previous observations, affirming the stability and reliability of the results (Supplementary Table 4).

The dose–response relationships between maternal albumin levels and fetal growth are displayed visually in Figure 1. After adjusting for various confounding factors, there was a notable decline observed in mid-term HC, BPD, and EFW measurements in women with elevated maternal albumin levels (Figure 1A). Furthermore, a significant inverse correlation was found between maternal serum albumin levels and neonatal growth status, including birthweight and birthweight z-score (Figure 1B). Gestational age showed a decrease in correlation with increasing levels of maternal albumin, albeit of a minor magnitude.

The associations of change in albumin level with birthweight and birthweight z-score were analyzed. The population were categorized into four groups based on the quartiles of early pregnancy albumin concentration (Q1-Q4). The observation reveals that the Q4 group required a more significant decline in albumin levels in the later pregnancy stages to achieve the same birthweight and z-score as the Q1 group. (Figure 2). Additionally, women in the Q4 group showed lower potential for fetal development in terms of birthweight compared to women in the Q1 group. (Figure 2).

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TABLE 2 Main fetal growth parameters of live born singletons stratified by quartiles of maternal albumin.

	Total (n=39200)	Quartiles of albumin level				P value
		Q1 (n=10269)	Q2 (n=9741)	Q3 (n=9707)	Q4 (n=9483)	
Mid-term fetal growth						
Biparietal diameter (mm)	55.81 ± 3.01	55.92 ± 3.00	55.91 ± 3.03	55.79 ± 3.02	55.60 ± 2.96	<0.001
Humerus length (mm)	36.22 ± 2.08	36.37 ± 2.08	36.33 ± 2.09	36.16 ± 2.06	36.01 ± 2.05	<0.001
Femur length (mm)	38.22 ± 2.19	38.38 ± 2.20	38.33 ± 2.21	38.15 ± 2.18	37.99 ± 2.13	<0.001
Head circumference (mm)	198.43 ± 9.57	198.66 ± 9.50	198.68 ± 9.71	198.48 ± 9.48	197.88 ± 9.57	<0.001
Abdominal circumference (mm)	173.81 ± 10.55	174.77 ± 10.80	174.28 ± 10.50	173.61 ± 10.51	172.50 ± 10.21	<0.001
Estimated fetal weight (g)	494.87 ± 63.78	500.75 ± 65.77	498.2 ± 64.29	493.28 ± 62.96	486.72 ± 60.93	<0.001
Neonatal outcomes						
Gestational age (weeks)	38.96 ± 1.36	38.92 ± 1.33	38.97 ± 1.33	39.00 ± 1.37	38.96 ± 1.38	0.001
PTB	1771 (4.5)	432 (4.2)	413 (4.2)	476 (4.9)	450 (4.7)	0.036
VPTB	124 (0.3)	35 (0.3)	30 (0.3)	28 (0.3)	31 (0.3)	0.921
Male sex	20014 (51.1)	4978 (48.5)	5067 (52.0)	4990 (51.4)	4979 (52.5)	<0.001
Body length (cm)	49.82 ± 1.36	49.89 ± 1.30	49.83 ± 1.34	49.82 ± 1.37	49.76 ± 1.41	<0.001
Birthweight (g)	3333.91 ± 430.08	3366.77 ± 425.99	3345.25 ± 426.32	3322.94 ± 430.74	3297.88 ± 434.51	<0.001
Birthweight z-score	0.22 ± 0.94	0.31 ± 0.95	0.24 ± 0.94	0.18 ± 0.94	0.12 ± 0.94	<0.001
LBW	1024 (2.6)	211 (2.1)	237 (2.4)	260 (2.7)	316 (3.3)	<0.001
Macrosomia	2097 (5.3)	620 (6.0)	530 (5.4)	505 (5.2)	442 (4.7)	<0.001
SGA	1899 (4.8)	407 (4.0)	438 (4.5)	485 (5.0)	569 (6.0)	<0.001
LGA	4863 (12.4)	1501 (14.6)	1229 (12.6)	1133 (11.7)	1000 (10.5)	<0.001
VSGA	458 (1.2)	92 (0.9)	98 (1.0)	118 (1.2)	150 (1.6)	<0.001
VLGA	1506 (3.8)	486 (4.7)	376 (3.9)	349 (3.6)	295 (3.1)	<0.001

All ultrasound measurements were performed at 21-23 weeks of gestation. Estimated fetal weight was computed from biparietal diameter, femur length, head circumference and abdominal circumference using a Hadlock formula. Data are presented as mean ± SD for continuous variables and n (%) for dichotomous variables.

Discussion

In this large cohort study, we investigated the impact of maternal serum albumin level on fetal growth in 39200 women with live-born singletons. Our findings indicated that a high maternal serum albumin level during early pregnancy was associated with poor fetal growth, with the detrimental effects becoming apparent as early as mid-gestation. Moreover, women with elevated albumin levels in early pregnancy required a more significant reduction in albumin levels during later stages of gestation to attain the optimal birth outcome. These findings provide vital information that could help clinicians to predict fetal growth velocity and identify cases with a high risk of adverse neonatal outcomes early in pregnancy.

To date, only a few studies have focused on the potential association between maternal albumin levels and fetal growth, and two earlier small-scale studies failed to find any correlation between neonatal outcomes and albumin concentrations (15, 18). In 1996, Hasin and colleagues analyzed 151 pregnant women from poor urban communities and found that serum albumin levels were significantly lower in the women who had low birthweight infants than in those who had normal weight infants (16). This was subsequently challenged by a prospective study, which reported an inverse association between maternal serum albumin level and birthweight when the measurements were done during the third trimester (25). However, during early pregnancy, no significant correlation was observed between maternal albumin levels and birthweight (25). More recently, a study involving 3065 term-born singletons revealed a reverse U-shaped relationship between the mid-trimester albumin level and fetal growth (17). Nevertheless, as the authors acknowledged, the missing data on pregnancy complications, such as GDM, limited the accuracy of their results (16).

Of note, gestation-adjusted z-scores were not calculated in the aforementioned literatures. Since the mean birthweight varied with race and region (26, 27), it is difficult to compare these studies. Moreover, none of these studies considered the possible adverse effects of pregnancy-induced hypertension, preeclampsia, and

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TABLE 3 Results of multiple linear regression analysis of main fetal growth parameters among live born singletons.

	Q1		Q2	Q3		Q4	
	β (95% CI)	P value		β (95% CI)	P value	β (95% CI)	P value
Mid-term ultrasound measurements							
Biparietal diameter	0.00(-0.01,0.01)	0.364	Reference	-0.01(-0.02,-0.00)	0.004	-0.03(-0.04,-0.02)	<0.001
Humerus length	0.00(-0.00,0.01)	0.803	Reference	-0.02(-0.02,-0.01)	<0.001	-0.03(-0.03,-0.02)	<0.001
Femur length	0.00(-0.00,0.01)	0.335	Reference	-0.02(-0.02,-0.01)	<0.001	-0.03(-0.04,-0.02)	<0.001
Head circumference	-0.00(-0.03,0.02)	0.813	Reference	-0.02(-0.05,0.01)	0.140	-0.08(-0.11,-0.05)	<0.001
Abdominal circumference	0.03(-0.00,0.06)	0.079	Reference	-0.05(-0.08,-0.02)	0.001	-0.14(-0.17,-0.11)	<0.001
Estimated fetal weight	1.41(-0.37,3.20)	0.119	Reference	-4.18(-5.97,-2.39)	<0.001	-9.36(-11.23,-7.49)	<0.001
Neonatal outcomes							
Gestational age	0.01(-0.03,0.05)	0.592	Reference	-0.00(-0.04,0.03)	0.909	-0.03(-0.07,0.00)	0.082
Body length	0.04(0.01,0.08)	0.024	Reference	-0.01(-0.05,0.03)	0.630	-0.06(-0.10,-0.02)	0.006
Birthweight	9.12(-2.72,20.96)	0.131	Reference	-17.32(-29.23,-5.41)	0.004	-36.89(-49.32,-24.45)	<0.001
Birthweight z-score	0.03(0.00,-0.06)	0.023	Reference	-0.04(-0.06,-0.01)	0.004	-0.09(-0.11,-0.06)	<0.001

Analyses were adjusted for age, BMI, gravidity, parity, educational level, alcohol and cigarette consumption before pregnancy, ALT, AST, gestational age at sampling, pregnancy induced hypertension and gestational diabetes mellitus.

TABLE 4 Crude and adjusted ORs for adverse neonatal outcomes in singleton births by maternal albumin levels.

	Q1	P value	Q2	Q3	P value	Q4	P value
PTB							
OR (95% CI)	0.99 (0.86,1.14)	0.903	Reference	1.17 (1.02,1.33)	0.027	1.13 (0.98,1.29)	0.092
AOR (95% CI)	0.95 (0.83,1.10)	0.502	Reference	1.21 (1.05,1.38)	0.007	1.16 (1.01,1.34)	0.038
LBW							
OR (95% CI)	0.84 (0.70,1.01)	0.070	Reference	1.10 (0.92,1.32)	0.279	1.38 (1.17,1.64)	<0.001
AOR (95% CI)	0.85 (0.71,1.04)	0.108	Reference	1.14 (0.95,1.36)	0.173	1.41 (1.18,1.69)	<0.001
Macrosomia							
OR (95% CI)	1.12 (0.99,1.26)	0.062	Reference	0.96 (0.85,1.09)	0.497	0.85 (0.75,0.97)	0.016
AOR (95% CI)	1.03 (0.91,1.17)	0.604	Reference	0.97 (0.86,1.11)	0.670	0.88 (0.77,1.01)	0.072
SGA							
OR (95% CI)	0.88 (0.76,1.01)	0.060	Reference	1.12 (0.98,1.28)	0.102	1.36 (1.19,1.54)	<0.001
AOR (95% CI)	0.98 (0.85,1.13)	0.755	Reference	1.08 (0.95,1.24)	0.246	1.27 (1.11,1.45)	0.001
LGA							
OR (95% CI)	1.19 (1.10,1.29)	<0.001	Reference	0.92 (0.84,1.00)	0.048	0.82 (0.75,0.89)	<0.001
AOR (95% CI)	1.09 (1.00,1.18)	0.051	Reference	0.94 (0.86,1.02)	0.149	0.85 (0.78,0.94)	0.001

Analyses were adjusted for age, BMI, gravidity, parity, educational level, alcohol and cigarette consumption before pregnancy, ALT, AST, gestational age at sampling, pregnancy induced hypertension and gestational diabetes mellitus. OR Odd ratio, AOR adjusted odd ratio.

GDM on fetal growth. Thus, the reliability of the studies reported so far on this topic is limited. Most importantly, while anthropometric measurements taken at birth can reflect the culminative effect of an aberrant intrauterine environment on fetal growth, it does not provide a picture of specific changes in the fetal growth trajectory.

The present study, aiming to improve on the limitations of the previous studies described above, examined the precise role of maternal serum albumin level in fetal growth. Our results, based on the records of 39200 women with live-born singletons, clearly demonstrated that a high maternal serum albumin level had an

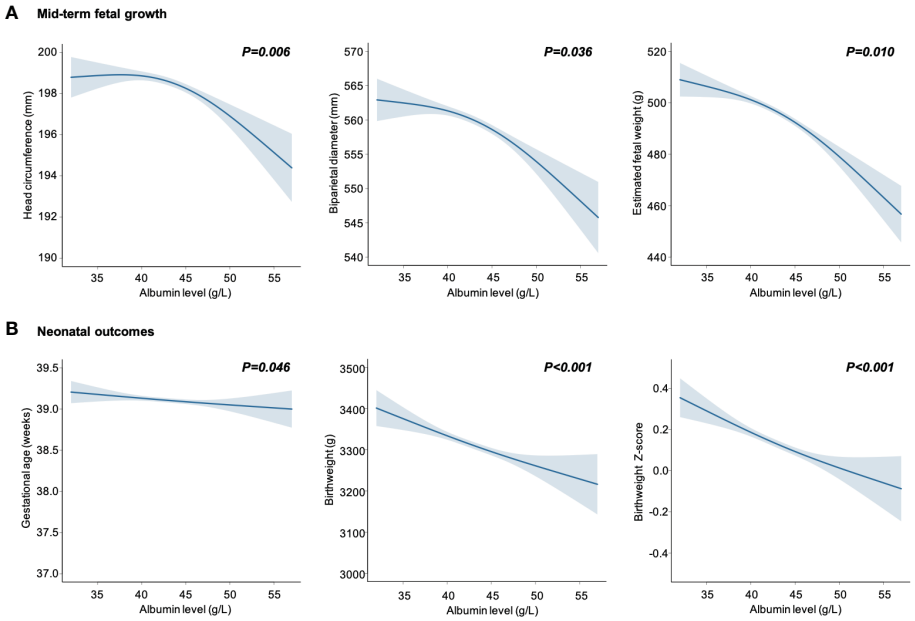

FIGURE 1
Maternal serum albumin in relation to mid-term fetal growth **(A)** and neonatal outcomes **(B)**. Data are presented as estimated mean with 95% CIs (shaded areas), adjusted for age, body mass index, gravidity, parity, educational level, alcohol and cigarette consumption before pregnancy, alanine transaminase, aspartate aminotransferase, gestational age at sampling, pregnancy induced hypertension and gestational diabetes mellitus.

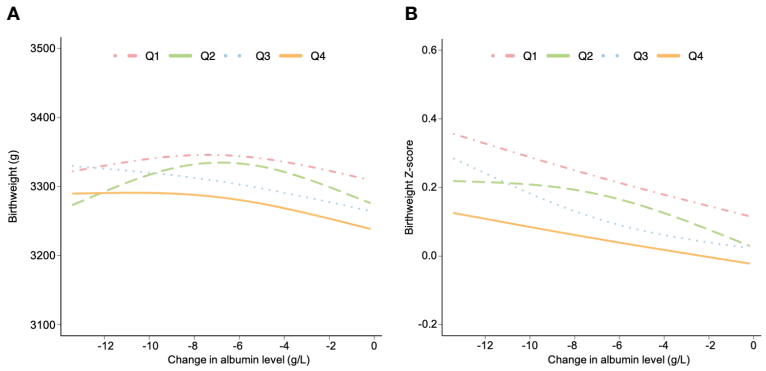

FIGURE 2
The associations between change in albumin level and birthweight **(A)** and birthweight z-score **(B)** stratified by albumin level in early pregnancy. Data are presented as estimated mean, adjusted for age, body mass index, gravidity, parity, educational level, alcohol and cigarette consumption before pregnancy, alanine transaminase, aspartate aminotransferase, gestational age at sampling and pregnancy induced hypertension and gestational diabetes mellitus. All women were grouped based on the quartile of albumin level during early pregnancy (Q1, Q2, Q3 and Q4).

adverse impact on fetal growth that initiated during the mid-gestational period and enduring until the late stages of pregnancy.

The reason why a high maternal serum albumin level leads to poor fetal growth is unclear. It is speculated that the resulting difference in plasma volume may play a role. Plasma volume expansion is a central physiological regulatory mechanism in pregnancy that begins as early as 6 to 8 gestational weeks. Although the mechanism underlying its role remains unclear, it has been suggested that reduced blood viscosity may favor blood flow in the maternal intervillous space (28). In addition, hemodilution in pregnancy is believed to prevent thrombosis in the uteroplacental circulation and further promote fetal development (29). Thus, plasma volume expansion is important for fetal growth. In fact, there is growing evidence that inadequate plasma volume expansion is associated with increased rates of intrauterine growth restriction and PTB (12, 30). This implies that the failure of plasma volume expansion in women with high levels of serum albumin may have implications for fetal growth and ultimately influence birthweight of infants. In the context of laboratory preparation for biochemical assays, the introduction of an anticoagulant, followed by centrifugation, yields a specimen known as plasma. Conversely, when an anticoagulant is omitted, blood naturally coagulates, resulting in the formation of serum upon centrifugation. Plasma typically exhibits a total protein

concentration that is approximately 0.2-0.5g/dL higher than that of serum. This distinction primarily arises from fibrinogen, a protein component that is consumed during the coagulation process. Despite the minor difference in total protein concentration between plasma and serum, clinically significant serum albumin levels provide a direct reflection of changes in plasma albumin concentration. Consequently, serum albumin levels serve as an indicator of how pregnant women respond to fluctuations in plasma volume throughout various stages of pregnancy.

Previous studies on this topic were largely carried out in women with term delivery, thus the possible influence of maternal albumin level on gestational age could not be fully explored. Only one study demonstrated a weak positive correlation between mid-pregnancy albumin level and birth duration, but it was limited by the absence of data on confounding factors (25). Contrary to their results, the current study found that elevated albumin levels were associated with decreased gestational age after adjusting for potential confounders. Also, women in the third and fourth quartiles were associated with a higher PTB rate compared to the lowest quartile.

Detailed information on fetal development across pregnancy trimesters provides important information for clinical practice. Ultrasound measurements during mid-pregnancy can reflect fetal growth and the internal processes during the entire duration of pregnancy. Hence, it elucidates the underlying biological mechanisms that contribute to the observed association between maternal serum albumin levels and fetal growth. Therefore, it is of vital importance to further explore the fetal growth trajectory with the use of ultrasound measures during pregnancy. The results of the current study indicated that albumin levels were inversely related to BPD, HL, FL, HC, and AC, which may have an influence on the subsequent health and development of the infant. For instance, BPD and HC, as indicators of head size, are correlated with cognitive achievement during childhood (31). Further, AC is a critical indicator of fetal liver size and subcutaneous fat deposition, and is also associated with cardiometabolic status later on in life (32), and FL has been reported to be associated with economic productivity in adulthood (33). In the present study, impaired fetal development appeared as early as the second trimester and persisted until delivery. Thus, to prevent poor fetal growth, it is of vital importance to monitor serum albumin levels from early pregnancy and carry out appropriate clinical interventions simultaneously. Such monitoring can serve as an invaluable tool for both doctors and pregnant women in identifying pregnancy-related risks at an early stage, facilitating timely interventions for enhanced outcomes. For the treatment of hypoproteinemia, if there are no contraindications related to the primary disease, a high-protein, high-calorie diet can be administered, ensuring an appropriate protein intake, providing sufficient calorie supply, and simultaneously supplementing with an adequate amount of vitamins.

During pregnancy, there is a normal reduction of serum albumin concentrations along with the increase of plasma volume (25). Our study, for the first time, discovered an inverted U-shaped relationship between change in albumin level and fetal development. The results demonstrated that both excessive and inadequate reductions in albumin levels had adverse effects on fetal development. For women with higher albumin levels in early pregnancy, it was necessary for them to further reduce their albumin levels during subsequent pregnancy stages in order to reach the highest developmental potential. The data from this study highlighted the importance of monitoring maternal serum albumin levels throughout the entire duration of pregnancy.

The main strength of the current study was the large sample size. To the best of our knowledge, this is the largest study assessing the effect of maternal serum albumin level on fetal growth. Another strength is that a number of relevant confounders that might otherwise have caused a bias in the results were adjusted for in this study, especially pregnancy complications. Moreover, ultrasound measurements during pregnancy combined with birth weight assessments were used to evaluate the trajectory of fetal growth, and this provided a better understanding of the underlying mechanisms that potentially drive the observed associations. Additionally, the laboratory conditions and clinical procedures remained unchanged during the study period. For example, the ultrasound measurements were performed by the same group of trained sonographers, and this reduced any intra-observer variability. Despite these advantages, the present study is limited by its retrospective nature. In our study, we excluded cases with missing essential data, such as albumin levels, birthweight and gestational age, which may introduce some degree of bias. To address this concern, we conducted a comparative analysis of baseline characteristics between the excluded and included cases. The results revealed that the excluded group had, on average, a slightly higher age (0.5 years) and a slightly higher BMI (0.1 kg/m2) compared to the included group. Furthermore, the excluded group exhibited a higher proportion of cesarean section deliveries and a greater incidence of gestational diabetes and pregnancy-induced hypertension. This may be attributed to a significant portion of the excluded population undergoing assisted reproductive technology (9839 individuals), a group characterized by older age and a higher incidence of pregnancy complications (34, 35). In addition, in an attempt to overcome any biases associated with the study design, we carefully reviewed the data according to strict criteria and conducted sensitivity analysis to reinforce the robustness of our findings.

Conclusions

In conclusion, the present large retrospective study showed that a high maternal serum albumin concentration was associated with impaired fetal growth in singletons. Thus, maternal serum albumin level may be used as an indicator in the clinic, based on which maximum fetal growth potential can be maintained in early pregnancy. Further studies are needed to verify our results.

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 Institute Medical Ethics Committee of the International Peace Maternity 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

JW: Writing – original draft. XiL: Writing – review & editing. CQ: Formal analysis, Writing – original draft. JZ: Formal analysis, Writing – original draft. XuL: Formal analysis, Writing – original draft. JH: Formal analysis, Writing – original draft. FW: Formal analysis, Writing – review & editing. CC: Project administration, Writing – review & editing. YL: Conceptualization, Funding acquisition, Writing – original draft.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1266669/full#supplementary-material

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University of Nottingham, United Kingdom

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Yafeng Liu,
Anhui University of Science and Technology, China
Cheng Zhan,
Fudan University, China

*CORRESPONDENCE
Yanwei Ren
263809227@qq.com
Shengjun Wu
3202107@zju.edu.cn

†These authors have contributed equally to this work

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Development and validation of nomograms to predict clinical outcomes of preeclampsia

Yan Xia[1,2†], Yao Wang[1,2†], Shijin Yuan[3], Jiaming Hu[1,2], Lu Zhang[1,2], Jiamin Xie[1,2], Yang Zhao[1,2], Jiahui Hao[4], Yanwei Ren[5*] and Shengjun Wu[1,2*]

[1]Department of Clinical Laboratory, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China, [2]Key Laboratory of Precision Medicine in Diagnosis and Monitoring Research of Zhejiang Province, Hangzhou, China, [3]Department of Medical Oncology, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China, [4]School of Medical Technology and Information Engineering, Zhejiang Chinese Medical University, Hangzhou, China, [5]Department of Gynaecology and Obstetrics, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China

Background: Preeclampsia (PE) is one of the most severe pregnancy-related diseases; however, there is still a lack of reliable biomarkers. In this study, we aimed to develop models for predicting early-onset PE, severe PE, and the gestation duration of patients with PE.

Methods: Eligible patients with PE were enrolled and divided into a training (n = 253) and a validation (n = 108) cohort. Multivariate logistic and Cox models were used to identify factors associated with early-onset PE, severe PE, and the gestation duration of patients with PE. Based on significant factors, nomograms were developed and evaluated using the area under the curve (AUC) and a calibration curve.

Results: In the training cohort, multiple gravidity experience (p = 0.005), lower albumin (ALB; p < 0.001), and higher lactate dehydrogenase (LDH; p < 0.001) were significantly associated with early-onset PE. Abortion history (p = 0.017), prolonged thrombin time (TT; p < 0.001), and higher aspartate aminotransferase (p = 0.002) and LDH (p = 0.003) were significantly associated with severe PE. Abortion history (p < 0.001), gemellary pregnancy (p < 0.001), prolonged TT (p < 0.001), higher mean platelet volume (p = 0.014) and LDH (p < 0.001), and lower ALB (p < 0.001) were significantly associated with shorter gestation duration. Three nomograms were developed and validated to predict the probability of early-onset PE, severe PE, and delivery time for each patient with PE. The AUC showed good predictive performance, and the calibration curve and decision curve analysis demonstrated clinical practicability.

Conclusion: Based on the clinical features and peripheral blood laboratory indicators, we identified significant factors and developed models to predict early-onset PE, severe PE, and the gestation duration of pregnant women with PE, which could help clinicians assess the clinical outcomes early and design appropriate strategies for patients.

KEYWORDS

pre-eclampsia, nomogram, biomarker, predictive model, peripheral biomarkers

1 Introduction

Preeclampsia (PE), which typically occurs after 20 weeks of gestation, is one of the most severe pregnancy-related diseases. It is characterized by sudden-onset hypertension and is accompanied by at least one of the following complications: proteinuria and maternal organ dysfunction (1). Globally, there are an estimated 4 million women newly diagnosed with PE each year, resulting in the death of more than 70,000 women and 500,000 newborns, making it the leading cause of maternal and perinatal morbidity and mortality (2, 3).

The heterogeneity of PE as clinical presentation and outcome varies between different subtypes. Patients with early-onset PE (<34 weeks of gestation) always present more severe clinical complications and an enrichment of metabolism-related pathways in the transcriptional profile compared to those with late-onset PE (≥34 weeks of gestation) (4, 5). Patients with PE are also at risk of rapid deterioration and severe disease, including eclampsia, stroke, HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome, placental abruption, renal function failure, and pulmonary edema, without receiving timely treatment (6). The management of PE consists of monitoring perinatal blood pressure and controlling complications through pharmacological intervention. Currently, timely delivery of the fetus is the only definitive treatment for PE; however, it may cause the babies of women with early-onset PE or severe symptoms to have increased risks of preterm birth, perinatal death, neurodevelopmental delay, and later cardiovascular and metabolic diseases (2). Therefore, early identification of the occurrence of PE, especially early-onset PE and severe PE, and prediction of gestation duration are of utmost importance to minimize adverse perinatal events both in pregnant women and in fetuses.

Three checklists from the International Society for the Study of Hypertension in Pregnancy (ISSHP) (3), the American College of Obstetricians and Gynecologists (ACOG) (7), and the National Institute for Health and Care Excellence (NICE) (8) are broadly used in clinical practice to assess the risk of PE occurrence; however, all risk factors derived from clinical features and their predictive power for PE are weak (9). Recently, increased numbers of biomarkers from peripheral blood have been identified to predict pregnant women with a high risk of PE at an early stage. Soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) are a pair of anti- and pro-angiogenic factors (respectively) found significantly unbalanced in PE (10). The PROGNOSIS trial demonstrated that, in women with a sFlt-1/PlGF ratio lower than 38, the likelihood of developing PE over the next week could accurately be ruled out, with a 99.3% negative predictive value (11). In addition, a series of novel placental- and endothelial-derived nucleic acid (mainly RNA) and proteins were also discovered for PE, including extravillous trophoblast signature (*MMP11*, *SLC6A2*, and *IL18BP*) (12), the chromosome 19 miRNA cluster (combination of miR-517-5p, miR520a-5p, and miR-525-5p) (13, 14), placental protein 13 (PP13) (15), pregnancy-associated plasma protein A (PAPP-A) (16), and vascular cell adhesion molecule-1 (VCAM-1) (17). However, the efficacy of a single biomarker from peripheral blood in the accurate diagnosis of PE is inadequate, and the majority of studies lacked validation. Hence, the development and validation of a predictive model based on multiple indicators consisting of clinical characteristics and laboratory parameters could be helpful in clinical practice. In the recent decade, several predictive models have been developed based on a series of risk factors. For example, by combining gestational age, chest pain or dyspnea, oxygen saturation, platelet count, and the creatinine and aspartate transaminase concentrations, the fullPIERS model could identify the risk of fatal or life-threatening complications in women with PE within 48 h of hospital admission (18). In addition, another study constructed a machine learning model for the prediction of PE in the first trimester based on the mean arterial blood pressure, uterine artery pulsatility index, PlGF, and PAPP-A (19). However, these models could not provide an exact probability for PE occurrence and the delivery of pregnant women at a certain time. A nomogram is a predictive tool to evaluate the clinical outcomes of patients by quantifying the probability based on easily accessed variables, which is widely used in patients with cancer and other chronic diseases, even in patients with coronavirus disease 2019 (20–22).

In this study, based on the clinical characteristics and peripheral blood laboratory indicators of patients with PE, we aimed to identify biomarkers for the early diagnosis of PE with early-onset and severe symptoms, predict the gestation duration, and construct a model for each clinical outcome, which could help clinicians recognize and manage patients with PE in the early stage of the

disease and improve the clinical prognosis for pregnant women and infants.

2 Methods

2.1 Study population

This study retrospectively enrolled patients from Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, China, in January 2017 and December 2022. Eligible populations were diagnosed with PE according to the "Diagnosis and treatment of hypertension and preeclampsia in pregnancy: a clinical practice guideline in China (2020)" (23). The detailed diagnostic criteria for PE were as follows: pregnant women with a systolic blood pressure higher than 140 mmHg and/or a diastolic blood pressure higher than 90 mmHg after 20 weeks of gestation, accompanied by any of the following symptoms: 1) urine protein ≥0.3 g/24 h or a urine protein/creatinine ratio ≥0.3 and 2) any dysfunction of important organs such as the heart, lung, liver, kidney, blood system, digestive system, and nervous system or involvement of placenta–fetus. The exclusion criteria were as follows: 1) patients with preexisting hypertension, immune disorders, and maternal organ dysfunction (such as hematopoietic, hepatic, and renal dysfunction) and 2) patients without complete maternal or infant records.

A total of 361 eligible patients were enrolled. This study was approved by the Ethics Committee of Sir Run Run Shaw Hospital (approval no. 2023-0248).

2.2 Data collection

Data on the clinical characteristics and laboratory indicators were collected from the electronic medical record of each patient. Clinical characteristics included age, history of parity, gravidity and abortion, multiple pregnancy, and regularity of the menstrual cycle. Laboratory indicators were collected at 20 weeks of gestation, which included white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb), hematocrit, mean corpuscular volume (MCV), red blood cell distribution width (RDW), absolute neutrophil count (ANC), absolute lymphocyte count (ALC), absolute monocyte count (AMC), absolute eosinophil count (AEC), absolute basophil count (ABC), platelet count (PC), platelet distribution width (PDW), mean platelet volume (MPV), thrombocytocrit, thrombin time (TT), prothrombin time (PT), activated partial thromboplastin time (APTT), international normalized ratio (INR), fibrinogen, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin (ALB), lactate dehydrogenase (LDH), serum amyloid A (SAA), total bile acid (TBA), and C-reactive protein (CRP).

2.3 Clinical outcomes

According to the gestational age at PE diagnosis, the patients were classified into an early-onset PE (<34 weeks) and a late-onset PE (≥34 weeks) group (24). The diagnostic criteria for severe PE were as follows: 1) continuously increasing blood pressure (systolic pressure ≥160 mmHg and/or diastolic pressure ≥110 mmHg); 2) persistent headache, visual disturbance, or other central nervous system abnormalities; 3) persistent upper abdominal pain, subcapsular hematoma, or liver rupture; 4) abnormal elevation of the AST and ALT levels; 5) impaired renal function—urinary protein quantification ≥2 g/24 h, oliguria, or serum creatinine level >106 μmol/L; 6) hypoproteinemia with ascites, pleural effusion, or pericardial effusion; 7) PC decreasing continuously and lower than 100×10^9/L, microvascular hemolysis, anemia, elevated LDH level, or jaundice; 8) heart failure; 9) pulmonary edema; and 10) fetal growth restriction, oligohydramnios, intrauterine fetal death, and placental abruption. A PE patient with one of the above symptoms was diagnosed as severe PE (23). The gestation duration was defined as the period between the last menstrual period and delivery.

2.4 Statistical analysis

Normality of the continuous variables was assessed using the Shapiro–Wilk test. Normally distributed variables were expressed as the mean ± standard deviation (SD), with significance analyzed using Student's *t*-test. Non-normally distributed variables were expressed as the median and interquartile range (IQR), with significance analyzed using the Mann–Whitney *U* test. Categorical variables were expressed as frequency and percentage, with significance analyzed using the chi-square test.

The whole study population was randomly divided into a training cohort and a validation cohort at a 7:3 ratio using a random sampling method ("Caret" R package). In the training cohort, univariate and multivariate logistic regression models (forward) were used to identify significant variables related to early-onset PE and severe PE ($p < 0.05$), and odds ratios (ORs) and 95% confidence intervals (CIs) were calculated ("glmnet" R package). Univariate and multivariate Cox proportional hazard regression models (forward) were used to identify significant variables related to gestation duration, and hazard ratios (HRs) and 95% CIs were calculated ("survival" R package). The results of the multivariate model were visualized using the "forestplot" R package. The curve of gestation duration was constructed using the Kaplan–Meier method and the log-rank test.

Based on the significant variables in the multivariate model, three nomograms were developed to predict the probability of early-onset PE, severe PE, and delivery at 26, 28, 30, 32, 34, 36, and 38 weeks for each patient with PE ("rms" R package). The predictive performance and the discriminative ability of each nomogram were assessed using 1,000 bootstrap resamples to obtain the concordance index (C-index) and the area under the receiver operating characteristic (ROC) curve (AUC). Calibration curves were used to evaluate the consistency between the predicted probabilities of the nomograms and the actual clinical outcomes. Decision curve analysis (DCA) was performed to evaluate the clinical utility of the nomograms. The above methods were then applied to validate the performance of the nomograms in the validation cohort.

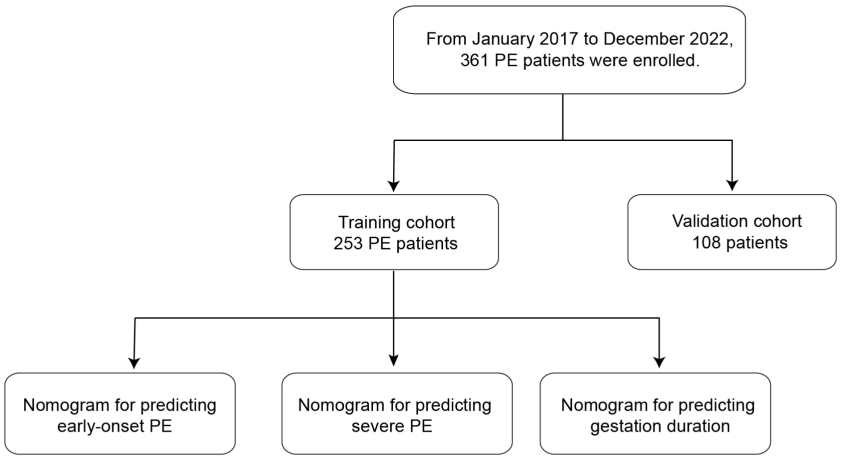

FIGURE 1
Workflow of the study.

All statistical analyses were performed using R software version 3.6.0. All tests were two-sided, and a $p < 0.05$ was considered statistically significant.

3 Results

3.1 Patient characteristics

The workflow is shown in Figure 1. After random sampling, a total of 361 patients with PE were allocated to the training cohort ($n = 253$) and the validation cohort ($n = 108$). The clinical characteristics and laboratory indicators between the two cohorts were basically balanced (Table 1). In the training cohort, the median age was 32.0 years (range, 29.0–35.0 years). A total of 135 patients (53.4%) had multiple gravidity experience, while 188 patients (74.3%) were primiparas. There were 198 patients (78.3%) who had a single pregnancy, 116 patients (45.8%) had a history of abortion, and 221 patients (87.4%) had irregular menstruation.

3.2 Development of a nomogram for predicting early-onset PE

In the training cohort, 60 patients (23.7%) were diagnosed as early-onset PE. Univariate logistic analysis showed that patients with early-onset PE had multiple gravidity experience ($p < 0.001$); abortion history ($p < 0.001$); gemellary pregnancy ($p = 0.021$); elevated levels of MPV ($p = 0.031$), TT ($p < 0.001$), AST ($p < 0.001$), ALP ($p < 0.001$), LDH ($p < 0.001$), SAA ($p = 0.006$), and TBA ($p < 0.001$); and reduced levels of ALB ($p < 0.001$) (Supplementary Table S1). The above variables were incorporated into the multivariate logistic model and revealed that multiple gravidity experience (OR = 3.244, 95%CI = 1.420–7.411, $p = 0.005$), low ALB levels (32.3 *vs.* 35.7; OR = 0.745, 95%CI = 0.647–0.858, $p < 0.001$), and high LDH levels (192 *vs.* 150; OR = 1.020, 95%CI = 1.010–1.030, $p < 0.001$) were significantly associated with early-onset PE (Figures 2A–C). Based on these three variables, a nomogram was constructed to predict the probability of early-onset PE for each individual patient (Figure 2D). The C-index and AUC of the nomogram were both 0.843 (95%CI = 0.776–0.910), indicating good predictive performance. In addition, the calibration curve demonstrated good consistency between the probabilities predicted by the nomogram and the actual results (Figures 2E, F), and DCA showed that the nomogram offered a net benefit over the "treat-all" or "treat-none" strategy (Supplementary Figure S1A).

3.3 Development of a nomogram for predicting severe PE

In the training cohort, 132 patients (52.2%) were diagnosed as severe PE. Univariate logistic analysis showed that patients with early-onset PE had multiple gravidity experience ($p < 0.001$); abortion history ($p < 0.001$); elevated levels of Hb ($p = 0.023$), MPV ($p = 0.001$), TT ($p = 0.001$), AST ($p < 0.001$), ALP ($p < 0.001$), LDH ($p = 0.003$), and TBA ($p = 0.048$); and reduced levels of RDW ($p = 0.030$), PT ($p = 0.025$), and ALB ($p < 0.001$) (Supplementary Table S2). The above variables were incorporated into the multivariate logistic model and revealed that abortion history (OR = 2.057, 95%CI = 1.136–3.726, $p = 0.017$) and elevated levels of TT (15.9 *vs.* 15.1; OR = 1.934, 95%CI = 1.342–2.785, $p < 0.001$), AST (21.5 *vs.* 14.0; OR = 1.068, 95%CI = 1.024–1.113, $p = 0.002$), and LDH (167 *vs.* 144; OR = 1.017, 95%CI = 1.006–1.028, $p = 0.003$) were significantly associated with severe PE (Figures 3A–D). Based on these four variables, a nomogram was constructed to predict the probability of severe PE for each individual patient (Figure 3E). The C-index and AUC of the nomogram were both 0.814 (95%CI = 0.762–0.866), indicating good predictive performance. In addition, the calibration curve demonstrated good consistency between the probabilities predicted by the nomogram and the actual results (Figures 3F, G), and DCA showed that the nomogram offered a net benefit over the "treat-all" or "treat-none" strategy (Supplementary Figure S1B).

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TABLE 1 Baseline clinical characteristics and laboratory parameters.

Variable	Total patients (n=361)	Training cohort (n=253)	Validation cohort (n=108)	P-value
Clinical characteristics				
Age, years (IQR)	32.0 (29.0, 36.0)	32.0 (29.0, 35.0)	32.0 (28.0, 37.0)	0.641
Gravidity				1.000
1	168 (46.5%)	118 (46.6%)	50 (46.3%)	
≥2	193 (53.5%)	135 (53.4%)	58 (53.7%)	
Parity				0.176
Primipara	260 (72.0%)	188 (74.3%)	72 (66.7%)	
Multipara	101 (28.0%)	65 (25.7%)	36 (33.3%)	
Abortion				0.896
No	197 (54.6%)	137 (54.2%)	60 (55.6%)	
Yes	164 (45.4%)	116 (45.8%)	48 (44.4%)	
Gemellary pregnancy				1.000
No	283 (78.4%)	198 (78.3%)	85 (78.7%)	
Yes	78 (21.6%)	55 (21.7%)	23 (21.3%)	
Menstrual regularity				0.398
No	50 (13.9%)	32 (12.6%)	18 (16.7%)	
Yes	311 (86.1%)	221 (87.4%)	90 (83.3%)	
Early-onset PE				0.716
No	278 (77.0%)	193 (76.3%)	85 (78.7%)	
Yes	83 (23.0%)	60 (23.7%)	23 (21.3%)	
Severe PE				0.455
No	178 (49.3%)	121 (47.8%)	57 (52.8%)	
Yes	183 (50.7%)	132 (52.2%)	51 (47.2%)	
Laboratory parameters				
WBC, $\times10^9$/L	9.70 (8.50, 11.5)	9.80 (8.50, 11.5)	9.65 (8.65, 11.1)	0.958
RBC, $\times10^{12}$/L	3.82 (3.55, 4.09)	3.81 (3.58, 4.08)	3.83 (3.55, 4.12)	0.927
Hb, g/L	108 (12.0, 121)	108 (11.9, 121)	108 (12.0, 121)	0.921
Hematocrit, %	35.1 (33.0, 36.9)	35.2 (33.0, 36.9)	34.5 (33.0, 36.7)	0.454
MCV, fL	92.0 (88.7, 94.8)	91.7 (88.7, 94.6)	92.5 (88.7, 95.0)	0.652
PC, $\times10^9$/L	215 (178, 256)	212 (176, 253)	228 (185, 261)	0.187
ANC, $\times10^9$/L	7.33 (6.20, 8.84)	7.33 (6.20, 9.00)	7.30 (6.18, 8.52)	0.834
ALC, $\times10^9$/L	1.70 (1.45, 2.00)	1.70 (1.40, 2.00)	1.71 (1.50, 2.00)	0.343
AMC, $\times10^9$/L	0.51 (0.42, 0.65)	0.50 (0.40, 0.63)	0.52 (0.45, 0.66)	0.310
AEC, $\times10^9$/L	0.09 (0.04, 0.10)	0.08 (0.04, 0.10)	0.10 (0.06, 0.11)	0.234
ABC, $\times10^9$/L	0.02 (0.00, 0.03)	0.02 (0.00, 0.03)	0.02 (0.00, 0.03)	0.842
RDW, %	13.4 (13.0, 14.0)	13.5 (13.0, 14.0)	13.3 (12.9, 13.8)	0.302
PDW, %	16.7 (16.2, 17.3)	16.8 (16.3, 17.3)	16.6 (16.0, 17.2)	0.562
MPV, fL	8.50 (7.80, 9.30)	8.50 (7.80, 9.30)	8.45 (7.77, 9.15)	0.615
Thrombocytocrit, %	0.18 (0.16, 0.22)	0.18 (0.16, 0.21)	0.20 (0.16, 0.22)	0.203

(Continued)

TABLE 1 Continued

Variable	Total patients (n=361)	Training cohort (n=253)	Validation cohort (n=108)	P-value
PT, s	12.4 (12.1, 12.8)	12.4 (12.1, 12.8)	12.4 (12.1, 12.9)	0.476
INR	0.93 (0.90, 0.97)	0.93 (0.91, 0.96)	0.94 (0.90, 0.98)	0.428
APTT, s	31.5 (29.9, 33.0)	31.6 (29.9, 33.1)	31.4 (30.1, 32.8)	0.991
TT, s	15.4 (14.8, 16.1)	15.4 (14.8, 16.1)	15.4 (14.8, 15.9)	0.791
Fibrinogen, g/L	4.69 (0.77)	4.69 (0.76)	4.68 (0.79)	0.895
ALT, U/L	13.0 (10.0, 20.0)	13.0 (10.0, 20.0)	12.0 (9.00, 20.2)	0.345
AST, U/L	18.0 (15.0, 24.0)	18.0 (15.0, 25.0)	17.0 (15.0, 21.0)	0.608
ALP, U/L	72.0 (59.0, 89.0)	72.0 (61.0, 91.0)	73.0 (57.0, 87.0)	0.427
Albumin, g/L	34.6 (32.9, 36.5)	34.9 (32.9, 37.0)	34.2 (33.0, 35.7)	0.058
LDH, U/L	152 (137, 175)	154 (138, 178)	148 (134, 165)	0.115
SAA, mg/L	4.80 (3.10, 7.80)	4.80 (3.20, 7.80)	5.15 (3.00, 7.78)	0.852
TBA, μmol/L	2.48 (1.61, 3.68)	2.50 (1.51, 3.68)	2.40 (1.79, 3.65)	0.436
CRP, mg/L	3.00 (1.80, 5.60)	3.00 (1.80, 5.50)	3.10 (1.78, 5.95)	0.539

Data were expressed as *n* (%), mean (±SD), and median (interquartile range).
The *p*-values compared between the training and the validation cohort.

3.4 Development of a nomogram for predicting gestation duration

In the training cohort, the median gestation duration was 36.0 weeks (range, 34.0–38.0 weeks). The results of the univariate Cox analysis showed that higher age ($p < 0.001$); multiple gravidity experience ($p < 0.001$); abortion history ($p < 0.001$); gemellary pregnancy ($p < 0.001$); multipara ($p = 0.001$); elevated levels of MPV ($p < 0.001$), TT ($p < 0.001$), AST ($p = 0.002$), ALP ($p < 0.001$), LDH ($p < 0.001$), SAA ($p = 0.015$), TBA ($p = 0.007$), and CRP ($p = 0.004$); and reduced levels of ALB ($p < 0.001$) were associated with shorter gestation duration (Supplementary Table S3). The above variables were incorporated into the multivariate Cox model and revealed that abortion history (HR = 1.822, 95%CI = 1.398–2.374, $p < 0.001$); gemellary pregnancy (HR = 2.233, 95%CI = 1.605–3.106, $p < 0.001$); elevated levels of MPV (HR = 1.134, 95%CI = 1.026–1.253, $p = 0.014$), TT (HR = 1.351, 95%CI = 1.159–1.574, $p < 0.001$), and LDH (HR = 1.009, 95%CI = 1.006–1.013, $p < 0.001$); and reduced levels of ALB (HR = 0.912, 95%CI = 0.872–0.955, $p < 0.001$) were significantly associated with shorter gestation duration (Figures 4A–G). Based on these six variables, a nomogram was constructed to predict the probability of delivery at 26, 28, 30, 32, 34, 36, and 38 gestational weeks for each individual PE patient (Figure 4H). The C-index of the nomogram was 0.772 (95%CI = 0.740–0.804). The AUC of each time point of nomogram prediction demonstrated good performance and discrimination (26 weeks: 0.852, 95%CI = 0.757–0.914; 28 weeks: 0.892, 95%CI = 0.730–0.942; 30 weeks: 0.929, 95%CI = 0.792–0.981; 32 weeks: 0.900, 95%CI = 0.881–0.955; 34 weeks: 0.831, 95%CI = 0.721–0.916; 36 weeks: 0.854, 95%CI = 0.718–0.935; 38 weeks: 0.815, 95%CI = 0.701–0.924) (Figure 5A). The time-dependent ROC curve is shown in Supplementary Figure S2A. Furthermore, the calibration curve demonstrated good consistency between the nomogram's predicted probabilities and the actual results (Figures 5B–H), and DCA showed that the nomogram offered a net benefit over the "treat-all" or "treat-none" strategy at each time point (Supplementary Figures S1C–I).

3.5 Validation of the nomograms

In the validation cohort, the median age was 32.0 years (range, 28.0–37.0 years). There were 58 patients (53.7%) who had multiple gravidity experience and 72 patients (66.7%) who were primiparas. A total of 85 patients (78.7%) had a single pregnancy, 48 patients (44.4%) had a history of abortion, and 90 patients (83.3%) had irregular menstruation.

In the validation cohort, there were 23 patients (21.3%) with early-onset PE and 51 patients (47.2%) with severe PE. The AUCs of the nomogram predicting early-onset PE and severe PE were 0.828 (95%CI = 0.736–0.919) and 0.823 (95%CI = 0.746–0.901), respectively (Figures 5I, J). The median gestation duration in the validation cohort was 37.0 weeks (range, 35.0–38.0 weeks). The C-index of the nomogram was 0.741 (95%CI = 0.689–0.793). The AUC of each time point of nomogram prediction demonstrated good performance and discrimination (26 weeks: 0.935, 95%CI = 0.872–0.977; 28 weeks: 0.800, 95%CI = 0.734–0.901; 30 weeks: 0.866, 95%CI = 0.716–0.923; 32 weeks: 0.857, 95%CI = 0.696–0.953; 34 weeks: 0.832, 95%CI = 0.724–0.938; 36 weeks: 0.800, 95% CI = 0.749–0.910; 38 weeks: 0.851, 95%CI = 0.772–0.924) (Figure 5M). The time-dependent ROC curve in the validation cohort is shown in Supplementary Figure S2B. The calibration curves for each nomogram also demonstrated good consistency between the predicted probabilities and the actual results (Figures 5K, L, N–T), and DCA showed that the nomogram offered a net benefit over the "treat-all" or "treat-none" strategy at each time point (Supplementary Figures S1L–R).

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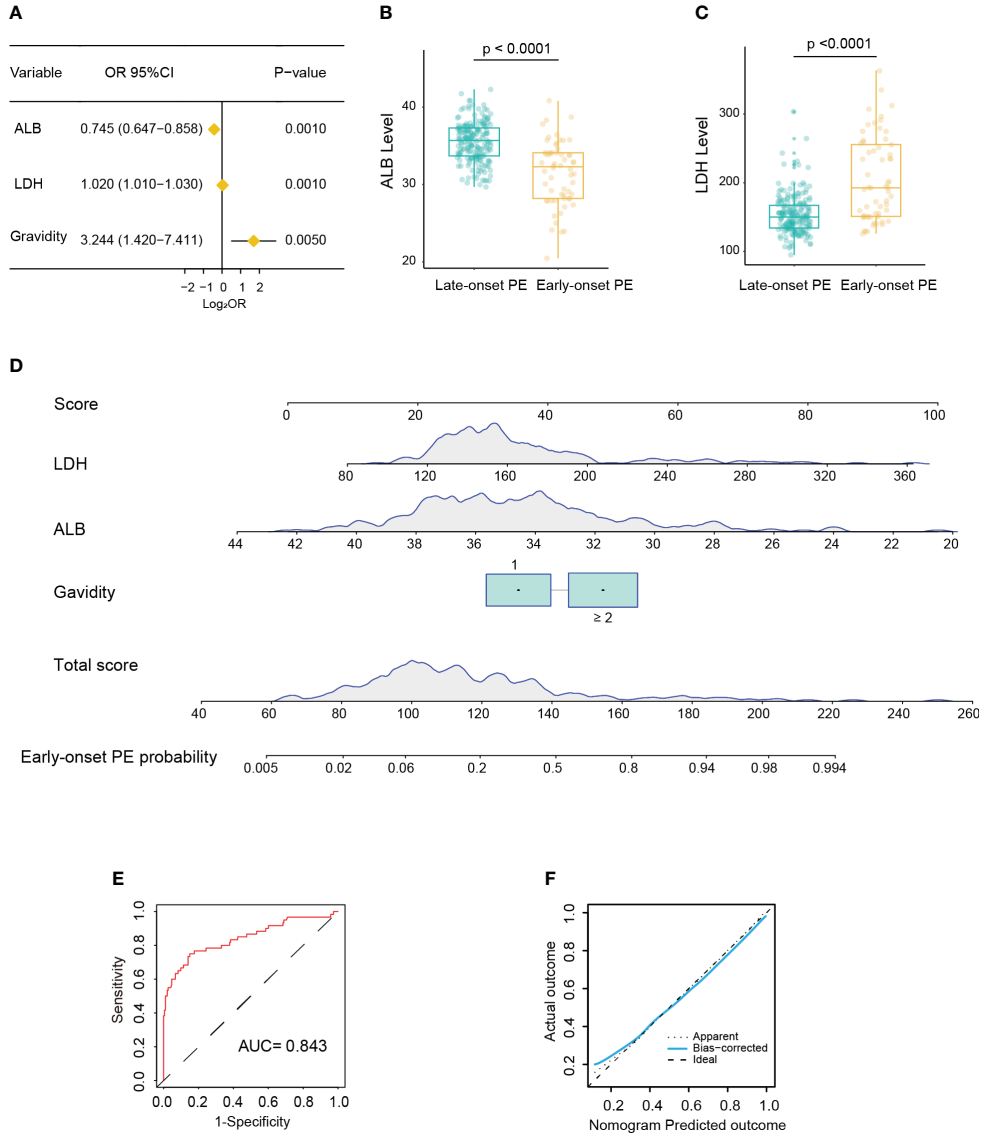

FIGURE 2
Development of a nomogram for predicting early-onset pre-eclampsia (PE). **(A)** Forest plot showing multiple gravidity experience, lower albumin (ALB), and higher lactate dehydrogenase (LDH) as significantly associated with early-onset PE in multivariable Cox regression analysis. **(B, C)** Box plot showing the distribution of the levels of ALB **(B)** and LDH **(C)** between the early-onset and late-onset PE groups (Wilcoxon test). **(D)** Nomogram for predicting early-onset PE probability in the training cohort. **(E)** Receiver operating characteristic (ROC) curve of the nomogram predicting early-onset PE probability in the training cohort. **(F)** Calibration curve of the nomogram predicting early-onset PE probability in the training cohort.

4 Discussion

In this study, based on the clinical characteristics and peripheral blood laboratory indicators, we identified a series of risk factors associated with early-onset PE, severe PE, and shorter gestation duration of patients with PE. In addition, three nomograms were developed to predict the probability of early-onset PE, severe PE, and delivery at different gestational weeks for each individual patient with PE, which could help clinicians manage patients with PE in the early stage of the disease and improve the clinical outcomes for pregnant women and infants.

The etiologies and pathogenesis of PE are complex and multisystemic, which involve placental dysfunction, immune system dysfunction, maternal metabolic disorder, and dysregulated endothelial function due to the release of a series of circulating factors including angiogenic proteins, pro-inflammatory cytokines, and small extracellular vesicles (25–30). Several risk factors of obstetric history have been identified as associated with PE from clinical guidelines, including previous PE, history of parity, gravidity, abortion, and multiple pregnancies, which were considered to lead to a weakened maternal immune tolerance to the placenta, thus increasing the risk of PE (3, 7, 8). A previous study found that multiple fetal pregnancies were associated with a significantly higher rate of PE than singleton pregnancies, with the rate increasing with the number of fetuses present (31). In this study, multiple gravidities, previous abortion history, and multiple

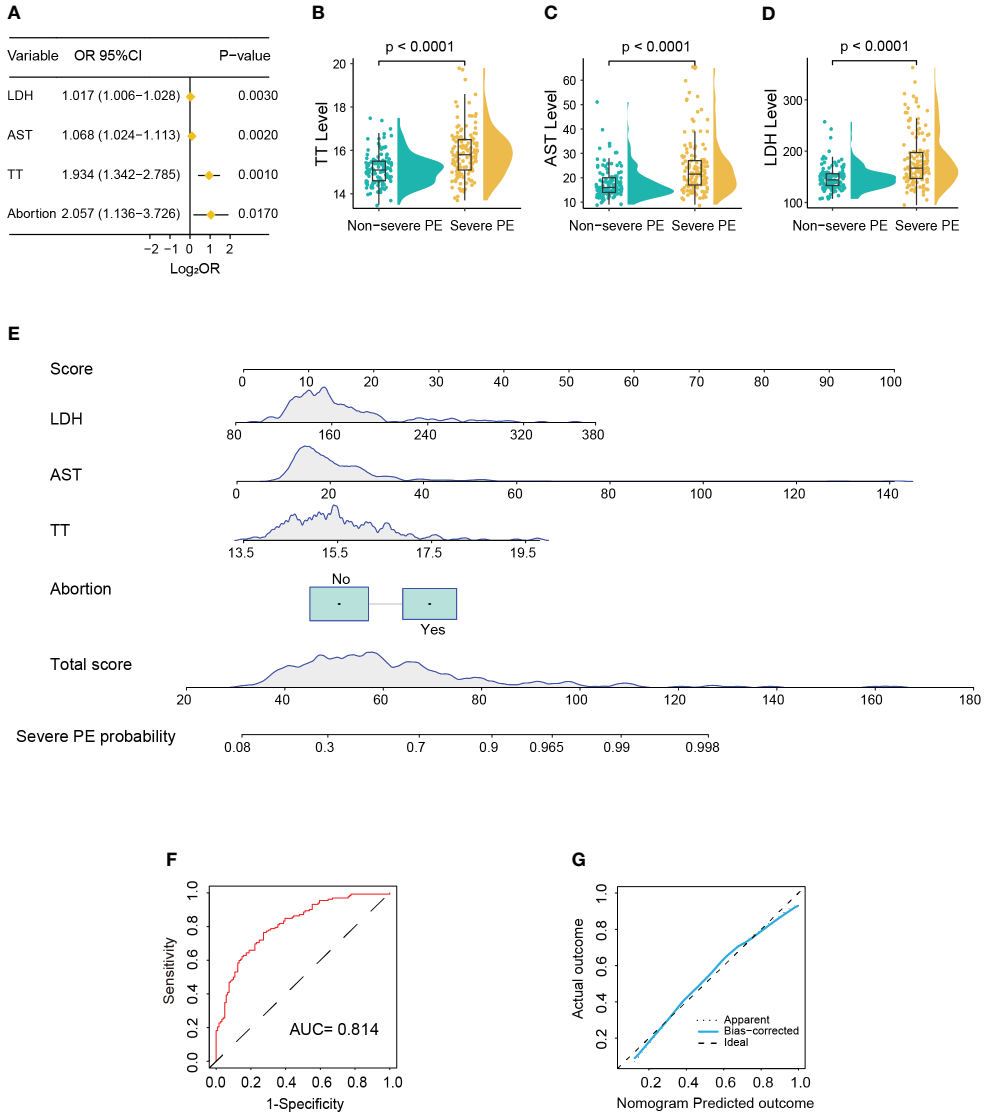

FIGURE 3
Development of a nomogram for predicting severe pre-eclampsia (PE). **(A)** Forest plot showing abortion history, prolonged thrombin time (TT), and higher aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) as significantly associated with severe PE in multivariable Cox regression analysis. **(B–D)** Violin box plots showing the distribution of the levels of TT **(B)**, AST **(C)**, and LDH **(D)** between the severe and non-severe PE groups (Wilcoxon test). **(E)** Nomogram for predicting severe PE probability in the training cohort. **(F)** Receiver operating characteristic (ROC) curve of the nomogram predicting severe PE probability in the training cohort. **(G)** Calibration curve of the nomogram predicting severe PE probability in the training cohort.

pregnancies were also found to be independent risk factors for inferior clinical outcomes of patients with PE, which is consistent with previous results.

Accumulating evidence suggested that impaired maternal metabolic function is associated with PE, which leads to inadequate adaptation to the demands of pregnancy. An altered metabolic function has been proposed to contribute to PE by causing reduced spiral artery remodeling and altered placental metabolic function (28). A previous study evaluated the role of LDH isozymes in the placenta between patients with PE and those with normal pregnancy and found that, compared to placentas from normal pregnancy, the mRNA and activity of LDH-A were increased in placentas from patients with PE, probably as a result of hypoxia (32). In this study, we found that a higher serum LDH level was related to early-onset PE, severe PE, and shorter gestation duration of patients with PE, indicating that LDH could serve as a marker for PE.

In addition, there was an increase of transaminases and hypoalbuminemia in PE patients with poor clinical outcomes, suggesting that an impaired liver function was associated with severe PE and shorter gestation duration of patients with PE. Similarly, several researchers also observed impaired liver function in patients with PE, including elevated AST and ALT and reduced ALB (33, 34). This elevation may be due to the systemic inflammatory response caused by placental ischemia, which then resulted in vasoconstriction and endothelial dysfunction and eventual liver dysfunctions.

Platelet activation occurred in the early stage of PE, which may be associated with platelet aggregation and depletion due to injury

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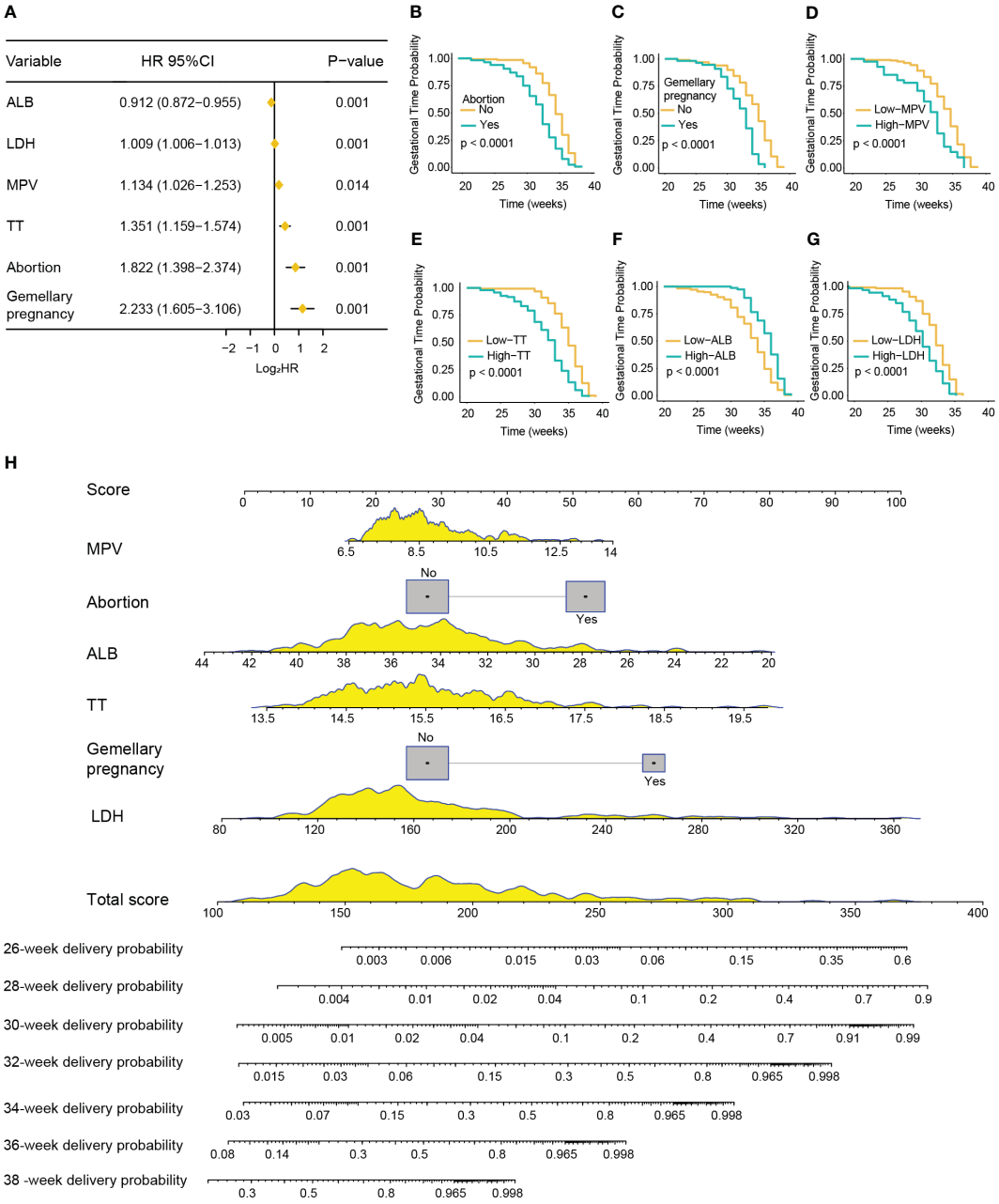

FIGURE 4
Development of a nomogram for predicting the gestation duration of patients with pre-eclampsia (PE). **(A)** Forest plot showing abortion history, gemellary pregnancy, prolonged thrombin time (TT), higher mean platelet volume (MPV) and lactate dehydrogenase (LDH), and lower albumin (ALB) as significantly associated with severe PE in multivariable Cox regression analysis. **(B–G)** Kaplan–Meier curves of gestation duration according to abortion history **(B)**, gemellary pregnancy **(C)**, MPV **(D)**, TT **(E)**, ALB **(F)**, and LDH **(G)** (log-rank test). **(H)** Nomogram for predicting the delivery probability of patients with PE in the training cohort.

to the vascular endothelium in patients with PE (35). In the present study, we found that a higher MPV was related to the shorter gestation duration of patients with PE. High levels of MPV represented a high platelet consumption status, and this aggregation and depletion would result in increased blood viscosity and the potential for microthrombosis, which could also lead to placental ischemia and hypoxia and further affect both maternal organ function and fetus growth. Moreover, we found that a prolonged TT was associated with the shorter gestation duration of patients with PE, suggesting that the coagulation function disorder could affect the severity of PE. Thrombin time refers to the time it takes for thrombin to convert fibrinogen into fibrin; the prolonged TT reflected the insufficiency of plasma fibrinogen or abnormal structure, or excessive anticoagulant substances in the body. A previous study identified that the levels of fibrinogen were significantly lower in placentas from women with early-onset PE compared with control placentas, indicating that a low fibrinogen level might be involved in the coagulation disorder in PE (36).

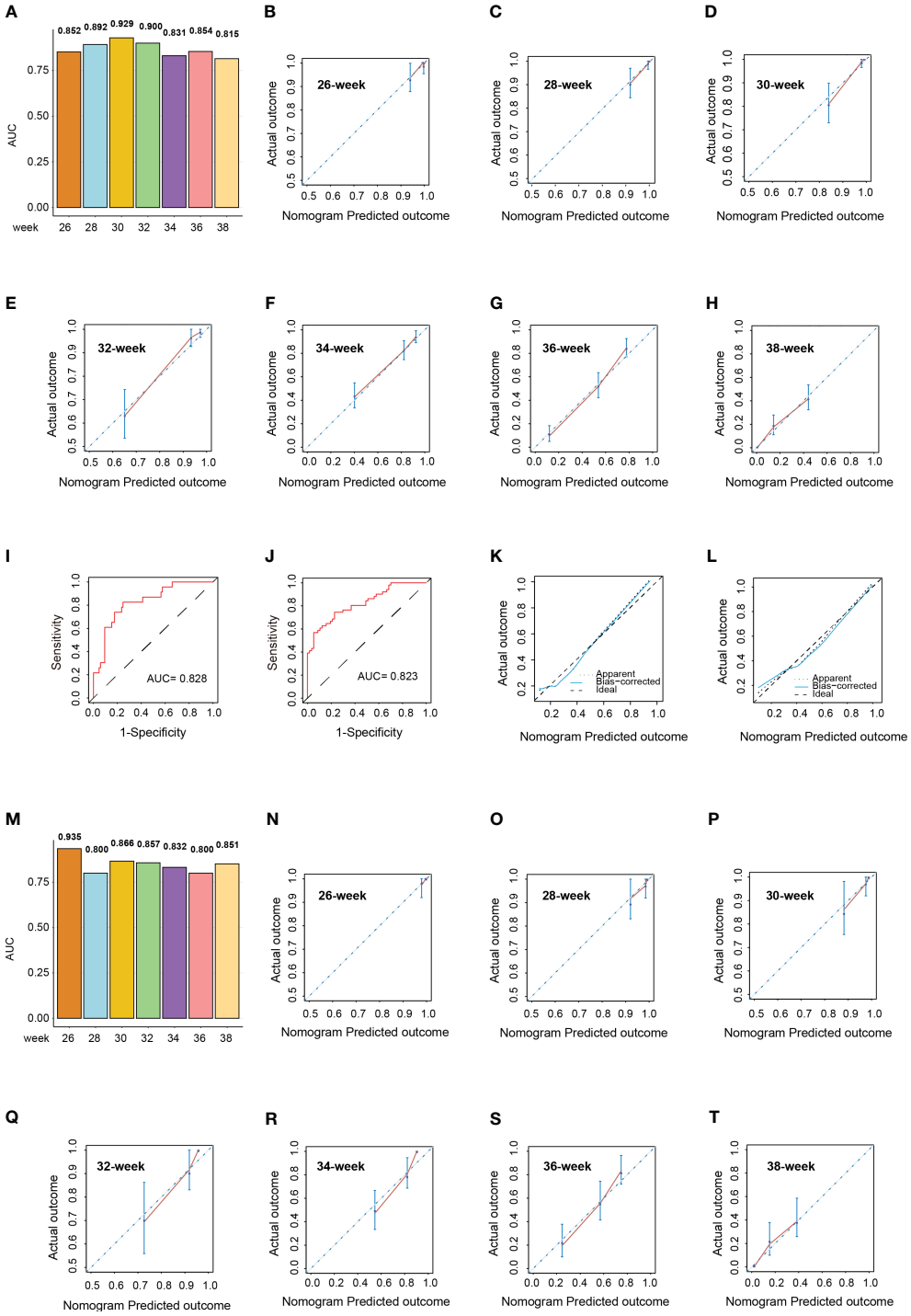

FIGURE 5
Validation of the nomograms in the validation cohort. **(A)** Area under the curve (AUC) of the nomogram predicting the delivery probability of patients with pre-eclampsia (PE) at 26, 28, 30, 32, 34, 36, and 38 weeks in the training cohort. **(B–H)** Calibration curves of the nomogram predicting the delivery probability of patients with PE at 26 **(B)**, 28 **(C)**, 30 **(D)**, 32 **(E)**, 34 **(F)**, 36 **(G)**, and 38 weeks **(H)** in the training cohort. **(I, J)** Receiver operating characteristic (ROC) curves of the nomograms predicting early-onset **(I)** and severe **(J)** PE probability in the validation cohort. **(K, L)** Calibration curves of the nomograms predicting early-onset **(K)** and severe **(L)** PE probability in the validation cohort. **(M)** AUCs of the nomogram predicting the delivery probability of patients with PE at 26, 28, 30, 32, 34, 36, and 38 weeks in the validation cohort. **(N–T)** Calibration curves of the nomogram predicting the delivery probability of patients with PE at 26 **(N)**, 28 **(O)**, 30 **(P)**, 32 **(Q)**, 34 **(R)**, 36 **(S)**, and 38 weeks **(T)** in the validation cohort.

Although numerous studies have explored a series of risk factors related to PE, none have constructed nomograms to accurately predict the probabilities of early-onset PE, severe PE, and the gestation duration of patients with PE. Here, based on significant clinical features and peripheral blood laboratory indicators, we constructed three nomograms to predict the probability of early-onset PE, severe PE, and delivery at 26, 28, 30, 32, 34, 36, and 38 weeks for pregnant women with PE. The AUC

of each nomogram presented good discriminative ability, and each nomogram was validated in the validation cohort. Although there were several flaws in the calibration curves at 26–30 weeks, we believe that the main reason was the improvements in medical condition and early intervention, and thus the delivery events were relatively low. However, the results of the AUC and C-index, as well as the DCA, demonstrated that our model still had reliability for prediction. Compared with other reported biomarkers or risk scores, the model developed in this study could quantify each predictive variable and provide a specific probability of the occurrence of severe and early-onset PE and the delivery time for each individual pregnant woman. Furthermore, this model could also help clinicians assess the clinical outcomes early and design an appropriate strategy for each patient. To our knowledge, this is the first study to construct three different nomograms to predict early-onset PE, severe PE, and the gestation duration of patients with PE based on clinical characteristics and laboratory parameters.

This study has some limitations. Firstly, it is a retrospective study with a relatively small sample size. Therefore, expanding the sample size and assessing the predictive performance of the nomograms in a larger prospective study are needed. Secondly, this study did not examine the serum sFlt-1, PlGF, PP13, PAPP-A, and VCAM-1, which are considered as important markers for PE.

5 Conclusion

Based on the clinical features and peripheral blood laboratory indicators, we identified significant factors and developed models to predict early-onset PE, severe PE, and the gestation duration of pregnant women with PE, which could help clinicians assess the clinical outcomes early and design an appropriate strategy for each patient.

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 the Ethics Committee of Sir Run Run Shaw Hospital (The Approval No. 2023-0248). 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

YX: Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft. YW: Data curation, Formal analysis, Methodology, Writing – original draft. SY: Formal analysis, Methodology, Writing – original draft. JHy: Data curation, Software, Writing – original draft. LZ: Data curation, Formal analysis, Software, Writing – original draft. JX: Data curation, Formal analysis, Software, Writing – original draft. YZ: Data curation, Software, Writing – original draft. JHa: Data curation, Formal analysis, Software, Writing – original draft. YR: Conceptualization, Supervision, Writing – review & editing. SW: Conceptualization, 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1292458/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Decision curve analysis of three nomograms. **(A, J)** Decision curve analysis of nomogram for predicting early-onset PE in training cohort **(A)** and validation cohort **(J)**. **(B, K)** Decision curve analysis of nomogram for predicting severe PE in training cohort **(B)** and validation cohort **(K)**. **(C–I, L-R)** Decision curve analysis of nomogram for predicting delivery probability of PE patients at 26-, 28-, 30-, 32-, 34-, 36-, and 38-week in training cohort **(C–I)** and validation cohort **(L–R)**. The y-axis indicates the net benefit, which is the sum of the benefits (true positives) minus harm (false positives). The x-axis indicates the threshold probability. The red line represents the nomogram net benefit. The green and black lines represent the hypotheses that all or no patients occurred end point event, respectively.

SUPPLEMENTARY FIGURE 2
The time-dependent ROC curve of nomogram for predicting delivery probability of PE patients. **(A, B)** The time-dependent ROC curve of nomogram for predicting delivery probability of PE patients at 26-38 week in training cohort **(A)** and validation cohort **(B)**.

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EDITED BY
Richard Ivell,
University of Nottingham, United Kingdom

REVIEWED BY
Amber Lockridge,
National Institute of Diabetes and Digestive and Kidney Diseases (NIH), United States
Shanshan Shao,
Shandong Provincial Hospital, China

*CORRESPONDENCE
Leying Zhang
zhangly056000@126.com
Ling Yang
yangling@hebeu.edu.cn

†These authors have contributed equally to this work and share first authorship

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Maternal nutrient metabolism in the liver during pregnancy

Hongxu Fang[1†], Qingyang Li[2†], Haichao Wang[1], Ying Ren[1], Leying Zhang[1*] and Ling Yang[1*]

[1]School of Life Sciences and Food Engineering, Hebei University of Engineering, Handan, China, [2]College of Life Sciences, Hebei Normal University, Shijiazhuang, China

The liver plays pivotal roles in nutrient metabolism, and correct hepatic adaptations are required in maternal nutrient metabolism during pregnancy. In this review, hepatic nutrient metabolism, including glucose metabolism, lipid and cholesterol metabolism, and protein and amino acid metabolism, is first addressed. In addition, recent progress on maternal hepatic adaptations in nutrient metabolism during pregnancy is discussed. Finally, the factors that regulate hepatic nutrient metabolism during pregnancy are highlighted, and the factors include follicle-stimulating hormone, estrogen, progesterone, insulin-like growth factor 1, prostaglandins fibroblast growth factor 21, serotonin, growth hormone, adrenocorticotropic hormone, prolactin, thyroid stimulating hormone, melatonin, adrenal hormone, leptin, glucagon-like peptide-1, insulin glucagon and thyroid hormone. Our vision is that more attention should be paid to liver nutrient metabolism during pregnancy, which will be helpful for utilizing nutrient appropriately and efficiently, and avoiding liver diseases during pregnancy.

KEYWORDS

glucose, hormone, lipid, liver, pregnancy, protein

1 Introduction

The liver is the largest gland of the mammalian body, and has thousands of vital functions, including efficient uptake of amino acids (AAs), carbohydrates, bile acids, cholesterol, proteins, lipids and vitamins for storage and metabolism (1). During normal pregnancy, there are essential adaptations in nutrient metabolism, which increase maternal energy reserves, in the form of glucose and lipids, to meet the maternal-fetal needs for advanced gestation (2). AA metabolism is downregulated in early and mid-pregnancy, but upregulated in late pregnancy in mice (3). Mammal nutrient supply is handled primarily by the gastrointestinal tract and the liver, and as a major metabolic hub, the liver is involved in nutrient metabolism and the synthesis of essential serum components (4). Moreover, the maternal liver systematically coordinates adaptations by activating the proneuronal

transcription factor Ascl1 in the maternal hepatocytes during second half of gestation, which allows for optimal placental development and growth, and ensures the health of mother and her infant during pregnancy in mice (5). The liver controls various pathways of glucose metabolism, including glycogenesis, glycogenolysis, glycolysis and gluconeogenesis, to maintain an individual's health by regulating several key transcription factors (6).

A successful pregnancy is dependent on correct hepatic adaptations in maternal nutrition. However, there is no systematic review that focuses on maternal liver nutrient metabolism during pregnancy. In this review, the latest information about hepatic nutrient metabolism, including glucose metabolism, lipid and cholesterol metabolism, and protein and AA metabolism, as well as maternal hepatic adaptations in nutrient metabolism during pregnancy, is discussed. In addition, the factors that regulate hepatic nutrient metabolism during pregnancy are reviewed.

2 Hepatic anatomy

Despite obvious differences in hepatic lobation and gallbladder between rodents and humans, but the microscopic architecture of the liver is generally similar in all mammals, and has a critical feature for liver function. The liver is divided into lobes (the anatomical sections of the liver), and lobe is made up of hepatic lobules. Furthermore, liver cells are organized around the functional structural unit of the liver — the lobule (microscopic building blocks of the liver) (7). Branches of the portal vein and the hepatic artery merge upon and entry into the liver lobule at the portal field for blood supply of the liver, and exits at the central vein (8). In general, a typical hepatic lobule contains the portal vein, hepatic artery, bile duct and hepatic sinusoid (Figure 1).

3 Liver nutrient metabolism

3.1 Glucose metabolism

The liver is the major site in the body for carbohydrate biosynthesis, and plays a central role in regulating systemic glucose metabolism, and maintaining blood glucose levels within a narrow range (9). The liver regulates the balance between the uptake and storage of glucose via glycogenesis and release of glucose via glycogenolysis and gluconeogenesis to maintain blood glucose homeostasis (10). Feeding enhances insulin-mediated signaling in the liver, which shifts from a mode of net output to net uptake of hepatic glucose. This requires the activation of glycogen synthase and inhibition of glycogen phosphorylase, as well as a decline in glucagon and an increase in insulin, which lead to a decrease in hepatic glucose output from glycogen stores and gluconeogenesis in hepatocytes (6, 7).

3.2 Lipid and cholesterol metabolism

The liver is involved in the uptake, synthesis, packaging, and secretion of lipids and lipoproteins. The major sources of hepatic fatty acids (FAs) are dietary lipids, adipose tissue derived FAs and *de novo*-synthesized FAs (11). FA synthesis and lipid circulation occur through lipoprotein in the liver, and lipid droplets accumulate in hepatocytes (12). The liver can utilize FAs as an internal energy source through oxidative pathways, and provide energy to other organs from ketogenic products (7). Under condition of increased FA uptake, the liver often produces large amounts of the ketone bodies, including β-hydroxybutyrate, acetoacetate, and acetone, and these ketone bodies circulate among extrahepatic tissues and are metabolized (13).

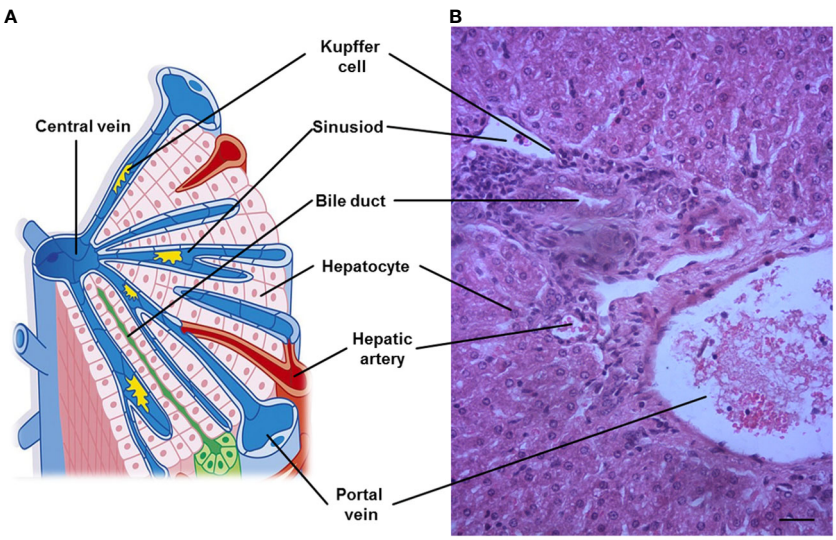

FIGURE 1
A representative hepatic lobule. **(A)** Representation of a hepatic lobule. **(B)** Hepatic tissue stained by hematoxylin and eosin. A portal triad is a component of the hepatic lobule, consists of proper hepatic artery, hepatic portal vein, small bile duct and hepatic sinusoid. Bar = 50 μm.

Dietary triglycerides are packaged into chylomicrons within the intestinal lumen, secreted into the lymphatic system and ultimately reach the plasma, and much of the chylomicron triglycerides are taken up by muscle and adipose tissue (11). The remaining triglycerides within the chylomicron remnants are taken up by receptor mediated endocytosis to the hepatocytes, and these particles are processed by lysosomes to release FAs (14). Triacylglycerols can also be exported as constituents of very low density lipoproteins (VLDL) that are synthesized and secreted by the liver, and released into the blood (12).

Dietary cholesterol is absorbed in the intestine, and incorporated into chylomicrons that are taken up by the liver through the bloodstream, and the majority of cholesterol catabolism and excretion is also the responsibility of the liver. Approximately half of this cholesterol is excreted in the feces, and the other half is reabsorbed in the large intestine and taken up by the liver (15). The liver also plays a buffering role in regulating cholesterol homeostasis of the whole body, both by controlling several cholesterol input and elimination pathways, and serving as a storage site for the cholesterol (16).

3.3 Protein and amino acid metabolism

The liver is responsible for 85-90% of circulating protein synthesis, including albumin, which is essential for the maintenance of blood volume and transporting a number of critical molecules (including lipids). In addition, the liver synthesizes many AAs, glucose, and glutathione, but is also the major organ that degrades AAs. The liver breaks down proteins and metabolizes AAs to provide energy for hepatocytes, and the carbon skeleton of specific AAs is incorporated into the tricarboxylic acid cycle (7). However, only a small amount of AAs is degraded in the liver if humans and animals take up optimal amounts of AAs (17).

4 Maternal hepatic adaptations in nutrient metabolism during pregnancy

Alterations in maternal metabolism meet the metabolic needs of the developing fetus, which is initiated very early in pregnancy. There are significant changes in hepatic nutrient metabolism in pregnant women compared with nonpregnant women, including increases in glucose metabolism, and lipid and cholesterol metabolism, protein synthesis, and a decrease AA catabolism (Figure 2).

4.1 Adaptations in hepatic glucose metabolism

The appearance rates of total glucose and total gluconeogenesis are increased, which are essential for the mother to adapt to the increasing fetal demands for glucose with advancing gestation (18). In addition, adjustments in glucose production and utilization in the maternal liver are necessary for the increased glucose requirements of the gravid uterus, which depends principally on

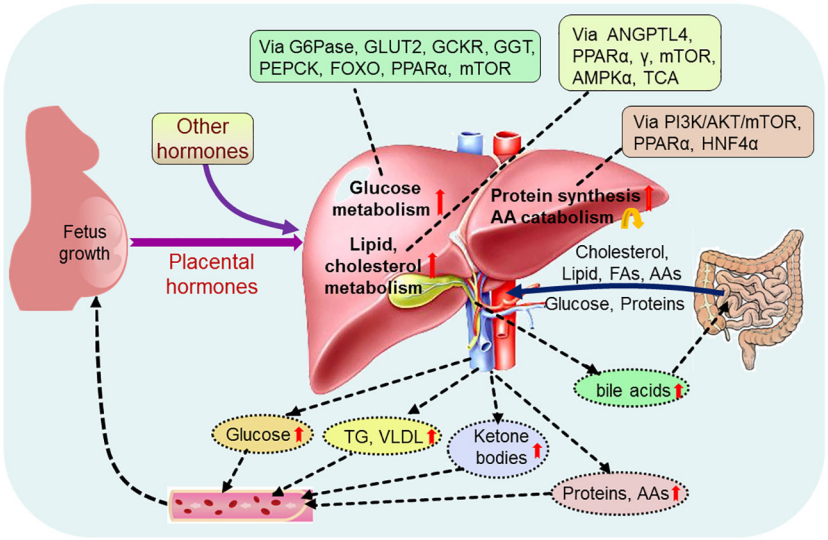

FIGURE 2
Pregnancy regulates maternal hepatic nutrient metabolism. During pregnancy, placental hormones and other hormones modulate hepatic glucose metabolism via glucose-6-phosphatase (G6Pase), glucose transporter 2 (GLUT2), glucokinase regulatory protein (GCKR), phosphoenolpyruvate carboxykinase (PEPCK), forkhead box protein O (FOXO), peroxisome proliferator-activated receptor α (PPARα) and mammalian target of rapamycin (mTOR), and regulate hepatic lipid metabolism via angiopoietin-like protein 4 (ANGPTL4), PPARα, γ, mTOR, adenosine 5'-monophosphate (AMP)-activated protein kinase α (AMPKα) and tricarboxylic acid cycle (TCA). In addition, hepatic protein metabolism, including protein synthesis and amino acid (AA) catabolism, is regulated via phosphoinositide 3-kinase (PI3K)/serine threonine kinase (AKT)/mTOR, PPARα and nuclear factor 4α (HNF4α). Glucose, cholesterol, lipid, proteins and other nutrients, including fatty acids (FAs) and amino acids (AAs), from the intestine enter into the liver, and pregnancy increases production of hepatic glucose, triglyceride (TG), very low density lipoproteins (VLDL), ketone bodies, proteins and AAs, which promote fetus growth and maternal nutrient store through the blood circulation. Furthermore, pregnancy enhances the production of hepatic bile acids, which improve lipid and glucose metabolism in the intestine.

hepatic gluconeogenesis for the glucose supply, as well as hepatic glycogen synthesis during late pregnancy in ruminants (19). Furthermore, pregnancy is characterized by progressive insulin resistance, and insulin action is lower in normal late pregnancy than nonpregnant women, which results in an increase in basal endogenous hepatic glucose production during normal pregnancy (20). Maternal glucose production is enhanced from early to late pregnancy by transiently modulating maternal hepatic insulin resistance to ensure sufficient glucose delivery to the fetus (21). Pregnancy induces insulin resistance, and improves the production of glucose through inhibition of glycogen synthesis (Figure 3).

4.2 Adaptations in hepatic lipid metabolism

The mother increases the utilization of lipids as an energy source with advancing gestation, and the liver plays a central role in lipid metabolism during pregnancy. There are significant increases in serum triglycerides and total low-density lipoprotein cholesterol (LDL-C) during pregnancy in women (22). Adipose tissue, liver and placenta secrete angiopoietin-like protein 4, which inhibit lipoprotein lipase activity and are related to placental FA transfer and fetal fat accumulation during pregnancy (23). It has been reported that peroxisome proliferator-activated receptor α (PPARα) and PPARγ regulate cellular fatty acid uptake, esterification and trafficking, as well as lipoprotein metabolism (24). Pregnancy leads to an increase in the transport of FAs from plasma and adipose tissue, as well as triglyceride and cholesterol transported to the liver (Figure 4).

4.3 Adaptations in hepatic protein metabolism

Concentrations of most plasma proteins, including clotting factors, albumin, and hormone binding proteins, are changed during pregnancy. These proteins are synthesized in the liver, which are related to the physiological alterations in hepatic metabolism during pregnancy (25). Albumin synthesis actually increases in the liver during pregnancy, but lower serum concentrations are the consequence of plasma volume expansion (26). Furthermore, in humans, the level of γ-glutamyl transferase (GGT), a liver enzyme, is elevated at 10-13 weeks of pregnancy, and GGT can increase the availability of AA for intracellular glutathione synthesis and play a crucial role in defense against oxidative stress (27).

Nuclear factor erythroid 2-related factor 2 is essential for regulating maternal hepatic adaptations to pregnancy by mammalian target of rapamycin signaling in mice, and modulates expression of genes encoding the denoted enzymes related to glycolysis, the pentose phosphate pathway, one carbon metabolism, nucleotide biosynthesis, glutaminolysis, fatty acid synthesis, and glutathione synthesis (28). Moreover, there are increases in the concentrations of many proteins produced by the liver, such as fibrinogen and other coagulation factors, including procoagulant factors, prothrombin fragments 1 + 2, tissue-plasminogen activator antigen and type 1 plasminogen activator inhibitor, but anticoagulants are reduced during pregnancy (29). Pregnancy results in insulin resistance, and increases protein synthesis from AAs, but a decrease in the production of glucose and urea from AAs (Figure 5).

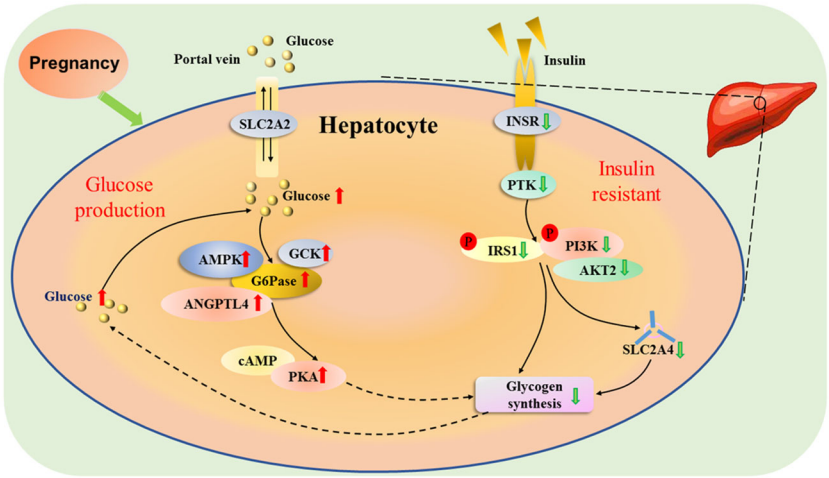

FIGURE 3
Hepatic glucose metabolic pathway during pregnancy. Pregnancy induces insulin resistance, and solute carrier family 2 member 2 (SLC2A2) mediates facilitated bidirectional glucose transport between portal vein and hepatocytes. During pregnancy, the increase in endogenous hepatic glucose production enhances the production of glucose-6-phosphatase (G6Pase), and 5'-prime-AMP-activated protein kinase (AMPK), angiopoietin like 4 (ANGPTL4) and glucokinase (GCK), which are involved in glucose homeostasis. In addition, there is an upregulation of cAMP-dependent protein kinase A (PAK), which inhibits the activity of glycogen synthesis. The glucose level increases in the liver, which reduces the activity of protein tyrosine kinase (PTK) by downregulating activity of insulin receptor (INSR). Furthermore, the phosphorylation of insulin receptor substrate 1 (IRS1) and phosphatidylinositol 3-kinase (PI3K) is inhibited, which downregulates the activity of AKT serine/threonine kinase 2 (AKT2), and reduces the ability of solute carrier family 2 member 4 (SLC2A4) transporter, resulting in a decrease in glycogen synthesis.

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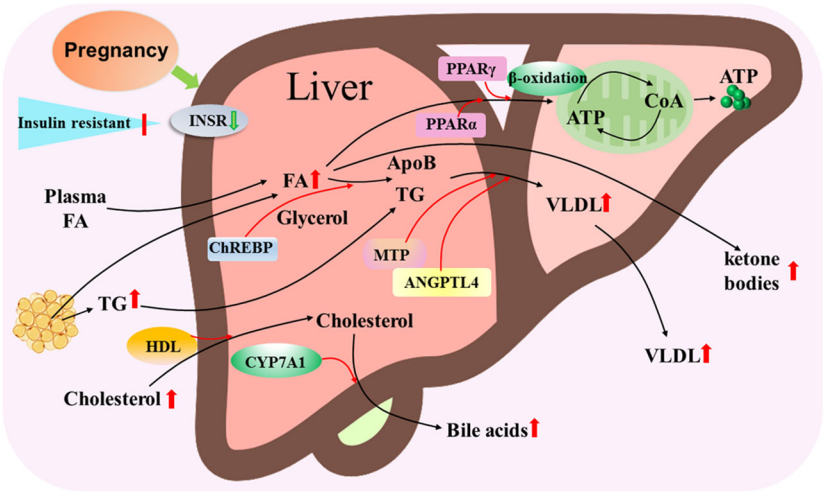

FIGURE 4
Hepatic lipid metabolic pathway during pregnancy. Pregnancy leads to insulin resistance and downregulation of insulin receptor (INSR), and increases in transport of fatty acids (FAs) from plasma and adipose tissue, triglyceride (TG) and cholesterol that are transported by high-density lipoproteins (HDL) particles to the liver. Carbohydrate-responsive element-binding protein (ChREBP) is involved in triglyceride synthesis from FAs and glycerol, and peroxisome proliferator activated receptor alpha (PPARα) and PPARγ regulate the β-oxidation of FA to release ATP through tricarboxylic acid cycle. Microsomal triglyceride transfer protein (MTP) and angiopoietin like 4 (ANGPTL4) modulate VLDL assembly by apolipoprotein B (ApoB) and TG, which result in an increase in VLDL release from the liver. In addition, cytochrome P450 family 7 subfamily A member 1 (CYP7A1) converts cholesterol to bile acids that are released from the liver to improve the digestion and absorption of lipids. Moreover, some free FAs are oxidized as ketone bodies that are released from the liver.

5 Factors that regulate maternal hepatic nutrient metabolism

Pregnancy induces maternal physiological changes by endocrine hormones and autocrine factors (30). The factors that regulate hepatic nutrition include follicle-stimulating hormone (FSH), estrogen, progesterone, growth hormone (GH)/insulin-like growth factor 1 (IGF-1), insulin, prolactin, aldosterone, adrenaline, thyroid stimulating hormone (TSH), thyroid hormone, melatonin, serotonin, glucagon and glucagon-like peptide-1 (GLP-1), leptin, prostaglandins (PGs) and fibroblast growth factor 21 (FGF21) (Figure 6).

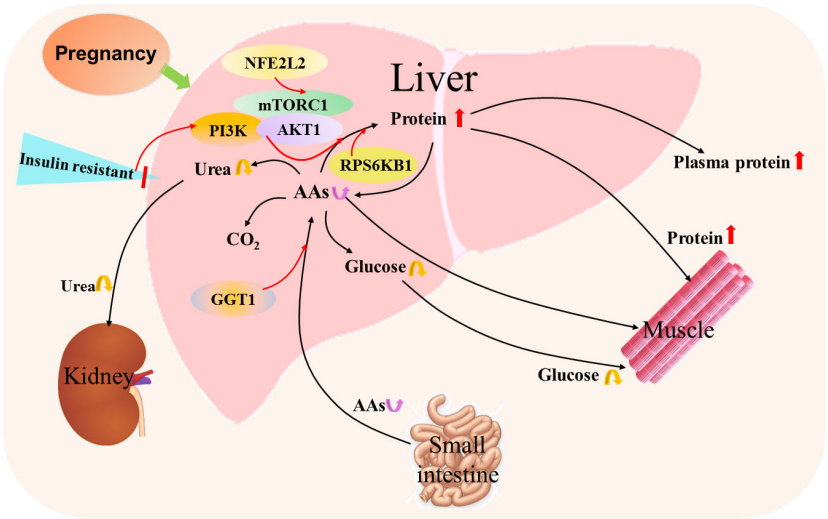

FIGURE 5
Hepatic protein and amino acid metabolic pathway during pregnancy. Pregnancy results in insulin resistance that increases protein synthesis from amino acids (AAs) by mammalian target of rapamycin (mTOR) complex 1 (mTORC1), PI3K phosphoinositide-3-kinase (PI3K) and AKT serine/threonine kinase 1 (AKT1) signaling, and ribosomal protein S6 kinase B1 (RPS6KB1) responds to mTOR signaling to promote protein synthesis. In addition, nuclear factor erythroid 2-related factor 2 (NFE2L2) is involved in the regulation of mTOR signaling. Furthermore, γ-glutamyl transferase (GGT) modulates the availability of AAs for intracellular glutathione synthesis. Moreover, the content of hepatic AAs from the small intestine through the blood circulation alters, which leads to changes in the production of glucose and urea from AAs.

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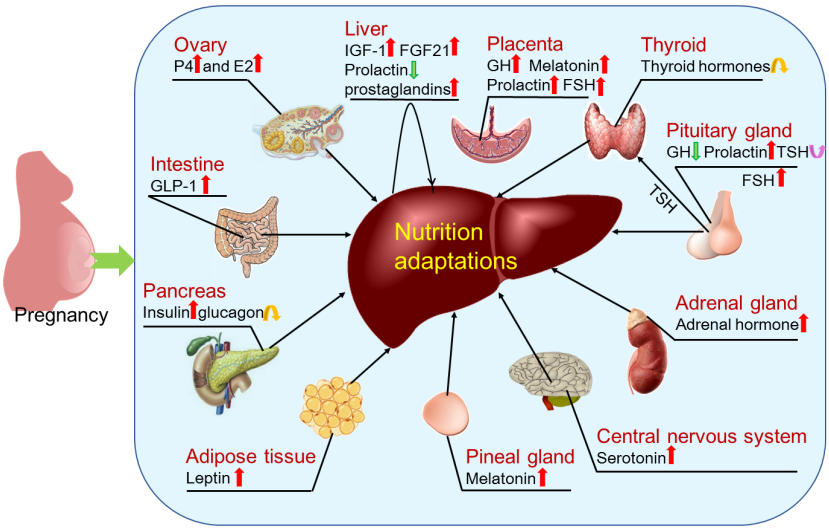

FIGURE 6
Factors that regulate hepatic adaptations in nutrient metabolism during pregnancy. During pregnancy, hepatic nutrient metabolism is modulated by factors that include estrogen (E2) and progesterone (P4) mainly produced by ovaries; insulin like growth factor 1 (IGF-1), prostaglandins and fibroblast growth factor 21 (FGF21) produced by the liver in a paracrine manner; thyroid hormones produced by thyroid; follicle-stimulating hormone (FSH), growth hormone (GH), prolactin and thyroid stimulating hormone (TSH) produced by pituitary gland; adrenal hormone mainly produced by adrenal glands; serotonin produced within the central nervous system; melatonin secreted by the pineal gland; leptin mainly synthesized in adipose tissue; insulin and glucagon secreted by pancreas; and glucagon-like peptide-1 (GLP-1) mainly released from intestinal L-cells. In addition, pregnancy regulates production of GH, melatonin, prolactin and FSH by the placenta.

5.1 FSH

FSH not only exert its effects on gonadal tissues, but FSH receptors (FSHRs) are also expressed in extragonadal tissues, including endothelium, monocytes, developing placenta, endometrium and fat (31). FSH can decrease body weight and modulate lipid metabolism, which are via downregulation of triglyceride concentration and PPARγ expression in the liver, and upregulation of PPARα protein level in liver and adipose tissue (32). FSH increases serum cholesterol level via inducing hepatic cholesterol biosynthesis, which is via binding to hepatic FSHRs, activating the Gi2α/β-arrestin-2/serine threonine kinase (AKT) pathway (33). FSH is related to the dysregulation of hepatic metabolism, and increased level of FSH has effects on the development of non-alcoholic fatty liver disease (34). In the placenta, vascular endothelial FSHR of fetal vessels mediates angiogenesis, and myometrial FSHR is related to the quieting of contractile activity required for successful implantation. However, the temporal upregulation of the FSHR at term pregnancy is necessary for the appropriate timing of parturition (35).

5.2 Estrogen

Increasing amounts of circulating estrogens suppress hepatic clearance function, which leads to increases in the levels of plasma sulfated and glucuronidated lipophilic endo- and xenobiotics. However, hepatic synthetic functions and cholesterol excretion into bile are enhanced during pregnancy (36, 37). During late pregnancy, phospholipids, cholesterol, and triglycerides increase in response to estrogen stimulation and insulin resistance, and free FAs are oxidized as ketone bodies in the maternal liver, which represent an alternative fuel source for the fetus (38). In addition, estrogen enhances liver VLDL production and decreases hepatic lipase activity during gestation in both women and experimental animals (39). Furthermore, in humans and mice, estrogen modulates liver gene expression through estrogen receptor α, β, and G-protein-coupled estrogen receptor, which is related to lipid metabolism (40).

5.3 Progesterone

There is an upregulation of serum progesterone levels during normal pregnancy, which suppresses hepatic clearance function, and increases hepatic synthetic functions (37). In addition, progesterone receptor is upregulated in the maternal liver during early pregnancy, which is involved in the regulation of maternal hepatic functions in an endocrine manner in an animal model (41). Progesterone reduces expression of phosphoenolpyruvate carboxykinase (PEPCK) to increase glucose uptake through transcription factor 7 like 2, a key regulator of glucose homeostasis, in liver cells (42). In addition, lipids and steroid hormones are closely linked, and steroid hormones regulate hepatic lipid production, and progesterone concentrations are related to cholesterol biosynthesis rates (43). During the latter half of pregnancy, progesterone improves the elaboration of ketones more promptly in the liver to meet the demands of advancing pregnancy (44), and there is also a rapid release of liver-synthesized triglycerides into the circulation (45).

5.4 Growth hormone/insulin-like growth factor 1

The pituitary gland produces GH, which modulates the production of IGF-1 via the GH receptor (GHR). GH improves body composition, fasting blood glucose levels, glucose tolerance and liver triacylglycerol levels (46), and maintains glucose metabolism through B-cell translocation gene 2 and the yin yang 1 signaling pathway in primary mouse hepatocytes and the liver (47). Hepatic GH signaling is essential for regulating intrahepatic lipid metabolism, and circulating IGF-1 can amplify the growth-promoting effects, and dampen the catabolic effects of GH (48). During pregnancy, there is an upregulation of the placental GH, which gradually replaces pituitary GH, and increases maternal IGF-1 levels (49).

IGF-1 binds to insulin receptors and stimulates insulin-like actions to regulate glucose homeostasis in the liver (50). On the other hand, IGF-1 can increase insulin resistance, and reduce triglyceride accumulation in hepatocytes (51). In addition, IGF-1 suppresses cholesterol accumulation in the liver by upregulating expression of ATP-binding cassette transporter A1 via the phosphoinositide 3-kinase (PI3K)/AKT/forkhead box protein O1 (FOXO1) signaling pathway in mice (52). IGF-1 suppresses the expression of B class scavenger receptors via PI3K/AKT pathways to participate in cholesterol metabolism in the liver (53). Moreover, IGF-1 improves VLDL assembly by upregulating mRNA abundances of apolipoprotein B (ApoB) 100, ApoE, microsomal triglyceride transfer protein, and low-density lipoprotein receptor (LDLR) in bovine hepatocytes (54).

5.5 Insulin

Insulin is produced by the pancreas, and as the master regulator of glucose, lipid, and protein metabolism, it can suppress hepatic glucose production and enhance hepatic glucose uptake, which results in inhibition of lipolysis and a decline in plasma free FA concentration (55). Insulin inhibits hepatic glucose production by directly acting on the liver and indirectly through its effects on the pancreas with a physiologic increase in insulin secretion (56). In addition, PPARγ coactivator 1 binds and coactivates FOXO1 to activate gluconeogenic gene expression and participate in insulin-regulated hepatic gluconeogenesis (57). Furthermore, TOX4, an insulin receptor-independent regulator of hepatic glucose production, and arrestin domain-containing 3 are involved in the modulation of insulin action and glucose metabolism in the liver (58, 59). Moreover, insulin directly controls lipid metabolism through hepatic insulin receptor and activation of AKT (60).

Insulin activates intracellular transport of lipid droplets into the smooth endoplasmic reticulum (ER) inside hepatocytes via phosphatidic acid and recruits kinesin-1, and catabolizes triglyceride-rich lipid droplets to supply FAs for producing lipoprotein particles (61). In addition, insulin regulates VLDL synthesis in hepatocytes and secretion from the liver, and FOXO1 acts on the liver to integrate hepatic insulin action on VLDL production (62). However, insulin resistance decreases catabolism of hepatic branched-chain AAs (63).

There is an increase in glucose-stimulated insulin secretion by maternal pancreatic β-cells during pregnancy (64). Therefore, insulin levels are elevated during pregnancy, which plays a vital role in promoting the uptake of glucose in insulin-sensitive tissues (the liver, muscle and fat) to ensure the growth and development of the fetus (65). However, for the obese women with gestational diabetes mellitus, insulin responses increase, but insulin sensitivity decreases with advancing gestation, which leads to an increase in basal glucose production (66).

5.6 Prolactin

Prolactin is primarily synthesized in the anterior pituitary gland (67), and has effects on liver gluconeogenic gene expression (68). In addition, prolactin downregulates hepatic triglyceride accumulation by downregulating stearoyl-coenzyme A desaturase 1, a rate-limiting enzyme in the biosynthesis of monounsaturated fats in female mice (69). It has been reported that early pregnancy inhibits protein expression of prolactin and prolactin receptor (PRLR) in the maternal liver of an animal model, and PRLR protein is located in the hepatocytes, endothelial cells of the proper hepatic arteries and hepatic portal veins (70). Pituitary prolactin cells increase during pregnancy, which enhance prolactin production in pituitary (71). Expression of placental lactogen increases with progression of pregnancy in the bovine placenta (72). Therefore, pregnancy regulates the expression of prolactin in the maternal liver, pituitary gland and placenta, and PRLR in the maternal liver, which has effects on hepatic triglyceride accumulation and liver gluconeogenesis.

5.7 Aldosterone and adrenaline

Adrenal glands mainly produce corticosteroid, aldosterone, cortisol and adrenaline type hormones. Aldosterone stimulates hepatic gluconeogenesis, and blunts the inhibitory effect of insulin (73). Furthermore, aldosterone improves the gene expression of hepatic gluconeogenic enzymes to affect the inhibitory effect of insulin on hepatic gluconeogenesis through the glucocorticoid receptor (GR) (74). On the other hand, epinephrine (adrenaline-type hormone) can promptly increase blood glucose concentration, which is mediated by a transient increase in hepatic glucose production through stimulating glycogenolysis and gluconeogenesis, but an inhibition of glucose disposal in insulin-dependent tissues (75).

In the liver, glucocorticoids (a type of corticosteroid) can inhibit the insulin receptor pathway and AKT activity, and induce FOXO1 activation via GR, which stimulates PEPCK and glucose-6-phosphatase (G6Pase) expression, hepatic glucose production and lipogenesis (76). On the other hand, upregulation of hepatic corticosterone concentration and nuclear GR activation are

induced by 5α-dihydrotestosterone treatment in an animal model, which stimulates triglyceride synthesis in the liver (77). Furthermore, there is an upregulation of hepatic lipid accumulation and plasma triglyceride levels during pregnancy in mice, which occurs via upregulation of hepatic CD36 through enhancing corticosterone/cortisol levels (78).

5.8 Thyroid stimulating hormone and thyroid hormone

TSH is produced by the anterior pituitary, and can stimulate thyroid hormone production by the thyroid gland. TSH stimulates expression of cAMP-regulated transcriptional coactivator 2, which leads to upregulation of hepatic gluconeogenic genes and gluconeogenesis (79). In addition, TSH promotes hepatic glucose production through upregulation of G6Pase and PEPCK, and downregulation of hepatic glucokinase in the liver (80). Furthermore, TSH enhances the expression of proprotein convertase subtilisin/kexin type 9, which leads to upregulation of LDL-C and degradation of LDLR (81).

TSH regulates lipid, glucose, and energy metabolism, and modulates hepatic bile acid homeostasis via a sterol regulatory element binding protein-1c (SREBP)-2/HNF-4α/cholesterol 7α-hydroxylase signaling pathway independent of thyroid hormones (82). Furthermore, thyroxine treatment enhances the relative rate of triacylglycerol synthesis from glycerol, and downregulates the accumulation of diacylglycerol in rat liver (83). Moreover, thyroid hormone plays essential roles in hepatic lipid synthesis and FA oxidation, which is dependent on the transcription factor carbohydrate-responsive element-binding protein in hepatocytes (84). There is a transient increase in free thyroxine levels, but a decrease in TSH concentrations during the first trimester. However, free thyroxine concentrations decrease approximately 10 to 15%, and serum TSH values return to normal after the first trimester (85).

5.9 Melatonin

The pineal gland secretes melatonin that acts directly on the liver to elevate the plasma glucose level via melatonin receptor 1B in mouse hepatocytes in a dose-dependent manner (86). Maternal melatonin and placental melatonin levels increase progressively until term during normal pregnancy (87). In addition, melatonin treatment inhibits glucose uptake and ATP production via downregulation of glucose transporter 3 and Yes-associated protein that are key regulators of Hippo signaling pathway in hepatocellular carcinoma cells (86). Furthermore, melatonin treatment improves hepatic insulin resistance and steatosis (88), attenuates lipid accumulation, and enhances the activity of AMP-activated protein kinase (AMPK) mediated by the melatonin receptor 1A signaling pathway in the liver of rats (89). Moreover, melatonin affects lipolysis by activating phosphorylation of AMPK, inactivating acetyl-CoA carboxylase, upregulating PPARα, but downregulates SREBP-1c, FA synthase, and stearoyl-CoA desaturase-1 in HepG2 cells (90). It has been reported that melatonin receptor 1A is upregulated in the maternal liver, but melatonin receptor 1B is downregulated during early pregnancy in an animal model (91), which may affect lipid and glucose metabolism during pregnancy.

5.10 Serotonin

Serotonin (5-hydroxytryptamine) produced within the central nervous system promotes energy expenditure via sympathetic drive, and is also secreted by peripheral tissues. Serotonin enters the bloodstream to promote the body for energy storage in the liver (92). In addition, serotonin functions as a hormone in central and peripheral systems to regulate systemic energy homeostasis, and participates in hepatic metabolism via its receptors (68). Furthermore, supplementation with 5-hydroxytryptophan improves expression of hepatic serotonin receptors, and glycolytic and gluconeogenic enzymes in dairy calves (93). Moreover, serotonin injection increases hepatic glycogen synthesis and concentrations, as well as hepatic cholesterol content, and stimulates the contraction of the gallbladder and excretion of bile (94). The concentration of hepatic serotonin, glucose transporters and expression of serotonin receptor are dynamic in the liver, suggesting that serotonin is essential for liver glucose homeostasis via its receptor in the liver during the transition from pregnancy to lactation in an animal model (95). There is an increase in serotonin expression in islets during pregnancy, which enhances glucose-stimulated insulin secretion, and contributes to maintaining glucose homeostasis and sensitivity in the liver (64).

5.11 Glucagon and glucagon-like peptide-1

Glucagon is a pancreas-derived hormone that exerts its function by binding to glucagon receptor (GCGR) that is mainly expressed in the hepatic periportal area (96). Glucagon enhances lipid oxidation and VLDL assembly via GCGR, and downregulates lipid synthesis in the hepatocytes, which stimulates the transportation of intracellular triglycerides, and downregulates liver fat accumulation in an animal model (97). In addition, glucagon can induce expression of gluconeogenic genes (G6Pase and PEPCK) through cAMP-response element-binding protein, and trigger a second delayed phase of FA oxidation gene expression to improve hepatic lipid homeostasis in mice (98). Glucagon-induced acetylation of cyclic AMP-responsive element binding protein, hepatocyte specific (CREBH) and SREBP-c1 suppresses hepatic lipid synthesis, and glucagon-induced acetylation of PPAR-α and FOXa2 enhances hepatic FA oxidation (99). Plasma glucagon level increases significantly between the 16th and the 28th week of gestation, but returns to normal at the last trimester of pregnancy in women (100).

GLP-1 is mainly released from intestinal L-cells in response to meal ingestion, and active GLP-1 can reach the liver through the circulation (101). GLP-1 receptor is located within the vicinity of the entrance of the hepatic portal vein, which is critically associated with portal glucose sensing (102). GLP-1 can directly stimulate insulin secretion and inhibit glucagon release, and has direct effects on

increasing the activity of glycogen synthase α, decreasing the activity of glycogen phosphorylase α to promote the incorporation of glucose into glycogen in hepatocytes independent of insulin and glucagon (103). In addition, GLP-1 analogs reduce hepatic endogenous glucose production, and *de novo* lipogenesis, but increase hepatic insulin sensitivity (104). Furthermore, GLP-1 analogs bind to their receptors to enhance hepatic insulin sensitivity, and modulate gene expression involved in FA oxidation and *de novo* lipogenesis in the liver (105). There is an increase in the production of islet-derived GLP-1 during pregnancy (106).

5.12 Leptin

Leptin is mainly synthesized in white adipose tissue, and secreted from the placenta during pregnancy, which plays an important role in the maintenance of maternal and fetal glucose metabolism (107). Leptin decreases blood glucose level, and modulates glucose and lipid metabolism in the liver (108). In addition, leptin enhances hepatic acetyl-coenzyme A carboxylase phosphorylation, FA oxidation and ketogenesis (109), and increases microsomal triglyceride transfer protein expression in hepatic cells via leptin receptors, which are involved in lipid transportation from the liver to peripheral tissues (110). Furthermore, hepatic Kupffer cells facilitate the effects of leptin on upregulation of hepatic FA oxidation and downregulation of triglycerides dependent on PI3K activity via leptin receptor in the liver (111).

Maternal obesity during pregnancy increases the risk of offspring developing obesity, and obesity is characterized by elevated levels of leptin in pregnant females (112). A state of leptin resistance in the liver during mid-pregnancy helps maintaining lipogenic metabolism, but an opposite pattern in late pregnancy favors catabolic metabolism in the liver, which is independent of progesterone and prolactin (113). Therefore, the mechanism by which leptin regulates maternal hepatic lipid metabolism requires investigation in the future.

5.13 Prostaglandins

PGD2 can regulate glucose homoeostasis and/or other specific metabolic processes inside parenchymal liver cells (114). In addition, PGs mediate intercellular communication between liver cell populations in regulating liver carbohydrate metabolism, and PGF2α, PGD2 or PGE2 treatment increases glucose output (115). Furthermore, PGs, mainly PGD2, from Kupffer and endothelial cells can influence glucose release from hepatic parenchymal cells (116), and PGE2 can attenuate fat deposition in mouse primary hepatocytes (117). In addition, cyclooxygenase-2, PGE synthase and PGF synthase are upregulated in the maternal liver during early pregnancy, which are related to maternal hepatic function adjustment during early pregnancy in an animal model (118).

5.14 Fibroblast growth factor 21

FGF21 is mainly secreted by the liver, and modulated by glucosamine to improve hepatic glucose metabolism via the nuclear factor kappa B, p38 and protein kinas pathways (119). In addition, Th2 cytokines interleukin-4 (IL-4) and IL-13 enhance expression of FGF21 to modulate energy metabolism via the IL-4/IL-13-signal transducer and activator of transcription 6 axis in the liver (120). Furthermore, adiponectin couples FGF21 actions to mediate hepatic glucose homeostasis and insulin sensitivity, which attenuates hepatic steatosis and obesity-induced impairment in mice (121). Moreover, exenatide and liraglutide induce hepatic FGF21 synthesis, which suppresses the activities of G6Pase and PEPCK induced in a paracrine manner in hepatocytes, and controls energy homeostasis in an endocrine manner (122).

Hepatic FGF21 is a critical regulator of lipid homeostasis, which is modulated by PPARα to change expression of key genes for governing lipid and ketone metabolism in the liver (123). In addition, FGF21 can directly regulate lipid metabolism and reduce lipid accumulation to reverse nonalcoholic fatty liver disease through an insulin-independent pathway, which is associated with retinoid-related orphan receptor response element, PPARγ coactivator-1α, and retinoid acid receptor-related orphan receptor α in hepatocytes (124). Furthermore, expression of FGF21 gene is increased in the liver of dairy cows during the transition from pregnancy to lactation (125), and the liver is the major source of plasma FGF21 in late pregnancy (126). Moreover, there is increased FGF21 secretion and hepatic triglyceride content during pregnancy, indicating that FGF21 is involved in balancing lipid homeostasis and meeting maternal and infant energy requirements in late pregnancy (127).

6 Conclusions

It is a worldwide problem that half of pregnant women suffer from maternal obesity and other pregnancy-associated liver diseases (128). It is reviewed in this paper that pregnancy induces insulin resistance, and improves the production of glucose, increases the transport of FAs, triglyceride and cholesterol, protein synthesis from AAs, but decreases the production of glucose and urea from AAs. In addition, some endocrine hormones and autocrine factors have effects on glucose metabolism, lipid and cholesterol metabolism, and protein metabolism. Therefore, more studies are necessary to determine food-based dietary guidelines for the pregnant women and the regulatory mechanisms of maternal hepatic nutrition adaptations depending on the pregnancy stages. Furthermore, success in these areas will bring new hopes for pregnancy precision nutrition and prevention of pregnancy-related nutrition diseases.

Author contributions

HF: Investigation, Writing – original draft. QL: Investigation, Writing – original draft. HW: Writing – review & editing, Validation, Software. YR: Writing – review & editing, Validation, Software. LZ: Writing – review & editing, Supervision, Conceptualization. LY: Writing – review & editing, Supervision, Conceptualization.

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Conflict of interest

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

Publisher's note

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EDITED BY
Enrique Podaza,
NewYork-Presbyterian, United States

REVIEWED BY
Vipin Alukkal Vidyadharan,
Baylor College of Medicine, United States

Sandeep Moothedath Subrahmanian,
The Pennsylvania State University,
United States

*CORRESPONDENCE
Jardena Jacqueline Puder
jardena.puder@chuv.ch

†These authors have contributed equally to this work and share first authorship

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Association between maternal and fetal inflammatory biomarkers and offspring weight and BMI during the first year of life in pregnancies with GDM: MySweetheart study

Maria-Christina Antoniou[1†], Dan Yedu Quansah[2†], Leah Gilbert[2,3], Amar Arhab[2], Sybille Schenk[2], Alain Lacroix[2,4], Bobby Stuijfzand[2], Antje Horsch[4,5] and Jardena Jacqueline Puder[2*] on behalf of MySweetheart Research group

[1]Unit of Pediatric Endocrinology and Diabetology, Pediatric Service, Woman-Mother-Child Department, Lausanne University Hospital, Lausanne, Switzerland, [2]Obstetric Service, Woman-Mother-Child Department, Lausanne University Hospital, Lausanne, Switzerland, [3]Nepean Clinical School, Faculty of Medicine and Health, The University of Sydney, Penrith, NSW, Australia, [4]Institute of Higher Education and Research in Healthcare (IUFRS), University of Lausanne, Lausanne, Switzerland, [5]Neonatology Service, Woman-Mother-Child Department, Lausanne University Hospital, Lausanne, Switzerland

Background: Gestational Diabetes Mellitus (GDM) is frequently associated with chronic, low-grade inflammation. Whether this environment affects offspring anthropometry during early childhood remains to be elucidated. The aim of this study was to investigate the associations between maternal and fetal (cord blood-umbilical artery) inflammatory biomarkers and offspring weight and BMI up to 1 year in pregnancies with GDM.

Methods: In this prospective secondary analysis of the MySweetheart study, we included 193 women with GDM and their offspring. Maternal and fetal (N=39) predictors included serum levels of inflammatory biomarkers including CRP, IL-6, and TNF-α at 24-32 weeks of gestational age (GA) and in the cord blood. Offspring outcomes were small and large for gestational age (SGA, LGA), sex- and age-adjusted weight, and BMI at birth and at 1 year. Univariate and multivariate regression models were performed. Associations were adjusted for maternal pre-pregnancy BMI, age, and ethnicity.

Results: Mean maternal age was 33.6 ± 4.8 years, and pre-pregnancy BMI 25.9 ± 5.6 kg/m^2. Their mean gestational age at the 1st GDM visit was 29 ± 2.4 weeks. Gestational age at delivery was 39.7 ± 1.1 weeks, with a mean birthweight of 3.4 ± 0.46 kg; 11.8% of offspring were LGA and 10.8% were SGA. At 1 year of age, mean offspring weight was 9.8 ± 1.2 kg and BMI z-score 0.23 ± 1.1 kg/m^2. In the models including only maternal predictors, TNF-α at 24-32 weeks of GA was positively associated with SGA and inversely with offspring weight and BMI at birth and at 1 year ($p \leq 0.034$). In the models including only fetal predictors and the combined model, CRP was inversely associated with BMI at 1 year ($p \leq 0.020$).

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Conclusions: In women with GDM, maternal and fetal inflammatory biomarkers distinctively influenced offspring anthropometry during the first year of life, independent of maternal age, prepregnancy BMI and ethnicity. These results suggest that low-grade inflammation during pregnancy may affect the developing offspring by leading to a decrease in weight and BMI and may have implications for future personalized follow-up of women with GDM and their offspring.

KEYWORDS

gestational diabetes, offspring anthropometry, perinatal inflammation, cord blood CRP, maternal pro-inflammatory cytokines

1 Introduction

Inflammation is associated with an increased risk of insulin resistance, hyperglycemia, metabolic syndrome, and cardiovascular disease (1–3). Increased inflammation during pregnancy caries an increased risk for short-term complications in the mother and the offspring, including miscarriage, gestational diabetes mellitus (GDM), preeclampsia, preterm birth, intrauterine growth restriction, and birth defects (4–8). Long-term complications in the offspring such as specific behavioral complications, and psychiatric disorders have also been recently evoked (9). Maternal GDM may be associated with a state of chronic, low-grade inflammation, which often precedes its diagnosis (10). The complex relationship between gestational diabetes and inflammation is underscored by evidence of increased plasma levels of pro-inflammatory cytokines, including plasma C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and/or IL-1a, in women with GDM and their fetuses (umbilical cord) in some, but not all studies (11–16).

To the best of our knowledge, the association between maternal inflammatory biomarkers during pregnancy and offspring weight or BMI during the 1st year of life in pregnancies with GDM has not been previously investigated. Most of the studies in the general population as well as a study including a large population with a wide spectrum of glucose tolerance (Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study), found an inverse association between pro-inflammatory biomarkers during pregnancy and weight and adiposity at birth (17–21). In the general pregnant population, this association was not significant (22). Beyond birth, 2nd trimester plasma CRP in healthy pregnancies has been associated with a higher childhood fat mass index (FMI) and trunk FMI in preschoolers (23). Regarding fetal predictors, existing studies in different populations (the general pregnant population, and populations with high prevalence of GDM), found no association between cord blood inflammatory biomarkers, such as CRP, IL-6 and TNF-α, and offspring weight and adiposity at birth (18, 24–26). However, the association between both maternal or fetal (cord blood) inflammatory biomarkers, including CRP, IL-6, and TNF-α has not been studied in populations with GDM (18, 21). There is also a lack of data on the impact of inflammatory biomarkers during the first year of life beyond birth in this population. Obesity and aging are associated with low-grade inflammation (27–29). Inflammatory biomarkers vary across different ethnic groups, underscoring the intricate interplay of genetic, environmental and social factors in shaping health disparities (30) A recent study in the general population found that maternal obesity-related inflammation during pregnancy increased the risk of childhood obesity in an ethnic-specific manner (31). However, it is unclear if this low-grade inflammation environment in pregnancies with GDM influences offspring anthropometry and contributes to the development of metabolic health disorders in offspring during early childhood as well as later in life.

The aims of this study were: 1) to investigate the associations between maternal and fetal inflammatory biomarkers on offspring anthropometric parameters at birth and at 1 year in a population of women with GDM; 2) to determine if the proposed associations are independent of maternal pre-pregnancy BMI, age, and ethnicity.

Abbreviations: AGA, Appropriate for Gestational Age; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; DS, standard deviation, FMI, fat mass index; GA, Gestational age; GDM, gestational diabetes mellitus; GWG, gestational weight gain; HAPO, Hyperglycemia and Adverse Pregnancy Outcome; HbA1c, glycated hemoglobin; IADPSG, International Association of Diabetes and Pregnancy Study Group; IL, interleukin; IOM, institute of medicine; LGA, Large for gestational age; N, Number; oGTT, oral glucose tolerance test; OR, odds ratio; SGA, Small for gestational age; TNF-α, tumor necrosis factor alpha.

2 Materials and methods

2.1 Study design and informed consent

The present study is a secondary data analysis of the *MySweetheart trial*, a randomized-controlled intervention trial of 211 women ≥18 years with GDM and their offspring (Clinicaltrials.gov NCT02890693)

(32), followed during pregnancy until one year postpartum in the Diabetes and Pregnancy Unit in the Lausanne University Hospital, Switzerland. GDM was diagnosed between 24 and 32 weeks of gestational age (GA), according to the International Association of Diabetes and Pregnancy Study Group (IADPSG Criteria) (33). Details of the study protocol have been previously described (32). Women on strict bed rest, with a severe mental health disorder, pre-existing diabetes, and women who did not understand English or French were excluded from the study. Signed informed consent was obtained from all participating women. The study was conducted in accordance with the guidelines of the declaration of Helsinki and good clinical practice. The Human Research Ethics Committee of the Canton de Vaud approved the study protocol (study number 2016-00745). Included women were randomized to the usual care or intervention group after the baseline visit and signing of an informed consent.

2.2 Allocation groups

2.2.1 Usual care group

Women randomized to usual care received treatment based on the American Diabetes Association and on the Endocrine Society guidelines for the management of GDM (34, 35). They had regular appointments every 1-3 weeks with a physician or a diabetes-specialist nurse and a dietician after the GDM diagnosis, and were encouraged to increase physical activity (34). During the 1st visit at 24-32 weeks of GA, they were taught how to perform self-monitoring of blood glucose (4 times during the day-fasting and 2 hours post-prandial). If glucose values remained above targets two or more times during a 1 to 2-week period (fasting glucose >5.3 mmol/l, 1-h postprandial glucose >8 mmol/l and/or 2-h postprandial glucose >7 mmol/l) despite lifestyle changes, insulin treatment or very rarely metformin was introduced depending on patient's glucose values and preferences. After delivery, glucose controls and glucose-lowering treatments were stopped. Women saw a physician and a dietician at the 6-8 week postpartum visit after an oGTT test to discuss further management.

2.2.2 Intervention group

Women randomized to the intervention group received a multidimensional, interdisciplinary lifestyle and psychosocial intervention on top of usual care, centered on eating behavior, a balanced food intake, as well as physical activity and breastfeeding. The intervention also included a psychosocial component, including the assessment and treatment of depression during and after pregnancy. During pregnancy and up to 1 year postpartum, patients were supported by a lifestyle coach [see (32] for more details).

2.3 Follow-up

For the current analysis, we used maternal data from their 1st GDM visit at 24-32 weeks of GA, and offspring data at birth, and at 1 year. At the 1st GDM visit, information on maternal socio-demographic characteristics were collected and maternal anthropometric parameters and inflammatory biomarkers were measured in the serum. Immediately after birth, blood was drawn from the umbilical cord to measure inflammatory biomarkers. Newborn anthropometric parameters were obtained from the hospital birth record. At 1 year postpartum, the offspring's anthropometric measures including weight and length were collected.

2.4 Maternal, fetal and offspring parameters

2.4.1 Maternal descriptive and confounder variables

Maternal socio-demographic parameters, including age, ethnicity, and parity were collected during the 1st GDM visit. Ethnicity was classified into Low (Europe, North America) and High Metabolic Risk (Asia, Central and South America, Africa, Oceania) ethnic groups (35). Pre-pregnancy BMI was calculated using weight information from medical charts and height measured during the 1st visit at the GDM clinic. In rare circumstances when pre-pregnancy weight was not mentioned in the chart, it was self-reported during the 1st GDM visit. Height was measured at the 1st GDM visit to the nearest 0.1 cm with a regularly calibrated Seca® height scale. GWG was determined as the difference between the weight at the end of pregnancy and pre-pregnancy weight. Glucose-lowering maternal medical treatment for GDM was classified into two categories (no treatment, treatment with insulin and/or very rarely metformin). HbA1c using a chemical photometric method (conjugation with boronate; Afinion®).

2.4.2 Maternal and fetal (cord blood) inflammatory predictor variables

At the 24-32 weeks of GA visit, maternal inflammatory parameters, including CRP, IL-6, and TNF-α were measured in maternal serum. At birth, CRP, IL-6, and TNF-α were again measured in the cord blood (umbilical artery) (36). CRP was analyzed at the Lausanne University Hospital in serum aliquots using a latex-enhanced immunoturbidimetric assay on a Cobas 8000 autoanalyzer (Roche Diagnostics, Mannheim, Germany) with assay characteristics as reported by the manufacturer. We also measured IL-6 (U-PLEX Human IL-6 Antibody Set) and TNF-α (U-PLEX Human TNF-α Antibody Set) using ELISA according to the manufacturer's instructions.

2.4.3 Offspring descriptive and outcome variables

At birth, weight (g) and length (cm) were documented; percentiles and z-scores for these parameters were calculated using the Intergrowth 21st newborn size application tool (37). BMI was also calculated. LGA was defined as birth weight >90th percentile and SGA as birth weight <10th percentile for sex and gestational age. Gestational age was calculated according to the date of the last menstruation, or as assessed by the fetal ultrasound in the cases where gestational age was adapted during the early in-utero ultrasound evaluation. Neonatal antropometric parameters were

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obtained from patient medical charts. If the birth took place in another hospital or clinic, they were provided by the respective hospital.

At the 1 year visit, offspring weight (kg) and length (cm) were measured, using standardized methods (38). BMI was calculated. Z-scores for weight, length and BMI were calculated using the WHO Anthro Survey Analyser tool -Offline version (39).

2.4.4 Predictors and outcomes

Predictors comprised maternal (1st GDM visit) and fetal (cord blood), including CRP, IL-6, and TNF-α. Outcomes included offspring anthropometric parameters at birth and 1 year. More precisely, birth outcomes included weight, BMI, LGA, and SGA, and outcomes at 1 year, weight, and BMI.

2.5 Statistical analysis

Data analysis was performed using Stata/SE 16.0 (StataCorp LLC, TX, USA). The normality of continuous variables were assessed using histograms and Q-Q plots. Outcomes variables were normally distributed. Continuous variables were described as means and standard deviations and binary outcomes as N (percentages) (Table 1). Comparisons between the intervention and control group were conducted using the unpaired t-test for normally distributed continuous variables, the Mann-Whitney test for continuous variables with non-normal distribution and the Fisher's exact test for binary variables. In all analyses, predictors and outcomes did not differ in the respective allocation groups (intervention vs usual care) and the effect sizes were similar. Thus, women from both groups were pooled together and we adjusted for group allocation in all analyses. Where appropriate, all analyses were also adjusted for infant age and sex.

We performed a Spearman's rank correlation coefficient test to investigate the correlation between maternal and fetal cord inflammatory parameters and the presence of collinearity (Supplementary Table 1). No collinearity was found between predictors (r_s < 0.6). We then conducted univariate linear and logistic regression analyses using offspring outcomes as the dependent variables (Supplementary Tables 2, 3). Maternal and fetal predictors with a p-value < 0.05 in univariate analysis were included in stepwise multiple regression analyses models. We performed different multivariate models. In terms of predictors, three multivariate models were used, a first model including only maternal predictors, a second model including only fetal predictors and a third model including both maternal and fetal predictors. Fetal predictors were available for N = 39 participants. In terms of adjustments, the above models were adjusted for maternal age, pre-pregnancy BMI, and ethnicity in addition to the already mentioned adjustments (group allocation, offspring age and sex). The analyses and adjustments were performed in order to identify the most significant maternal and fetal predictors of infant anthropometric parameters at birth and 1 year, and to determine the extent of the impact of these predictors independent of maternal confounder variables, i.e., ethnicity, age, and obesity; known to be associated with low-grade inflammation (27–29) (Table 2). For all analyses, β-coefficients (for continuous outcomes) and adjusted odds ratios (aORs-for binary outcomes) are reported along with their 95% confidence intervals (CIs), and statistical significance was set at p<0.05.

TABLE 1 Maternal and offspring characteristics.

Maternal Characteristics		Infant characteristics	
Number of patients (N)	193	**Birth anthropometric parameters**	
Age (years)	33.6 ± 4.8	Number of patients	190 (Male:52%)
High risk ethnicity (yes; N(%)	39 (22.7%)	Gestational age (weeks)	39.7 ± 1.1
Pre-pregnancy BMI (kg/m^2)	25.9 ± 5.6	Weight (kg)	3.4 ± 0.46
Gestational weight gain (kg)	12.6 ± 6.5	Weight z-score (SD) [1]	0.18 ± 1.1
Glucose lowering medical treatment	85 (46.5%)	Length (cm)	49.6 ± 2.4
Gestational age at the at the 1st GDM visit (weeks)	29 ± 2.4	Length z-score (SD) [1]	0.10 ± 1.4
HbA1c at the 1st GDM visit (%)	5.1 ± 0.31	BMI (kg/m^2)	13.7 ± 1.7
(mmol/mol)	32.2 ± 2.0	LGA [1,2]	22 (11.8%)
Maternal inflammatory parameters		SGA [1,3]	20 (10.8%)
CRP at the 1st GDM visit (mg/L)	4.5 ± 3.8	**1 year anthropometric parameters**	
IL-6 at the 1st GDM visit (pg/ml)	1.0 ± 1.3	Number of patients	170 (Male:52%)
TNF-α at the 1st GDM visit (pg/ml)	0.74 ± 0.76	Age (months)	12.4 ± 1.0
Fetal parameters		Weight (kg)	9.8 ± 1.2
Number of patients	39	Weight z-score (SD) [4]	0.32 ± 0.91
Cord blood CRP (mg/L)	0.29 ± 0.51	Length z-score (SD) [4]	0.27 ± 1.2
Cord blood IL-6 (pg/ml)	7.21 ± 7.55	BMI (kg/m^2)	16.9 ± 1.6
Cord blood TNF-α (pg/ml)	1.5 ± 0.46	BMI z-score (SD) [4]	0.23 ± 1.1

BMI, body mass index; CRP, C-reactive protein; GDM, gestational diabetes mellitus; HbA1c, glycated hemoglobin; IL-6, interleukin 6; LGA, large for gestational age; SD, standard deviation; SGA, small for gestational age; TNF-α, tumor necrosis factor alpha.
[1]according to the Intergrowth 21st newborn size application tool (37).
[2]LGA: birth weight >90th percentile for sex and gestational age using the Intergrowth 21st newborn size application tool (37).
[3]SGA: birth weight <10th percentile for sex and gestational age using the Intergrowth newborn size application tool (37).
[4]according to the WHO Anthro Survey Analyser tool (39).

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3 Results

The initial population included 211 women with GDM. As previously described (38), one woman was excluded as the diagnosis of GDM was done too early (< 13 weeks of gestation), and 17 were excluded due to multiple gestation, and/or because their offspring were premature (gestational age (GA) < 37 weeks. Thus, 193 women and their offspring were included in the analyses.

3.1 Maternal, fetal and infant characteristics

Table 1 describes the maternal characteristics, fetal inflammatory parameters, and offspring anthropometry at birth and 1 year. In summary, mean maternal age was 33.6 ± 4.8 years, and pre-pregnancy BMI was 25.9 ± 5.6 kg/m^2. Their mean gestational age at the 1st GDM visit at 24 to 32 weeks of GA was 29 ± 2.4 weeks. Gestational age at delivery was 39.7 ± 1.1 weeks, with a mean birthweight of 3.4 ± 0.46 kg; 11.8% of offspring were LGA and 10.8% were SGA. At 1 year of age, mean offspring weight was 9.8 ± 1.2 kg and BMI z-score 0.23 ± 1.1 kg/m^2. Maternal and fetal (cord blood) inflammatory biomarkers, including CRP, IL-6, and TNF-α were not significantly correlated (Supplementary Table 1).

3.2 Associations between maternal and fetal predictors and offspring anthropometry at birth and 1 year in univariate analyses

3.2.1 Birth

TNF-α at the 1st GDM visit was inversely associated with offspring weight [β-coefficient= -0.107 (CI: -0.189; -0.026), p=0.010] and BMI at birth [β-coefficient= -0.501 (CI: -0.816; -0.186), p=0.002] and positively with SGA [OR= 0.492 (CI: 0.056; 0.927), p=0.027], and cord blood CRP was inversely associated with offspring weight [β-coefficient= -0.370 (CI: -0.659; -0.081), p=0.015] and BMI at birth [β-coefficient= -1.052 (CI: -1.900; -0.205), p=0.017]. No association was found between maternal and fetal inflammatory biomarkers and LGA (all p ≥0.199, Supplementary Table 2).

3.2.2 One year

TNF-α at the 1st GDM visit was inversely associated with offspring weight [β-coefficient= -0.376 (CI: -0.625; -0.128), p=0.003] and BMI at 1 year pβ-coefficient= -0.641 (CI: -1.003; -0.279), p=0.001], and cord blood CRP and TNF-α were inversely associated with offspring BMI at 1 year [β-coefficient= -2.566 (CI: -3.666; -1.465), p=0.000] and [β-coefficient= -2.177 (CI: -3.671; -0.684), p=0.006], respectively, Supplementary Table 3).

3.3 Associations between maternal and fetal predictors and offspring anthropometry at birth and 1 year in multivariate analyses

The significant results of all multivariate analyses are shown in Table 2.

3.3.1 Birth

In the models including only maternal predictors, TNF-α at the 1st GDM visit was inversely associated with offspring weight [β-coefficient= -0.090 (CI: -0.170; -0.010), p=0.028] and BMI [β-coefficient= -0.455 (CI: -0.773; -0.137), p=0.005], and positively

TABLE 2 Maternal serum and fetal cord blood predictors of offspring anthropometric outcomes in multivariate regression analyses.

Offspring outcomes	Predictors	OR[1]/ β-coefficient	95% CI		p-value
Birth					
	Maternal				
Weight (kg)	TNF-α at the 1st GDM visit (pg/ml) [2]	-0.090	-0.170	-0.010	0.028
BMI (kg/m2)	TNF-α at the 1st GDM visit (pg/ml) [2]	-0.455	-0.773	-0.137	0.005
SGA [3]	TNF-α at the 1st GDM visit (pg/ml) [2]	1.609 [1]	1.036	2.500	0.034
1 year					
	Maternal & Fetal				
BMI (kg/m2)	Cord blood CRP (mg/L)	-2.838	-4.029	-1.646	0.001
	Maternal				
Weight (kg)	TNF-α at the 1st GDM visit (pg/ml) [2]	-0.411	-0.659	-0.163	0.001
BMI (kg/m2)	TNF-α at the 1st GDM visit (pg/ml) [2]	-0.702	-1.069	-0.335	0.000
	Fetal				
BMI (kg/m2)	Cord blood CRP (mg/L)	-2.838	-4.029	-1.646	0.001

BMI, body mass index; CI, Confidence Interval; CRP, C-reactive protein; GDM, gestational diabetes mellitus; HbA1c, glycated hemoglobin; IL-6, interleukin 6; LGA, large for gestational age; OR, Odds Ratio; SD, standard deviation; SGA, small for gestational age; TNF-α, tumor necrosis factor alpha.
[1]this value corresponds to an OR.
[2]the mean gestational age at the 1st GDM visit was 29 ± 2.4 weeks.
[3]SGA: birth weight < 10th percentile for sex and gestational age using the Intergrowth 21st newborn size application tool (37).
Stepwise multiple logistic regression analyses. All analyses were adjusted for maternal age, pre-pregnancy BMI, and ethnicity (high/low risk), allocation group (intervention/usual care), and infant sex and age (where appropriate). Outcomes are only shown if at least one predictor is found. Only significant results are displayed (defined significance, p-value <0.05, see text). Three distinct sub-models were performed (combined model, including maternal and fetal predictors, model including only maternal or only fetal predictors), and results are displayed separately.

with SGA [OR= 1.609 (CI: 1.036; 2.500), p=0.034]. No significant associations were found between maternal and fetal inflammatory biomarkers and offspring anthropometry in models including only fetal predictors or in the combined model ($p \geq 0.05$).

3.3.2 One year

In models including maternal predictors, TNF-α at the 1st GDM visit was inversely associated with offspring weight [β-coefficient= -0.411 (CI: -0.659; -0.163), p=0.001] and BMI at 1 year [β-coefficient= -0.702 (CI: -1.069; -0.335), p=0.000]. In models including fetal predictors as well as in the combined model, cord blood CRP showed an inverse association with BMI at 1 year [β-coefficient= -2.838 (CI: -4.029; -1.646), p=0.001].

4 Discussion

This prospective, secondary analysis of the MySweethheart study, of women with GDM and their offspring showed that maternal and fetal inflammatory biomarkers predicted offspring weight and BMI during the 1st year of life, independent of maternal age, pre-pregnancy BMI, and ethnicity. In the adjusted analyses, maternal TNF-α at 24-32 weeks GA was positively associated with SGA and inversely with offspring weight and BMI at birth and at 1 year, whereas. CRP was inversely associated with offspring BMI at 1 year independent of maternal predictors. Thus, while maternal cytokines predicted lower weight and BMI, both at birth and up to 1 year, the impact of fetal CRP was observed at 1 year.

4.1 Impact of maternal and fetal inflammatory parameters on offspring anthropometry at birth

In our study, maternal serum concentrations of TNF-α at the 1st GDM visit at 24-32 weeks of GA were negatively associated with offspring weight and BMI at birth and positively with SGA. This is consistent with a recent study in a healthy population that found a negative correlation between inflammatory biomarkers such as CRP, and IL-6, and birthweight (17). Other studies have evaluated the impact of maternal inflammatory parameters and offspring birth outcomes in healthy pregnant women, as well as in populations with some degree of glucose intolerance (19, 20, 22, 40, 41). Thus, maternal CRP levels in early pregnancy were associated with higher rates of SGA in the general population (40), and those during the 3rd trimester were inversely associated with offspring weight and sum of skinfolds at birth in the general population and in a subpopulation of the HAPO Study (19, 20). No correlations between maternal 3rd trimester TNF-α, and IL-6 values and fetal adiposity or birthweight were found in a healthy pregnant population of women without GDM (22), whereas maternal 2nd and 3rd trimester IL-6 values were inversely associated with weight and sum of skinfolds at birth in a study including women with GDM and without GDM (41). A study performed in women with a previous history of having a macrosomic infant, but without GDM, found no association between maternal IL-6 and TNF-α and birthweight and adiposity measures in the total or male cohort; however, an inverse association was found between maternal 3rd trimester IL-6 levels and the sum of skinfolds at birth in the female cohort (18). Thus, most of the previous studies have shown an inverse association between maternal pro-inflammatory markers, including CRP, IL-6 and/or TNF-α, and offspring anthropometry at birth. For the first time, our data extends this now to a metabolically high-risk population of women with GDM. Evidence indicates a U-shaped curve relating the size at birth with long-term cardiometabolic disease, highlighting the significance of optimal fetal growth for long-term health outcomes (42, 43). The impact of maternal pro-inflammatory factors on fetal growth may be mediated by their direct and indirect effects on the placental development, function and immunomodulatory activity, as well as on the hormone synthesis and action in the materno-fetal unit (41, 44, 45). The absence of association between IL-6 levels and BMI or weight in our study may be possibly explained by the fact that IL-6 correlates more closely with visceral adiposity (46), which was not specifically evaluated.

We found no associations between cord blood inflammatory biomarkers and offspring anthropometry at birth. Similarly, studies in the general population as well as in populations with high prevalence of GDM, and/or pre-existing diabetes, found no association between cord blood IL-6, CRP or increased CRP [CRP >0.3 mg/l (2.9 nmol/l)] levels and one or more anthropometric/adiposity measures at birth (including weight, weight z-score, LGA, fat mass, % body fat, sum of skinfold thickness) (21, 24–26).During the process of birth, there is a physiological increase in the cord blood pro-inflammatory marker concentration and this increase may be further influenced by other parameters, including the duration of labor and the mode of delivery (47, 48).Therefore, cord blood inflammatory biomarkers may be a complex reflexion of both the prenatal and the perinatal fetal inflammatory milieu, explaining the correlation between inflammatory biomarkers in the cord blood and offspring anthropometry later in life (at 1 year) but not at birth. Yeung et al, found an association between cord blood CRP levels and DNA methylation in the cord blood, particularly in gene regions associated with angiogenic and inflammatory pathways, which in turn could have an impact on future cardio-metabolic risk (49). Recent research suggests that methylation in specific inflammation-related genes in the cord blood is associated with adiposity measures later in the development, such as in early childhood (50). Additional investigation is warranted to elucidate the complex mechanisms that underlie the impact of pro-inflammatory cytokines in the cord blood on offspring body composition and metabolic profile during childhood.

4.2 Impact of maternal and fetal metabolism on infant weight and BMI during childhood

In our population, maternal serum concentrations of TNF-α during the 3rd trimester were inversely associated with offspring weight and BMI at 1 year. Our results are in agreement with a study in a population of women with a relatively low GDM prevalence

(4.5%), where higher 2nd trimester CRP levels were associated with a higher childhood fat mass index (FMI), and trunk FMI, but this later in the development, i.e. in early (3-5 years) and mid childhood (7-10 years) (23). Our results are, however, contrary to a study performed in the general population where 1st trimester IL-6 and TNF-α were not associated with offspring adiposity measures, including weight, BMI z-scores at 2-6 years (31). In another study, 1st trimester IL-6 and TNF-α were not associated with weight and adiposity measures at 6 months of age in the total and male cohort, but only with adiposity measures in the female cohort (18). In a large, Danish general population, CRP, TNF-α, IL-6, and IL-1b measures in the 3rd trimester was not associated with offspring BMI, waist circumference, blood pressure, glucose metabolism measures, or lipid profile at the age of 20 years (51).

Data on the association between fetal (cord blood) inflammatory parameters and offspring growth during the first years of life are scarce. The above mentioned study of women with a history of macrosomia in the absence of GDM, found no association between cord blood IL-6, and TNF-α and offspring weight or adiposity measures at 6 months in their cohort (18). Regarding fetal predictors, in a cohort of children born extremely premature (< 28 weeks gestational age), showed that elevated IL-6 on day 1 in the newborn was associated with an increased risk for obesity at 2 years in multivariate models (52).

4.3 Speculations on the mechanisms of impact of maternal and fetal metabolism on offspring anthropometry during the first years of life in women with GDM

In pregnancies with GDM, the impact on offspring anthropometry is multifaceted, involving intricate mechanisms that span maternal, placental, and fetal domains (38, 53, 54). These include various factors such as maternal insulin resistance, elevated cytokines, and subsequent effects on placental function (41, 44). Inflammation and hyperglycemia lead to disruptions in insulin-like growth factors and adiponectin influencing fetal growth patterns and adipose tissue development (55, 56). Exposure to GDM may induce epigenetic modifications, affecting gene expression linked to fetal development and could be a mechanism leading to metabolic dysfunction later in life (57). Additionally, maternal obesity and excessive gestational weight gain, prevalent in GDM, independently contribute to adverse offspring outcomes (58, 59). This collective interplay underscores the complexity of factors influencing offspring anthropometry in the context of GDM, emphasizing the need for a comprehensive understanding of both inflammatory and metabolic pathways in order to identify potential therapeutic targets.

4.4 Strengths and limitations

To our knowledge, this is the first study assessing the impact of maternal and fetal inflammatory biomarkers on offspring anthropometric parameters at birth and up to 1 year of life in a metabolically high-risk population of women with GDM and their offspring. Another strength of our study is the prospective design. Nevertheless, some limitations ought to be mentioned. Maternal inflammatory biomarkers were measured only once during gestation. Moreover, offspring predictors did not include height and head circumference as we opted to focus on metabolic health-related anthropometric parameters. As the focus of this study was to investigate the relationship between biomarkers and offspring metabolic health-related offspring in a metabolically high-risk population, we lack a proper control group of healthy participants. Cord blood (umbilical artery) parameters were only available for 39 patients; which could influence our results, especially the correlations. Additionally, due to the small sample size of cord blood parameters, separate analyses for the intervention and usual care group, as well as infant sex could not be performed. However, maternal and fetal predictors and infant anthropometry did not differ between both groups and we always adjusted for group allocation.

4.5 Conclusions

Maternal and fetal inflammatory biomarkers including TNF-α at 24-32 weeks of GA and cord blood CRP distinctively influenced offspring weight and BMI during the first year of life, and this independent of maternal age, ethnicity, and pre-pregnancy BMI. The impact of fetal inflammatory biomarkers was not apparent at birth but was observed at 1 year. Further research is warranted to elucidate how the inflammatory milieu before and during birth might impact the developing offspring, and may in turn have implications for designing intervention strategies based on maternal and cord blood inflammatory biomarkers in order to decrease the risk of offspring metabolic dysfunction in the medium and long-term.

MySweetHeart Research Group

AA, Pascal Bovet, Arnaud Chiolero, Stefano Di Bernardo, Adina Mihaela Epure, Sandrine Estoppey Younes, LG, Justine Gross, AH, Stefano Lanzi, Seyda Mayerat, Yvan Mivelaz, JP, DQ, Jean-Benoit Rossel, Nicole Sekarski, Umberto Simeoni, BS, Yvan Vial.

Data availability statement

The datasets presented in this article are not readily available because many of them are still being worked on but are available from the corresponding author on reasonable request. Requests to access the datasets should be directed to jardena.puder@chuv.ch.

Ethics statement

The study was approved by the Human Research Ethics Committee of the Canton de Vaud (study number 2016-00745). The study was conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was obtained from all participating women (for themselves and their offspring).

Author contributions

M-CA: Formal analysis, Writing – original draft. DYQ: Investigation, Methodology, Writing – review & editing. LG: Investigation, Methodology, Writing – review & editing. AA: Investigation, Methodology, Writing – review & editing. SS: Methodology, Writing – review & editing. AL: Methodology, Writing – review & editing. BS: Methodology, Writing – review & editing. AH: Conceptualization, Funding acquisition, Writing – review & editing. JJP: Conceptualization, 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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Supplementary material

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EDITED BY
Claus Yding Andersen,
University of Copenhagen, Denmark

REVIEWED BY
Wenwei Pan,
The First Affiliated Hospital of Sun Yat-sen University, China
Dana Kimelman,
Centro de Esterilidad Montevideo, Uruguay

*CORRESPONDENCE
Paul L. Hofman
p.hofman@auckland.ac.nz

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Iodine and other factors associated with fertility outcome following oil-soluble contrast medium hysterosalpingography: a prospective cohort study

Divya M. Mathews[1,2], Jane M. Peart[3], Robert G. Sim[3], Neil P. Johnson[4,5,6], Susannah O'Sullivan[7], José G B Derraik[8,9,10] and Paul L. Hofman[1,2]*

[1]Liggins Institute, University of Auckland, Auckland, New Zealand, [2]Starship Children's Hospital, Health New Zealand | Te Whatu Ora, Auckland, New Zealand, [3]Auckland Radiology Group, Auckland, New Zealand, [4]Robinson Research Institute, University of Adelaide, Adelaide, SA, Australia, [5]Department of Obstetrics and Gynaecology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand, [6]Repromed Auckland, Auckland, New Zealand, [7]Endocrinology, Greenlane Clinical Centre, Auckland District Health Board, Auckland, New Zealand, [8]Department of Paediatrics: Child & Youth Health, Faculty of Medicine and Health Sciences, University of Auckland, Auckland, New Zealand, [9]Environmental–Occupational Health Sciences and Non-Communicable Diseases Research Group, Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand, [10]Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden

Objective: To examine factors associated with fertility following hysterosalpingography (HSG) using an oil-soluble contrast medium (OSCM).

Design: In a prospective cohort study on 196 women undergoing OSCM HSG, we showed that iodine excess was almost universal (98%) and mild subclinical hypothyroidism was frequent (38%). Here, we report the analyses of secondary outcomes examining factors associated with the likelihood of pregnancy following the HSG.

Setting: Auckland, New Zealand (2019–2021).

Sample: 196 women with primary or secondary infertility who underwent OSCM HSG.

Methods: Baseline and serial urine iodine concentrations (UIC) and thyroid function tests were measured over six months following the HSG. Pregnancy and treatment with levothyroxine during the study period were documented.

Results: Following OSCM HSG, pregnancy rates were 49% in women aged <40 years (77/158) but considerably lower (16%) among those ≥40 years (6/38). Similarly, live birth rates were markedly lower in women ≥40 years (17%; 1/6) versus <40 years (73%; 56/77). 29% of participants were iodine deficient at baseline despite advice recommending iodine fortification. Following HSG, the likelihood of pregnancy in women with moderate iodine deficiency was 64% higher than in women with normal iodine levels (p=0.048). Among women aged <40 years who had subclinical hypothyroidism (n=75), levothyroxine treatment was associated

with higher pregnancy rates compared to untreated women [63% (26/48) vs 37% (10/27), respectively; p=0.047].

Conclusion: OSCM HSG was associated with higher pregnancy rates in women ≤40 than in those aged >40 years. Iodine deficiency was relatively common in this cohort, and increased iodine levels from OSCM exposure may contribute to the improved fertility observed with this procedure.

Trial registration: This study is registered with the Australian New Zealand Clinical Trials Registry (ANZCTR: 12620000738921) https://anzctr.org.au/Trial/Registration/TrialReview.aspx?ACTRN=12620000738921.

KEYWORDS

fertility, hysterosalpingography, iodine, oil-soluble contrast medium, pregnancy, age, tubal patency, hypothyroidism

Introduction

Hysterosalpingography (HSG) with oil-soluble contrast medium (OSCM) is known to improve pregnancy rates in women with infertility (1–3). Pregnancy rates of 39.7% and live birth rates of 38.8% were reported in the H2Oil study, the large multicentre trial that confirmed the fertility enhancement with OSCM HSG (3). Although the improvement in pregnancy rates was reported within the initial six months of the procedure (3–5), little is known about the characteristics of those women who achieved the greatest fertility benefit. A secondary outcome analysis of the H2Oil study could not identify any characteristics of women who would benefit from OSCM HSG (6). The paucity of data in this area partially reflects our lack of understanding of the mechanism(s) underlying the improved fertility observed with OSCM HSG.

Nonetheless, several mechanisms have been proposed, including a mechanical flushing effect (7), an immune-biological peritoneal bathing effect (8), and an immune-biological uterine bathing effect (9). The other hypothesis is that iodine in OSCM could contribute to this fertility improvement (10). The reasons behind this postulation are the association between iodine deficiency and infertility and the iodine excess state produced by OSCM exposure. OSCM, such as Lipiodol, contains approximately 480 mg/ml of iodine (11) and has a reported half-life of approximately 50 days (12), creating severe and prolonged iodine excess for six months post-procedure. Recently published research from our group suggested almost universal (98%) iodine excess following an OSCM HSG, leading to the frequent occurrence of subclinical hypothyroidism (38%; 71/188) and an occasional occurrence of late-onset hyperthyroidism (5%; 9/196) (Supplementary Table 1) (13).

While iodine uptake via sodium-iodide symporters occurs mainly in the thyroid, other tissues also actively take up iodine from circulation. Two such examples are ovaries and endometrium, which have relatively high levels of sodium-iodide symporters (14, 15). The effect of iodine on the function of the ovaries and endometrium remains unclear. Still, it seems likely to have an important role, as iodine deficiency and insufficiency are well-established causes of subfertility (16). This study aimed to examine factors associated with increased fertility and live births following OSCM HSG, particularly the potential effects of iodine status on pregnancy rates before and after the HSG.

Methods

The SELFI (Safety and Efficacy of Lipiodol in Fertility Investigations) Study was a prospective cohort study conducted in the Auckland region, New Zealand (2019–2021) (17). 196 consecutively consenting women who underwent OSCM HSG were followed for 6 months. The study's primary outcome was the development of subclinical hypothyroidism, and our findings on iodine excess and thyroid dysfunction following OSCM HSG have been published (13). Secondary outcomes related to fertility are discussed in this article.

The inclusion and exclusion criteria are listed in Supplementary Table 2. Details of the HSG protocol and investigations are available in the published protocol (17). Clinical parameters assessed at baseline (before the HSG) included urine iodine concentration (UIC), and serum concentrations of thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), and anti-mullerian hormone (AMH). The OSCM used in the HSG procedure was Lipiodol Ultrafluide (Guerbet, Aulnay-Sous-Bois, France). Following the HSG, participants had UIC measured at weeks 1, 4, 12, and 24, and thyroid function tests (TSH, Free T4 and Free T3) done at weeks 1, 4, 8, 12, 16, 20, and 24 (Supplementary Figure 1). Biochemical pregnancy was defined as a positive beta human

chorionic gonadotropin (β-hCG) test. Live births were recorded, and any thyroxine treatment initiated by their primary clinician during the study period was documented.

The associations between clinical parameters and the likelihood of biochemical pregnancy were assessed with generalised linear models using a modified Poisson procedure with robust error variances (18). Model outcomes were reported as the unadjusted relative risk (RR) or the adjusted relative risk (aRR) and their respective 95% confidence intervals (CI). Models were adjusted for TSH levels and UIC at baseline, woman's age (<35 years/35–39.9 years/≥40 years), and the instilled OSCM volume. UIC AUC calculations and data analyses were performed using SAS v9.4 (SAS Institute, Cary, NC, USA). Figures were created in GraphPad Prism v8.2.1 (GraphPad Software Inc., San Diego, CA, USA). All statistical tests were two-tailed, with statistical significance maintained at the 5% level, with no adjustments for multiple comparisons (19). There was no imputation of missing values.

Results

Study population

Table 1 describes the demographic characteristics of the study population at baseline (n=196). Participants had a median age of 36.2 years (range 26 to 49 years), with 38 (19%) women aged ≥40 years.

Based on WHO definitions of iodine status (21), 55% of participants were iodine sufficient, 29% were deficient, and 16% had iodine excess (Table 1). Among those who were iodine deficient, most (77%) had mild deficiency, and the rest (23%) had moderate deficiency (Table 1).

Pregnancy rates following OSCM HSG

Overall, 83 participants (42%) had a biochemical pregnancy (i.e., a positive serum β-hCG result), while 57 (29%) had an ongoing pregnancy that progressed to a live birth. The other 26 participants (13%) had a miscarriage, usually in the first trimester. When only women aged 40 years or below were considered, 49% (77/158) conceived and 73% of them had a live birth (56/77), which equated to 37% of women in this age group (56/158).

The timing of conception and subsequent miscarriages in association with the OSCM HSG procedure are itemised in Figure 1. Nearly half of all conceptions (45%; 37/83) were recorded within 8 weeks of the HSG, and more than three quarters (77%; 64/83) had occurred by week 16 (Figure 1). Notably, the vast majority of ongoing pregnancies (88%; 50/57) were recorded by week 16 (Figure 1).

An exploratory analysis showed no association between infertility cause and biochemical pregnancy rates following OSCM HSG (Supplementary Table 3). In addition, baseline iodine status did not differ between women with different infertility causes (Supplementary Table 4), and there was no evidence that iodine status differentially affected pregnancy rates in these groups (Supplementary Table 5). Similarly, there was no evidence that BMI (Supplementary Table 6) or assisted reproductive technologies (i.e., intrauterine insemination or *in vitro* fertilisation) (Supplementary Table 7) affected pregnancy rates.

Woman's age at baseline

Pregnancy rates were similar among women aged <35 and 35–39.9 years, but there was a marked decline in fertility rates among participants aged ≥40 (Figure 2; Supplementary Table 8). Only 16% (6/38) of the latter became pregnant compared to 51% (40/79) and 47% (37/79) of women aged <35 years and 35–39.9 years, respectively (p<0.001) (Figure 2; Supplementary Table 8). Thus, in comparison to the women aged ≥40 years, those aged <35 years were 3 times more likely to become pregnant [aRR=3.03 (95% CI 1.43, 6.45); p=0.004] and women aged 35–39.9 years 2.9 times more likely [aRR=2.92 (95% CI 1.37, 6.25); p=0.009]. The rate of miscarriage was 30% (25/83), and this rate progressively increased with the woman's age, so that 83% of those aged ≥40 years (5/6) experienced pregnancy loss (Figure 2; Supplementary Table 8).

Iodine status

Overall, lower iodine levels at baseline were associated with a greater likelihood of pregnancy. Women who became pregnant had baseline UIC 21% lower than those who did not become pregnant (95% CI -38%, -1%; p=0.042) (Supplementary Table 9), with an adjusted mean difference slightly greater [-23% (95% CI -40%, -2%); p=0.033]. As a result, a 10-fold lower UIC at baseline was associated with a 77% increase in the likelihood of pregnancy [aRR 1.77 (95% CI 1.11, 2.81); p=0.017].

Reflecting the above-described associations, pregnancy rates progressively decreased from the group of women with moderate iodine deficiency at baseline (58%) to those with excess iodine (31%) (Figure 3). Thus, the likelihood of pregnancy in women with moderate deficiency at baseline was 64% higher than in women with normal iodine levels [aRR 1.64 (95% CI 1.01, 2.67); p=0.048] and more than 2-fold higher than those with iodine excess [aRR 2.13 (95% CI 1.09, 4.14); p=0.026]. These data indicate that women with iodine deficiency treated by iodine exposure from OSCM HSG had improved pregnancy rates compared to those who were iodine-sufficient or had excess iodine. Interestingly, we also noted a higher pregnancy rate (43%) in the iodine-sufficient group compared to women with iodine excess (Figure 3).

Iodine levels after the HSG

Iodine excess (UIC ≥300 µg/L) after the OSCM HSG was almost universal among our participants (98%) and was often marked (90% had UIC ≥1000 µg/L and 17% had UIC >10,000 µg/L) and prolonged (67% had UIC ≥1000 µg/L lasting at least three months)

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TABLE 1 Demographic and clinical characteristics of the SELFI Study participants at baseline prior to hysterosalpingography.

Characteristic	Parameter	Level	
n			196
Demography	**Age (years)**		36.2 [32.8, 39.3]
	Ethnicity	**NZ European/European**	118 (60.2%)
		Indian	37 (18.9%)
		Other Asian	32 (16.3%)
		Māori	5 (2.6%)
		Pacific	4 (2.0%)
Clinical	**BMI (kg/m^2)** [1]		23.9 [21.9, 27.2]
	BMI status [1,2]	**Normal weight**	95 (62.5%)
		Overweight	12 (7.9%)
		Obesity	45 (29.6%)
	TSH (mIU/L)		1.8 [1.3, 2.5]
	Urine iodine (µg/L) [3]		152 [89, 228]
	Infertility cause	**Idiopathic**	130 (66.3%)
		Endometriosis	37 (18.9%)
		PCOS	15 (7.7%)
		Other	14 (7.1%)
	Infertility type [4]	**Primary**	147 (75.0%)
		Secondary	49 (25.0%)
	Fertility treatment [5]	**None**	141 (71.9%)
		IVF	31 (15.8%)
		IUI	19 (9.7%)
		Unknown	5 (2.6%)
	Iodine status [3,6]	**Deficiency**	52 (29.2%)
		Severe	nil
		Moderate	12 (23.1%)
		Mild	40 (76.9%)
		Normal	98 (55.1%)
		Excessive	28 (15.7%)

Data are n (%) or median [Q1, Q3]. BMI, body mass index; IUI, intra-uterine insemination; IVF, *in vitro* fertilisation; OSCM, oil-soluble contrast medium; PCOS, polycystic ovarian syndrome; TSH, thyroid-stimulating hormone.
[1]n=152.
[2]Normal weight was defined as a BMI ≥18.5 to <25 kg/m^2; overweight as ≥25 to <30 kg/m^2; and obesity as ≥30 kg/m^2.
[3]n=183.
[4]Primary and secondary infertility were defined as at least 12 months of unsuccessfully attempting pregnancy with no previous live births and with a previous live birth, respectively (20).
[5]Treatment (if any) was undertaken after OSCM HSG.
[6]Iodine status was defined as per WHO criteria (21) using urine iodine concentrations: deficiency (<100 µg/L), severe deficiency (<20 µg/L), moderate deficiency (≥20 to <50 µg/L), mild deficiency (≥50 to <100 µg/L), normal (≥100 to <300 µg/L), and excessive (≥300 µg/L).

(13). However, in contrast to baseline iodine status, UIC after HSG did not seem to influence the likelihood of conception, with the UIC time-weighted area under the curve similar in women who did and did not conceive [45.4 mg/L/week (95% CI 35.2, 58.4) vs 42.5 mg/L/week (95% CI 34.9, 51.8); p=0.69].

AMH levels at baseline

AMH concentrations (reflecting ovarian reserve) were correlated with the women's age (r=-0.39; p<0.0001). Thus, AMH steadily declined with increasing age (Supplementary Figure 2).

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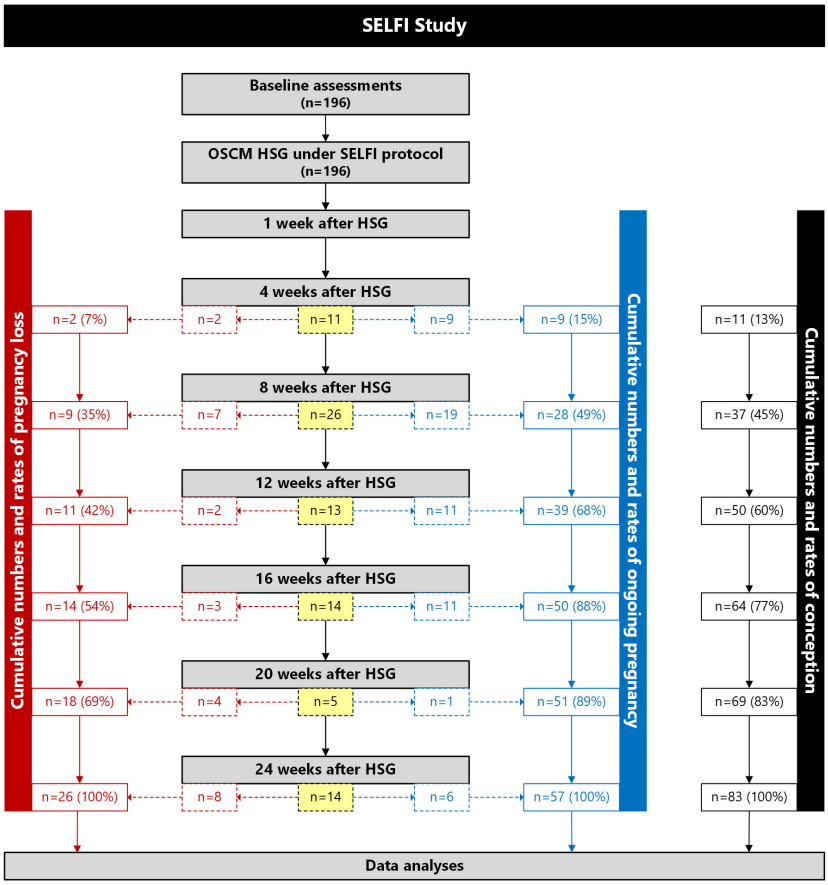

FIGURE 1
Flow diagram showing the numbers and cumulative rates of conception (black), pregnancy loss (red), and ongoing pregnancies (blue) among women who underwent hysterosalpingography (HSG) with an oil-soluble contrast medium (OSCM) in the SELFI Study. Values within boxes with dashed lines are the numbers and rates of new conceptions, pregnancy losses, and ongoing pregnancies at a given time point since HSG. Values within boxes with solid lines are the cumulative numbers and rates of conceptions, pregnancy losses, and ongoing pregnancies at a given time point since HSG. The number in bold font (n=196) indicates the number of participants who underwent OSCM HSG and completed study (i.e., there were no participants lost to follow-up).

Women who became pregnant during the study had higher AMH levels compared to those who did not (22.4 vs 17 µg/L, respectively; p=0.021) and were nearly 2 years younger on average (p=0.003). There was no observed effect of the HSG on AMH levels.

Treatment of subclinical hypothyroidism

Mild subclinical hypothyroidism (TSH 4-10 mIU/L with normal FT4) was the most common thyroid dysfunction in the SELFI cohort. The treatment of mild subclinical hypothyroidism with thyroxine is controversial (22), and during the SELFI Study, an individualised treatment decision was made by the participant's primary clinician. There was a trend suggesting that women treated with levothyroxine were more likely to conceive compared to untreated women [54% vs 35%, respectively (Table 2); aRR 1.66 (95% CI 0.97, 2.84); p=0.063]. Notably, when only women aged <40 years at baseline were considered, the pregnancy rate after levothyroxine treatment was higher than that of untreated women (63% vs 37%; p=0.047; Table 2), with a 75% increase in the likelihood of pregnancy [aRR 1.75 (95% CI 1.01, 3.02); p=0.046].

Tubal patency

The data on tubal patency status for our study participants are provided in Supplementary Table 10. Notably, 3 out of 16 women (19%) with radiological evidence of bilateral tubal obstructions had spontaneous pregnancy (Supplementary Table 10). Thus, the findings of no patency did not exclude the chance of pregnancy.

Discussion

Main findings and interpretation

Our study confirms that OSCM HSG is followed by high pregnancy rates in women under 40 years. The proportion of women (40 years and below) who conceived within 6 months of OSCM HSG and successfully progressed to a live birth in our study was similar to that of the H2Oil trial, which used this age limit (36.6% vs 38.8%) (3). Similarly, the timing of pregnancy following the HSG was also consistent with previous studies (23, 24).

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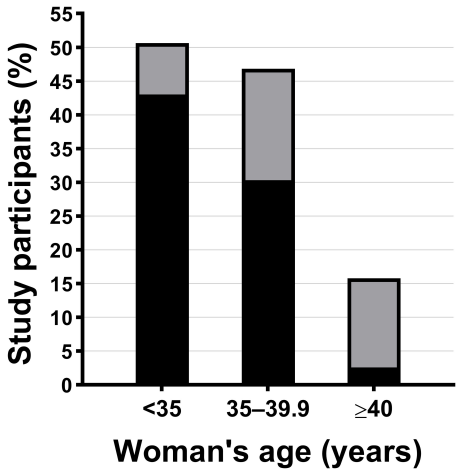

FIGURE 2
Biochemical pregnancy (based on beta human chorionic gonadotropin positivity) according to the woman's age at baseline: <35 years (n=40), 35–39.9 years (n=37), and ≥40 years (n=6). The bands in each bar represent the percentage of women who conceived and either had a miscarriage (grey) or delivered a live baby (black).

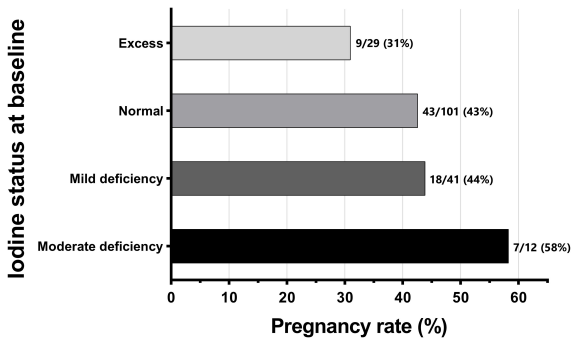

FIGURE 3
Frequencies and rates of biochemical pregnancy [based on beta human chorionic gonadotropin (β-hCG) positivity] according to the women's urine iodine status at baseline (n=183). Iodine status was classified according to WHO criteria: moderate deficiency (≥20 to <50 µg/L), mild deficiency (≥50 to <100 µg/L), normal (≥100 to <300 µg/L), and excess (≥300 µg/L) (21). No woman in the study had severe iodine deficiency (<20 µg/L).

Interestingly, almost 30% of our cohort had iodine deficiency or insufficiency. The New Zealand soil is deficient in iodine (25), and fortification of bread with iodized salt is mandatory (26). However, mild iodine deficiency persists in the New Zealand population, especially women (27, 28), and iodine supplementation is recommended for women trying to conceive (29). In our study, we allowed the women to continue the iodine supplements (150 µg iodine/tablet) or multivitamin supplements (220 µg iodine/tablet) as advised by their respective fertility specialists. Our observations of baseline iodine status in this cohort suggest that despite the iodine fortification programmes in New Zealand (30) and the recommendations for iodine supplementation in women planning pregnancy (29), this issue remains an aspect of antenatal care that needs to be addressed. The pivotal role of iodine in conception and successful pregnancy progression, and the importance of achieving at least normal iodine levels has been demonstrated in other studies (16, 31, 32). It seems possible that iodine deficiency was one of the factors contributing to idiopathic infertility in this cohort of women. Thus, additional approaches to improve iodine status should be considered, including prescribing oral iodine supplements to women who are trying to conceive and adopting methods that can improve adherence, such as the use of one-dose or long-acting iodine replacements [e.g., oral OSCM (11)].

Not only were there persistent and high iodine levels following OSCM HSG, but those women with lower iodine levels at baseline were more likely to conceive following the procedure, indicating that treating iodine insufficiency/deficiency via the OSCM HSG's iodine load improved fertility. Of note, the magnitude of iodine excess post-HSG did not correlate with pregnancy success, and we hypothesize that the correction of this iodine deficiency is more important than the extremely high levels subsequently achieved following the OSCM HSG. The reduced pregnancy rate in women with iodine excess at baseline was interesting and may reflect a cohort in whom other pathologies unaffected by iodine status are the cause of infertility. However, a better pregnancy rate in those who were iodine-sufficient (compared to the iodine-excess group) does raise the possibility that fertility can be enhanced with higher (supraphysiologic, yet not extreme) iodine levels in women with infertility. This question needs further exploration in future studies.

We also observed that women treated for mild SCH with levothyroxine during the six-month study period had higher pregnancy rates than those who were untreated. The treatment of

TABLE 2 Rates of biochemical pregnancy based on beta human chorionic gonadotropin (β-hCG) positivity according to the diagnosis of subclinical hypothyroidism (SCH), the timing of its onset, and any subsequent thyroxine treatment.

	Subclinical hypothyroidism			Thyroxine		Thyroxine (aged <40 years)	
	No SCH	SCH at baseline	SCH after HSG	Not treated	Treated	Not treated	Treated
n	117 (59.7%)[1]	8 (4.1%)[1]	71 (36.2%)[1]	31 (39.2%)[2]	48 (60.8%)[2]	27 (41.5%)[2]	38 (58.5%)[2]
β-hCG negative	71 (60.7%)	2 (25.0%)	40 (56.3%)	20 (64.5%)	22 (45.8%)	17 (63.0%)	14 (36.8%)
β-hCG positive	46 (39.3%)	6 (75.0%)	31 (43.7%)	11 (35.5%)	26 (54.2%)	10 (37.0%)	24 (63.2%) *

HSG, hysterosalpingography.
Unless otherwise stated, data are *n* and percentage within a given column.
[1]Percentages from all study participants.
[2]Percentages from participants who had SCH at some point during the study.
*p=0.047 from a Fisher's exact test.

mild SCH remains controversial (22). However, some studies suggest that SCH reduces fertility and that treatment improves pregnancy rates (33, 34). In this context, our findings suggest that women with mild SCH post-HSG who are attempting pregnancy may benefit from replacement therapy with levothyroxine.

This study demonstrates the limited fertility benefit of OSCM HSG in women aged 40 years or above, with only 16% conceiving and only one live birth recorded. This result is not surprising, reflecting the impact of aging and reduced follicular number. Indeed, age was the single most important factor in predicting pregnancy. The high miscarriage rates in this study should be interpreted in the context of an infertile cohort, which included women of older age, who had endometriosis, and/or experienced recurrent miscarriages. A previous large prospective Australian cohort study (5806 women, 31-36 years) had reported that the miscarriage rates varied highly between different groups of women, with a calculable rate of miscarriage ranging from 11.3 to 86.5 miscarriages per 100 live births (35). One study in younger women (18–33-year-olds) reported a lower miscarriage rate of 16% (36), whereas another earlier study including older women (aged 16–59 years) reported miscarriage rates of 33.4% (37). As expected, younger age was associated with higher AMH levels (38–40), a marker of follicular number, and predicted improved pregnancy rates following OSCM HSG. Thus, whilst OSCM HSG is a very good modality for augmenting fertility, the efficacy in those over 40 years appears limited.

Limitations

Potentially important factors such as BMI and infertility aetiology could not be obtained for all participants, as these data were extracted retrospectively from clinical charts. BMI in particular, is known to adversely affect both female (41, 42) and male (43) fertility. While we had no data on the male partner's BMI, among the 78% of study participants with BMI data, there was no evidence to suggest a BMI effect on fertility. Also, since 72% of study participants did not undergo any fertility treatment, it was not possible to carry out any robust analyses looking at the potential associations between assisted reproductive technologies and pregnancy rates. In addition, as most of our participants were recruited from private fertility clinics, disadvantaged groups were underrepresented, particularly women from Māori and Pacific communities. Thus, it is not possible to generalize our findings on iodine status to the entire female population of New Zealand or all women with infertility. Lastly, it is unknown if any of our study participants underwent transvaginal ultrasound or hysteroscopy before the OSCM HSG procedure to detect uterine or endometrial pathology. However, most study participants underwent transvaginal ultrasound following the OSCM HSG, and all of these were normal. Moreover, no uterine pathology was observed on fluoroscopy during the OSCM HSG.

Strengths

To our knowledge, this is the only study that has examined the associations between HSG and fertility accounting for the women's iodine levels before and after the procedure. This study highlights the caveats in iodine supplementation and the importance of ensuring prescription and compliance in women planning pregnancy. Our data suggest that iodine deficiency could contribute to some cases of unexplained infertility, and correction of iodine deficiency following OSCM exposure seems to be a contributing factor to improved fertility. Moreover, we show that fertility rates were markedly lower in women aged ≥40 years compared to younger women. These data provide additional evidence to fertility specialists and infertile couples for their decision-making process on whether to offer or undergo OSCM HSG, respectively.

Conclusions

This study confirmed that while pregnancy rates were similar to other recent studies using OSCM HSG, women over 40 years of age have poor fertility outcomes. Iodine deficiency was relatively common despite government-instituted iodine fortification programmes and recommendations for iodine supplements by the fertility specialists. Interestingly, the fertility improvement with OSCM HSG was greater in those who were iodine deficient. We hypothesise that increased iodine levels may contribute to this procedure's improved fertility. Treatment of the subclinical hypothyroidism that can occur following the OSCM HSG may also improve fertility rates further. Further studies are required to examine the potential effects of iodine deficiency on infertility, particularly the fertility improvement with OSCM HSG in iodine-deficient women. It would be interesting to determine if one oral or IM dose of OSCM is a suitable alternative to improve iodine levels and, subsequently, fertility. The benefit of OSCM HSG as a standalone fertility treatment and as an adjunct before intrauterine insemination or *in vitro* fertilization also needs to be explored further.

Data availability statement

The study data cannot be made available in a public repository due to the strict conditions of the ethics approval, as no consent was obtained from study participants to make their confidential health data publicly available, even if anonymised. Nonetheless, the anonymised data on which this study was based could be made available to other investigators upon bona fide request, following all the necessary approvals (including ethics approval) of the detailed study proposal and statistical analysis plan. Any queries should be directed to Prof Paul Hofman (p.hofman@auckland.ac.nz).

Ethics statement

Ethics approval for the SELFI (Safety and Efficacy of Lipiodol in Fertility Investigations) Study was granted by the Northern B Health and Disability Ethics Committee (19/NTB/52). 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

DM: Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing. JP: Conceptualization, Resources, Supervision, Writing – review & editing. RS: Conceptualization, Resources, Supervision, Writing – review & editing. NJ: Conceptualization, Resources, Supervision, Writing – review & editing. SO'S: Conceptualization, Resources, Supervision, Writing – review & editing. JD: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. PH: Conceptualization, Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing.

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Conflict of interest

NJ is involved in research with the University of Auckland and the University of Adelaide, which are funded by Guerbet. NJ has undertaken paid consultancies for Guerbet. DM and PH are involved with a University of Auckland study on Lipiodol safety through an unrestricted independent grant to the Liggins institute from Guerbet. PH has received fees for speaking in two webinars sponsored by Guerbet. RS and JP have been paid for presenting and being an advisory board member by Guerbet. RS, JP, and NJ undertake Lipiodol HSGs as a part of their profession.

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

The funder Guerbet had no role in the study design, conduction of the study, data analyses or interpretation, manuscript preparation, decision to publish it, or dissemination of study findings.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1257888/full#supplementary-material

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EDITED BY
Lisa Owens,
St. James's Hospital, Ireland

REVIEWED BY
Zainab M. Alawad,
University of Baghdad, Iraq
Zhiqin Bu,
Zhengzhou University, China

*CORRESPONDENCE
Qingyun Mai
maiqy@mail.sysu.edu.cn
Yizi Wang
wangyz3@mail3.sysu.edu.cn

†These authors have contributed equally to this work

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Causal relationships exist between polycystic ovary syndrome and adverse pregnancy and perinatal outcomes: a Mendelian randomization study

Yuanlin Ma[1,2,3†], Jiahao Cai[4†], Lok-Wan Liu[1,2,3†], Tianrui Wen[1,2,3], Weina Huang[1,2,3], Wenhui Hou[1,5], Zixin Wei[6], Yan Xu[1,2,3], Yanwen Xu[1,2,3], Yizi Wang[1,2,3*] and Qingyun Mai[1,2,3*]

[1]Reproductive Medicine Center, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China, [2]The Key Laboratory for Reproductive Medicine of Guangdong Province, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China, [3]Guangdong Provincial Clinical Research Center for obstetrical and gynecological diseases, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China, [4]Department of Neurology, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, Guangdong, China, [5]Reproductive Medicine Center, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China, [6]Department of Pulmonary and Critical Care Medicine, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China

Introduction: Previous observational studies have shown that polycystic ovary syndrome (PCOS) was associated with adverse pregnancy and perinatal outcomes. However, it remains controversial whether PCOS is an essential risk factor for these adverse pregnancy and perinatal outcomes. We aimed to use instrumental variables in a two-sample Mendelian randomization (MR) study to determine causality between PCOS and adverse pregnancy and perinatal outcomes.

Materials and methods: Summary statistics were extracted from a recent genome-wide association study (GWAS) meta-analysis conducted in PCOS, which included 10,074 cases and 103,164 controls of European ancestry. Data on Adverse pregnancy and perinatal outcomes were summarized from the FinnGen database of European ancestry, which included more than 180,000 samples. The inverse variance weighted (IVW) method of MR was applied for the main outcome. To assess heterogeneity and pleiotropy, we conducted sensitivity analyses, including leave-one-out analysis, weighted median, MR-PRESSO (Mendelian Randomization Pleiotropy RESidual Sum and Outlier), and MR-Egger regression.

Results: Two-sample MR analysis with the IVW method suggested that PCOS exerted causal effects on the risk of hypertensive disorders of pregnancy [odds ratio (OR) 1.170, 95% confidence interval (CI) 1.051–1.302, p = 0.004], in particular gestational hypertension (OR 1.083, 95% CI 1.007–1.164, p = 0.031), but not other pregnancy and perinatal diseases (all $p > 0.05$). Sensitivity analyses demonstrated pleiotropy only in pre-eclampsia or eclampsia ($p = 0.0004$), but not in other pregnancy and perinatal diseases (all $p > 0.05$). The results remained consistent after excluding two outliers (all $p > 0.05$).

Conclusions: We confirmed a causal relationship between PCOS and hypertensive disorders of pregnancy, in particular gestational hypertension, but no association with any other adverse pregnancy or perinatal outcome. Therefore, we suggest that women with PCOS who are pregnant should have their blood pressure closely monitored.

KEYWORDS

polycystic ovary syndrome, adverse pregnancy and perinatal outcomes, genetic role, Mendelian randomization, hypertensive disorders of pregnancy, gestational hypertension

1 Introduction

Polycystic ovary syndrome (PCOS) affects 10%–13% of reproductive-age women. It is characterized by anovulation, amenorrhea, hyperandrogenism, and polycystic ovary morphology (PCOM) (1). The pathophysiology of PCOS was associated with metabolic disorders, such as insulin resistance (IR), and endocrine-reproductive comorbidities (2), such as infertility, obesity, hirsutism, and cardiovascular problems (3). Women with PCOS often experience hyperandrogenism and IR, which have been associated with an increased risk of sporadic miscarriage and unfavorable obstetric outcomes during pregnancy (4). It has been well understood that the etiology of PCOS is the complex interplay of polygenetic and environmental elements (5). Previous reports have suggested that women with PCOS have an increased risk of maternal and fetal complications during pregnancy (4, 6–8).

Women with PCOS have reduced fertility potential, such as altered oocyte and endometrial competence and impaired endometrial–embryo cross-talk (9). In recent years, the reproductive outcomes of PCOS have become a research hotspot. Observational studies and meta-analyses have reported the relationship between PCOS and adverse pregnancy and perinatal outcomes (4, 6–8). It has been suggested that women with PCOS were at increased risk for miscarriage, gestational diabetes mellitus (GDM), gestational hypertension, and pre-eclampsia (4). A retrospective cohort study discovered that women with PCOS were more likely to experience preterm premature rupture of membrane (PPROM), preterm delivery, and placental abruption (8). However, the consensus on these effects is lacking. Cofactors related to PCOS, such as obesity, IR, glucose metabolism impairment, and metabolic syndrome, could influence endometrial competence, trophoblast invasion, placentation, pregnancy outcome, and even obstetric complications (9). Thus, the relationship between PCOS and pregnancy outcomes remains controversial because of confounding bias and methodological flaws in previous studies.

A Mendelian randomization (MR) study can estimate the causality between the exposure and outcome using instrumental variables (IVs) for the exposure and outcome. This method offers the advantage of reducing reverse causality and eliminating confounder bias (10, 11). In our MR study, the two-sample MR approach can be more efficient and powerful for exploring the "gene-risk factor" and "gene-outcome" relationship from two independent groups in the same ancestry compared to the one-sample MR approach (12). It was, therefore, useful to explain the relationship between PCOS and adverse pregnancy and perinatal outcomes in the genetic role (13).

The purpose of our study was to systematically investigate the causal effect of PCOS on adverse pregnancy and perinatal outcomes by conducting a two-sample MR analysis.

2 Material and methods

To evaluate the causative influence of PCOS on adverse pregnancy and perinatal outcomes, we conducted a two-sample MR study. Published genome-wide association study (GWAS) meta-analyses (14–16) provided the pooled data. Figure 1 illustrates the overview of the research design.

2.1 GWAS data for PCOS

Day et al. (14, 15) performed the biggest GWAS meta-analysis of PCOS in European ancestry, with 10,074 cases and 103,164 controls (Supplementary Table S1). The diagnosis of PCOS was according to the Rotterdam criteria (17), National Institutes of Health criteria (NIH/NICHD) (18), or self-report questionnaire (19). NIH/NICHD criteria were satisfied by the presentation of both hyperandrogenism, such as hirsutism or acne, and ovulatory

Abbreviations: PCOS, polycystic ovary syndrome; MR, Mendelian randomization; SNPs, single-nucleotide polymorphisms; GWAS, genome-wide association study; IVW, inverse variance weighted; MR-PRESSO, MR-pleiotropy residual sum and outlier; IR, insulin resistance; GDM, gestational diabetes mellitus; PPROM, preterm premature rupture of membrane; IVs, instrumental variables; NIH/NICHD, National Institutes of Health criteria; ICP, intrahepatic cholestasis of pregnancy.

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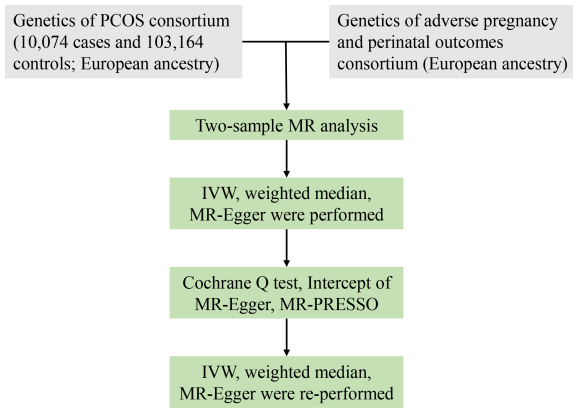

FIGURE 1
Workflow of MR study revealing causality from PCOS on adverse pregnancy and perinatal outcomes. PCOS, polycystic ovary syndrome; IVW, inverse variance weighted; MR, Mendelian randomization; MR-PRESSO, MR-pleiotropy residual sum and outlier; SNPs, single-nucleotide polymorphisms.

dysfunction, such as oligomenorrhea or amenorrhea, whereas the Rotterdam criteria required two out of three major features to be presented and the existence of PCOM. In the 23andMe (Mountain View, CA, USA) cohort, the self-reported diagnosis was employed; however, summary-level data from 4,890 cases and 20,405 controls included in this cohort were not available because of the data sharing policy. The GWAS meta-analysis elucidated shared genetic structure across the three diagnostic criteria (14).

2.2 IV selection

The instruments chosen for exposure (PCOS) had to satisfy the following criteria to ensure the validity of the IVs included in our MR study: single-nucleotide polymorphisms (SNPs) were associated with exposure at the threshold of genome-wide significance ($p < 5 \times 10^{-8}$) (20), all SNPs should follow the linkage equilibrium (pairwise $r^2 \leq 0.01$ in the current study), and *F* statistic above 10 was required for sufficient strength to limit the bias from weak IVs (21). We used $R^2 \times (N - k - 1)/[(1 - R^2) \times k]$ to calculate the *F* statistic, where N means the sample size of GWAS, k refers to the number of SNPs, and R^2 is the ratio of the variability of PCOS explained by each SNP. Specifically, R^2 is calculated using the formula $[2 \times \beta^2 \times (1 - EAF) \times EAF]/[2 \times \beta^2 \times (1 - EAF) \times EAF + 2 \times N \times SE^2 \times (1 - EAF) \times EAF]$, where EAF is the effect allele frequency, β is the estimate of the genetic effect of each SNP on PCOS, and SE is the standard error of beta (21). Supplementary Table S2 shows detailed genetic information on selected SNPs. SNPs linked to exposure were retrieved from outcome data (adverse pregnancy and perinatal outcomes). To reduce the possible bias from population heterogeneity, all the GWAS consortia employed in our MR study were restricted to those of European ancestry.

2.3 GWAS data for adverse pregnancy and perinatal outcomes

We examined associations with 14 outcomes: sporadic miscarriage, GDM, hypertensive disorders of pregnancy, gestational hypertension, pre-eclampsia or eclampsia, polyhydramnios, intrahepatic cholestasis of pregnancy (ICP), placenta disorder, placental abruption, placenta previa, premature rupture of membranes (PROM), postpartum hemorrhage, postpartum depression, and poor fetal growth. The definitions of these outcomes in FinnGen (16) are provided in Supplementary Table S1. The FinnGen study is a countrywide Finnish GWAS meta-analysis that includes nine biobanks and has minimal overlap with the PCOS GWAS, thereby reducing the potential bias arising from overlapping samples (22). FinnGen includes sporadic miscarriage (N = 15073 cases/135,962 controls), GDM (N = 11,279 cases/179,600 controls), hypertensive disorder of pregnancy (N = 13,071 cases/177,808 controls), gestational hypertension (N = 7,503 cases/176,113 controls), pre-eclampsia or eclampsia (N = 6,436 cases/176,113 controls), polyhydramnios (N = 1,049 cases/154,102 controls), ICP (N = 2,196 cases/188,683 controls), placenta disorder (N = 193 cases/154,102 controls), placenta previa (N = 1,076 cases/154,102 controls), placental abruption (N = 546 cases/154,102 controls), PROM (N = 6,129 cases/154,102 controls), postpartum hemorrhage (N = 7,221 cases/148,153 controls), postpartum depression (N = 13,657 cases/236,178 controls), and poor fetal growth (N = 3,056 cases/187,823 controls), and those outcomes were defined based on International Classification of Diseases (ICD) codes (16). In addition, hypertensive disorders of pregnancy encompass gestational hypertension, pre-eclampsia or eclampsia, chronic hypertension, and chronic hypertension with superimposed pre-eclampsia.

2.4 MR estimates

From the GWAS meta-analysis of the outcome, we retrieved and extracted IVs for PCOS. We ruled out SNPs linked to outcome (adverse pregnancy and perinatal outcomes) ($p < 5 \times 10^{-8}$) or absent in the outcome data pool. We harmonized the effect alleles across the GWASs of PCOS and pregnancy outcomes and then excluded those that were palindromic based on the information of EAF (default EAF > 0.42 of the "harmonisation" function in the "Two-Sample MR" package). We employed the inverse variance weighted (IVW) method as the major of MR estimation to examine the causality of PCOS on the risk of pregnancy outcomes. Based on the MR assumptions, this method supposed that all IVs were effective and combined the Wald ratio estimates of the causal effect by different SNPs to offer an identical assessment of the causal effect of PCOS on the pregnancy outcomes (12). Then, we obtained a *post-hoc* power calculation through the IVW model (https://shiny.cnsgenomics.com/mRnd/) (23).

2.5 Sensitivity analyses

In MR studies, sensitivity analysis has been proven crucial in detecting the pleiotropy and heterogeneity for MR estimations that may significantly violate the MR assumptions. We used Cochran's Q test to characterize potential heterogeneity derived from the IVW approach. The directional pleiotropy was shown by the intercept achieved from MR-Egger regression ($p < 0.05$ referred to as the existence of directional pleiotropy) (24). In addition, it is universal to employ MR-pleiotropy residual sum and outlier (MR-PRESSO) methods to evaluate and correct horizontal pleiotropy (25). MR-PRESSO included the following three contents: a) testing of significant results in the causal estimates before and after correction for outliers, b) correction for horizontal pleiotropy through outlier removal, and c) detection of horizontal pleiotropy. When the condition of parallel pleiotropy variants' percentage is <10%, it minimizes bias and has greater precision than IVW and MR-Egger (25). Moreover, we performed leave-one-out analyses to assess whether a single SNP could drive and influence the MR estimate.

The "Two-Sample MR" package (version 0.5.6) and "MR-PRESSO" package (version 1.0) were used to conduct all of the analyses in the R program (version 3.6.1). Results with *p*-value <0.05 were considered to be significant.

3 Results

The study includes 14 PCOS-related SNPs that met the threshold of genome-wide significance with LD $r^2 \leq 0.01$. However, two SNPs (rs11225154 and rs853854) were not directly matched in the outcome data and were therefore not used in further analysis. After ruling out SNPs significantly linked to adverse pregnancy and perinatal outcomes ($p < 5 \times 10^{-8}$), the remaining SNPs were used for analysis in our study. Only one excluded SNP (rs7563201) was strongly linked to gestational hypertension.

We discovered no relationships between the causal effect of PCOS and sporadic miscarriage [odds ratio (OR) 1.059, 95% CI 0.978–1.146, $p = 0.156$], GDM (OR 0.976, 95% CI 0.904–1.053, $p = 0.529$), pre-eclampsia or eclampsia (OR 1.137, 95% CI 0.961–1.346, $p = 0.134$), polyhydramnios (OR 1.075, 95% CI 0.849–1.360, $p = 0.548$), ICP (OR 0.848, 95% CI 0.696–1.034, $p = 0.104$), placenta disorder (OR 0.839, 95% CI 0.454–1.548, $p = 0.573$), placenta previa (OR 0.882, 95% CI 0.674–1.154, $p = 0.361$), placenta abruption (OR 1.092, 95% CI 0.761–1.567, $p = 0.631$), PROM (OR 0.974, 95% CI 0.865–1.097, $p = 0.668$), postpartum hemorrhage (OR 0.998, 95% CI 0.907–1.099, $p = 0.968$), postpartum depression (OR 1.034, 95% CI 0.963–1.110, $p = 0.354$), and poor fetal growth (OR 1.026, 95% CI 0.893–1.180, $p = 0.713$) by the IVW method (as shown in Figure 2). *Post-hoc* analyses revealed a power of 0.009–0.700 for the IVW model (Table 1).

We found a causal relationship between PCOS and hypertensive disorders of pregnancy (OR 1.170, 95% CI 1.051–1.302, $p = 0.004$) by the IVW method (as shown in Figure 2). As hypertensive disorders of pregnancy have several subtypes, further analyses revealed only causal effects of PCOS and gestational hypertension (OR 1.083, 95% CI 1.007–1.164, $p = 0.031$), but not pre-eclampsia or eclampsia (OR 1.137, 95% CI 0.961–1.346, $p = 0.134$) (Figure 2). *Post-hoc* analyses revealed power of 0.990 and 0.370 for the IVW model (Table 1).

We performed a sensitivity analysis using MR-Egger regression and weighted mean approaches. For most outcomes, consistent magnitude and direction of MR estimates were obtained (Figure 2). Further, no significant heterogeneity was observed with *p*-value >0.05 of IVW by Cochran's Q test, except for pre-eclampsia or eclampsia ($p = 0.0004$). The same conclusion was also gained using MR-PRESSO, with *p*-value >0.05, except for pre-eclampsia or eclampsia ($p = 0.001$) (Table 1). In addition, no evidence showed a significant intercept ($p > 0.05$), suggesting that no directional pleiotropy was observed. Some single SNPs affected the overall effect of PCOS on adverse pregnancy and perinatal outcomes in the leave-one-out sensitivity analysis (Supplementary Figure S1).

For pre-eclampsia or eclampsia, heterogeneity was also investigated using a standard Cochran's Q test, which derived a *p*-value <0.001 of IVW. MR-PRESSO also presented a similar result (global heterogeneity test $p = 0.001$). After weeding out two outliers (rs2271194 and rs7563201), the same MR approach followed by the IVW method was conducted again. As expected, further results demonstrated that the result was consistent with the previous (before correction, OR 1.137, 95% CI 0.961–1.346, $p = 0.134$ *vs.* after correction, OR 1.122, 95% CI 0.982–1.281, $p = 0.090$) (Figure 2).

4 Discussion

4.1 Principal findings

In the present study, a two-sample MR method was applied to assess whether PCOS adversely influenced pregnancy and perinatal outcomes in a causal effect. Our results showed that PCOS played a confirmative genetic role in the risk of hypertensive disorders of pregnancy, in particular gestational hypertension, but not pre-eclampsia or eclampsia.

4.2 Results in the context of what is known

PCOS has multiple etiologies associated with various genetic and environmental factors (1). It has many metabolic symptoms, such as central obesity, hyperandrogenism, elevated fasting blood glucose, and IR. PCOS and its comorbidities are linked to altered endometrial competence, oocyte quality, and impaired endometrial–embryo cross-talk, which increase the risk of infertility and early or late obstetric complications through abnormal trophoblast invasion and placentation (9). In addition, maternal exposure to 5α-dihydrotestosterone (DHT) and IR in a PCOS rat model changed the ferroptosis pathway in the gestational uterus and placenta, which was associated with increased necroptosis in the placenta and reduced the activation of apoptosis in the uterus, leading to miscarriage (26).

Reproductive outcome is one of the most essential concerns for women with PCOS in childbearing age. Therefore, in clinical studies, investigating the relationship between PCOS and adverse pregnancy and perinatal outcomes is necessary, but up to now, it

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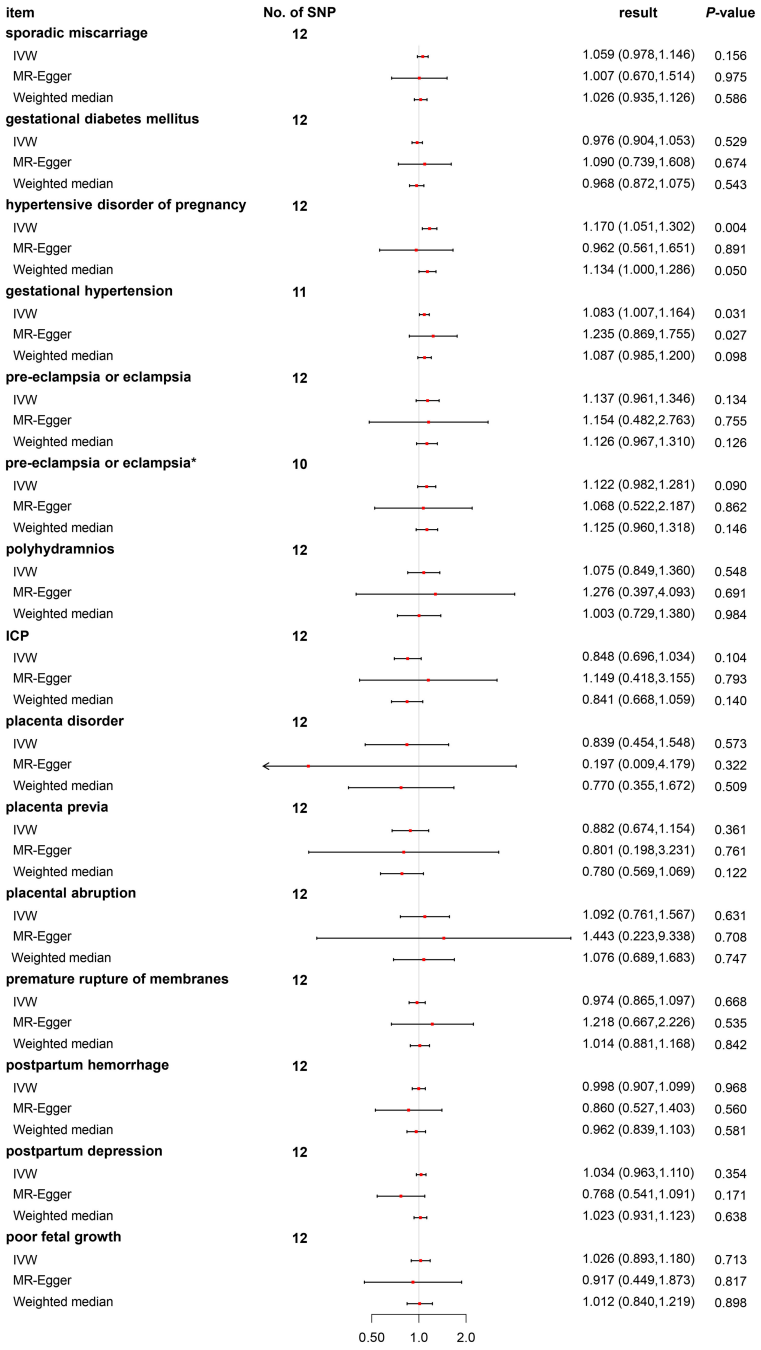

FIGURE 2
Odds ratio plot for PCOS and adverse pregnancy and perinatal outcomes. OR, odds ratio; PCOS, polycystic ovary syndrome. *Two outliers (rs11225154 and rs853854) were weeded out.

has remained unclear (4, 6–8). The previous observational studies had the limitation of possible bias from confounding factors. However, adequately powered and well-designed cohort studies or prospective trials with long-term follow-up would be very costly in terms of time, money, labor, and material resources. Moreover, findings from observational studies have not been sufficient to draw conclusions on cause–effect relationships. Compared with previous methods, MR is more effective and practical to comprehensively reveal these causalities.

We discovered a higher risk of hypertensive disorders of pregnancy, consistent with previous results (27). Hypertensive disorders of pregnancy encompass four subtypes. We tried to clarify which subtype was most likely to be affected. We discovered that PCOS only exerted causal effects on the risk of gestational hypertension (Figure 2), but not pre-eclampsia or eclampsia (Figure 2), chronic hypertension (Supplementary Table S3), and chronic hypertension with superimposed pre-eclampsia (Supplementary Table S3). Possibly, it was that just gestational

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TABLE 1 MR results of heterogeneity and directional pleiotropy.

Item	Power	Heterogeneity	Global heterogeneity test	Directional pleiotropy		
		p-Value	*p*-Value	Intercept	SE	*p*-Value
Sporadic miscarriage	0.380	0.138	0.155	0.006	0.026	0.810
GDM	0.090	0.416	0.416	−0.014	0.025	0.582
Hypertensive disorders of pregnancy	0.990	0.157	0.179	0.025	0.035	0.485
Gestational hypertension	0.370	0.468	0.477	−0.017	0.023	0.472
Pre-eclampsia or eclampsia	0.700	**0.0004**	**0.001**	−0.002	0.056	0.975
Pre-eclampsia or eclampsia*	/	0.107	0.112	0.007	0.048	0.894
Polyhydramnios	0.009	0.587	0.616	−0.022	0.748	0.775
ICP	0.380	0.135	0.144	−0.039	0.065	0.562
Placenta disorder	0.080	0.243	0.256	0.186	0.196	0.365
Placenta previa	0.150	0.199	0.224	0.012	0.089	0.892
Placenta abruption	0.080	0.260	0.281	−0.036	0.120	0.771
PROM	0.080	0.163	0.167	−0.029	0.039	0.475
Postpartum hemorrhage	0.050	0.358	0.363	0.019	0.031	0.557
Postpartum depression	0.140	0.741	0.759	0.038	0.022	0.121
Poor fetal growth	0.060	0.453	0.457	0.014	0.046	0.758

GDM, gestational diabetes mellitus; ICP, intrahepatic cholestasis of pregnancy; PROM, premature rupture of membranes; MR, Mendelian randomization. Bold text indicates statistical significance ($p<0.05$). "/" indicates that it is not calculated.
*Two outliers (rs11225154 and rs853854) were weeded out.

hypertension derived the causal relationship between PCOS and hypertensive disorders of pregnancy.

Several systematic reviews have summarized previous studies and come to different conclusions; nonetheless, the results of those pooled analyses suggested that women with PCOS were at increased risk of hypertensive disorders of pregnancy and pre-eclampsia (4, 6–8). Hyperinsulinemia and IR exacerbated endothelial injury and interfered with endothelium-dependent vasodilation, resulting in dyslipidemia and muscular hypertrophy of the vascular wall. High levels of free testosterone induced sympathetic and vascular hyperresponsiveness, both of which in PCOS were important for the occurrence and development of hypertensive disorders of pregnancy (28). Rs7563201, as one of the IVs of PCOS in our MR study, was associated with the expression of *THADA* (https://www.ncbi.nlm.nih.gov/snp/rs7563201). *THADA* was shown to have metabolic contributions to the pathophysiology of PCOS, such as disorders of glucose metabolism, hyperandrogenism, and dyslipidemia (29), which could also contribute to hypertensive disorders of pregnancy (28).

Possible factors were considered regarding our negative findings. First, the effect of PCOS on adverse pregnancy and perinatal outcomes was slightly lower than expected. In conventional regression analysis, we may ignore the bias from reverse causation or common risk factors. Second, vertical pleiotropy may exert efforts. Hyperandrogenism level and IR, which were genetically related, could lead to a more susceptible status in the evolution of PCOS. Thus, the detailed mechanisms underlying PCOS and pregnancy and perinatal outcomes were complicated and deserving of further investigation. Especially, as PCOS is a widely varying disease, the criteria for PCOS diagnosis should be restricted in future research.

4.3 Clinical implications

These findings suggested that PCOS was causally associated with hypertensive disorders of pregnancy, in particular gestational hypertension, which were among the idiopathic diseases of pregnancy, posing serious threats to the health of mothers and infants. It was suggested that the blood pressure of all pregnant women with PCOS should be closely monitored.

4.4 Strengths and limitations

Our study had several strengths. First, we effectively reduced the occurrence probability of reverse causality and confounding bias using the MR method, which genetically predicted phenotype as the exposure of interest. Second, the data we recruited were GWAS summary data, which came from the largest scale of recent meta-studies, which may, to a large extent, reduce the bias related to population heterogeneity in European people.

However, the study also had some limitations. First, GWAS data utilized in our study came from a European population. For this

reason, this kind of relationship needs to be confirmed in demographically different populations such as Asian individuals. Second, because there were three main diagnostic criteria of PCOS set by the NIH/NICHD (18), Rotterdam criteria (17), and the Androgen Excess and PCOS Society (30), we could not distinguish what kind of phenotypes were more influential. The Rotterdam criteria described four symptoms of PCOS, and there were differences in hormones and metabolism between these groups (11). Furthermore, since the associations between PCOS phenotype and adverse pregnancy and perinatal outcomes were untested, the manifestations of PCOS may present with variety, indicating that the effects from specific characteristics of PCOS subgroups may be ignored or defaulted. A third limitation was that we analyzed PCOS as a binary risk factor. However, the development of PCOS was progressive and successive. The *post-hoc* powers were low in many outcomes. Therefore, it was difficult to interpret our obtained effect estimate, as our included genetic variants did not represent all risks of different subtypes of PCOS. The MR study for a relationship between PCOS and adverse pregnancy and perinatal outcomes was still valid (31).

5 Conclusion

In this study, using MR analysis, we demonstrated a significant effect between PCOS and hypertensive disorders of pregnancy, in particular gestational hypertension, but found no association with any other adverse pregnancy or perinatal outcome. Therefore, we suggest that women with PCOS who are pregnant should have their blood pressure closely monitored.

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 in the article/Supplementary Material.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Approval by a formal institutional review board was not required because this analysis consisted of a collection of published studies. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

YM: Writing – review & editing, Writing – original draft. JC: Writing – original draft. LL: Writing – original draft, Writing – review & editing. TW: Writing – review & editing. WNH: Writing – original draft. WHH: Writing – review & editing. ZW: Writing – review & editing. YX: Writing – review & editing. YWX: Writing – review & editing. YW: Writing – review & editing. QM: 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.2024.1327849/full#supplementary-material

SUPPLEMENTARY FIGURE 1
MR results of leave-one-out sensitivity analysis.

SUPPLEMENTARY TABLE 1
Details of the GWAS included in the MR.

SUPPLEMENTARY TABLE 2
Details of instrumental variables included in the MR.

SUPPLEMENTARY TABLE 3
MR results of chronic hypertension and chronic hypertension with superimposed pre-eclampsia.

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EDITED BY
Richard Ivell,
University of Nottingham, United Kingdom

REVIEWED BY
Rui Zhao,
Shandong Provincial Qianfoshan Hospital, China
Giuseppe Basile,
IRCCS Istituto Ortopedico Galeazzi, Italy

*CORRESPONDENCE
Chunming Gu
guchunming0119@163.com

†These authors have contributed equally to this work and share first authorship

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Association between maternal lipid profiles and vitamin D status in second trimester and risk of LGA or SGA: a retrospective study

Xianhua Zheng†, Kefeng Lai†, Chengyi Liu, Yuan Chen, Xiaodan Zhang, Weixiang Wu, Mingyong Luo and Chunming Gu*

Department of Clinical Laboratory, Guangdong Women and Children Hospital, Guangzhou, China

Background: Accumulating evidence has linked dyslipidemia during pregnancy to the risk of delivering infants born either large for gestational age (LGA) or small for gestational age (SGA). However, the effects of the vitamin D status on these relationships require further investigation. This study investigated whether the relationship between lipid profiles and the risk of LGA or SGA was influenced by vitamin D levels during the second trimester.

Methods: Maternal lipid profile levels, including total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and vitamin D levels, were measured in a cohort of 6,499 pregnant women during the second trimester. Multivariate regression models and subgroup analyses were employed to evaluate the potential associations between maternal lipid profiles, vitamin D levels, and the risk of LGA or SGA.

Results: The prevalence of SGA infants was 9.8% (n=635), whereas that of LGA infants was 6.9% (n=447). Maternal TG levels were found to be positively associated with the risk of LGA (odds ratio [OR] = 1.41, 95% confidence interval [CI]:1.17–1.70), whereas a negative association was observed between maternal TG, TC, LDL-C levels, and risk of SGA. Additionally, mothers with higher HDL-C levels were less likely to give birth to an LGA infant (OR=0.58, 95% CI:0.39–0.85). Importantly, associations between TG, TC, LDL-c, and SGA as well as between TG and LGA were primarily observed among pregnant women with insufficient vitamin D levels. As for HDL-C, the risk of LGA was lower in mothers with sufficient vitamin D (OR = 0.42, 95% CI:0.18–0.98) compared to those with insufficient vitamin D (OR = 0.65, 95% CI:0.42–0.99).

Conclusion: Vitamin D status during the second trimester exerts a modifying effect on the association between lipid profiles and the risk of LGA and SGA infants.

KEYWORDS

pregnancy, lipid profile, vitamin D, large for gestational age, small for gestational age

1 Introduction

Adverse birth outcomes, including preterm birth (PTB), low birth weight (LBW), macrosomia, large for gestational age (LGA), and small for gestational age (SGA), have been identified as predictors of morbidity and mortality, as well as long-term health risks, such as metabolic syndrome, type II diabetes, and asthma (1–3). Numerous maternal factors, including gestational weight gain, pre-pregnancy body mass index (pre-BMI), and nutritional status during pregnancy, have been shown to be associated with adverse birth outcomes (4–6). Therefore, investigating the regulatory mechanisms underlying these outcomes during pregnancy is crucial for identifying potential preventive strategies.

During pregnancy, women undergo unique physiological changes and require increased nutrition and energy to support maternal metabolism and fetal growth. Maternal lipid profiles, including total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), play crucial roles in providing energy for placental development (7). However, studies on the relationship between maternal dyslipidemia and adverse birth outcomes have yielded inconsistent results. For instance, a prospective study found a positive association between maternal TG levels and the risk of LGA infants, independent of maternal pre-BMI (8). Conversely, a cross-sectional analysis conducted in Brazil did not find a significant association between lipid intake and LGA newborns (9).

Vitamin D deficiency is a global public health problem affecting various age groups, particularly in pregnant women. Emerging evidence on the physiological activities of vitamin D has highlighted its role in reducing hepatic TG synthesis, cholesterol conversion, and the promotion of fatty acid (FA) oxidation (10, 11). The expression of coenzyme A reductase (HMG CoA reductase), sterol regulatory element binding proteins (SREBPs), and peroxisome proliferators activated receptor (PPAR) regulated by vitamin D might account for the improvements of lipid profile *in vivo* and *in vitro* (12). The optimal level of vitamin D for pregnancy health was unclear, but a higher risk of adverse pregnancy outcomes is more likely to be related to vitamin D deficiency. The serum vitamin D levels have been found to correlate with profile levels, which are attributed to the increased metabolic demands of pregnancy (13). Furthermore, high vitamin D levels in the second trimester may improve the lipid profile and mitigate the elevation of C-reactive protein induced by hyperlipidemia (14). A meta-analysis suggested an inverse association between maternal vitamin D levels and the risk of LBW, PTB, and SGA (15). The adequate vitamin D status during pregnancy has been considered a protective factor against SGA and is associated with improved infant growth (16).

Abnormal lipid profiles in pregnant women are considered as risk factors for LGA or SGA infants, but the effects of vitamin D status on these relationships remain unclear. Therefore, this study was performed to investigate the association between vitamin D status, lipid profile during the second mid-pregnancy, and the risk of LGA or SGA infants in Chinese women.

2 Materials and methods

2.1 Study population

This retrospective study included pregnant Chinese women who received prenatal care and intended to give birth at the Guangdong Women and Children's Hospital (Guangzhou, China) between January 2020 and December 2021. This study was approved by the Ethics Committee of the Guangdong Women and Children's Hospital (reference number 202301269). All participants were provided detailed information about the study and provided written informed consent. Women who met any of the following criteria were excluded from the study: (1) multiple pregnancies or stillbirths (n = 2765), (2) preexisting diabetes (n = 327), (3) preexisting hypertension (n = 94), (4) *in vitro* fertilization (n = 2513), or (5) incomplete data on basic information or testing (n = 1651) (Figure 1). Ultimately, 6499 mother-fetus pairs were included in this study.

2.2 Measurement for lipid profiles and vitamin D in mid-pregnancy

Non-fasting plasma samples were obtained during mid-pregnancy by a trained nurse (median 17.43 weeks of gestation, 90% range [14.14 to 24.86]). The concentrations of serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were analyzed using an automatic analyzer (Beckman Coulter, Brea, CA, USA) and a commercial kit (Leadman, Beijing, China). The vitamin D concentration was determined using an electrochemiluminescence immunoassay (Abbott Laboratories, IL, USA). Internal quality and quality control measurements were performed for each batch of analyses, with inter- and intra-assay coefficients of variation (CVs) below 10%.

2.3 Birth outcome and covariates

Anthropometric data on infants and basic information on mothers were obtained from the medical records of the study hospital. Immediately after birth, obstetric nurses recorded the birth weight, length, and head circumference of newborns. Data on maternal age, gravidity, parity, education level, smoking and drinking status, pregnancy complications (gestational diabetes mellitus [GDM] and hypertensive disorders in pregnancy [HDP]), gestational age at lipid profile testing, and the season of vitamin D measurement were extracted from medical records as potential covariates. Seasons of vitamin D measurement were defined as winter (December, January, February), spring (March, April, May), summer (June, July, August) and fall (September, October, November). We adjusted for potential covariates in the regression models based on previous reports. The maternal vitamin D status was defined according to the Endocrine Society's Clinical Guidelines, with 25(OH)D levels below 75 nmol/L classified as non-sufficiency and levels equal to or above 75 nmol/L classified as sufficiency (17).

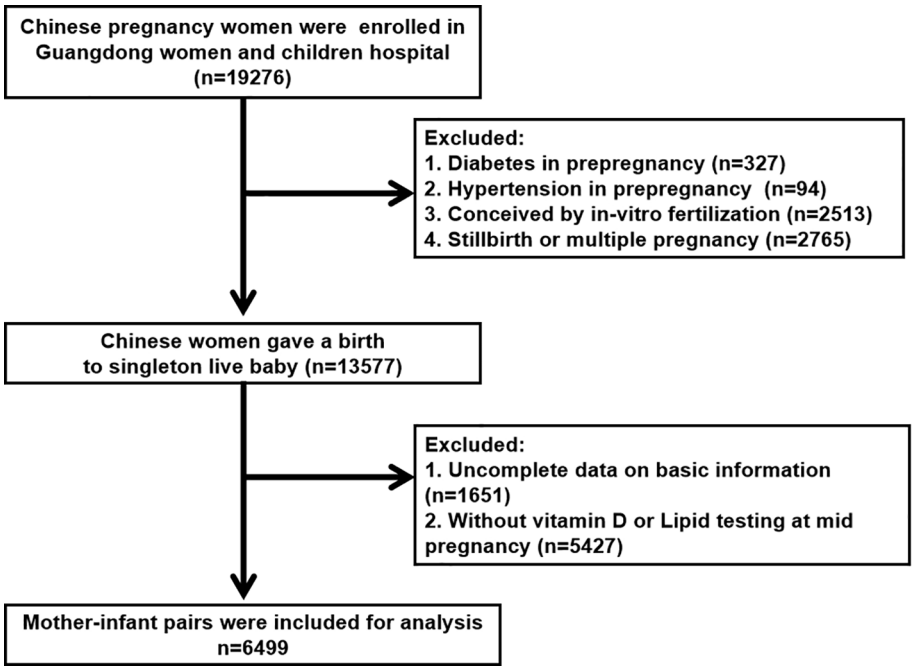

FIGURE 1
Flow chart for screening eligible participants.

Newborns were classified as appropriate for gestational age (AGA), small for gestational age (SGA), or large for gestational age (LGA) based on Neonatal Birth Weight for Gestational Age and Percentile in 23 Cities of China. LGA was defined as birth weight above the 90th percentile, SGA as birth weight below the 10th percentile, and AGA as birth weight between the 10th and 90th percentiles (18).

2.4 Statistical analyses

Descriptive statistics were used to summarize the baseline data of the study participants. Continuous variables are reported as mean (standard deviation, SD) or median (interquartile range, IQR), while categorical variables are expressed as percentages. Non-parametric tests were used to compare continuous variables, and chi-square tests were used to compare categorical variables.

The Shapiro Wilk normality test was performed to verify the distribution of vitamin D and lipid profiles, which were right-skewed. To achieve a normal distribution, the raw values were log2-transformed. Spearman's correlation coefficients (rs) were calculated to analyze the correlations between the log2-transformed concentrations of vitamin D and lipids.

Multivariate linear and logistic regression analyses were conducted to evaluate the association between serum lipid profiles and vitamin D concentration or status during mid-pregnancy. For LGA and SGA infants, multiple logistic regression analyses were performed to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) based on TC, TG, HDL-C, LDL-C, and vitamin D concentrations. Pregnant women of normal weight served as the reference group. Regression models included potential covariates based on relevant reports. Subgroup analyses were conducted according to the maternal vitamin D status. Furthermore, the combined effects of vitamin D status and lipid concentration (TC, TG, HDL-C, and LDL-C) in the second trimester on LGA and SGA infants were investigated by adding the product interaction term of vitamin D status × lipid concentration to the models. A p-value for interaction less than 0.15 was used as a cutoff to explore the potential effect modification through stratification (19, 20).

All statistical analyses were performed using SPSS (version 26.0; SPSS, Chicago, IL, USA) and R version 3.3.3 (R Foundation for Statistical Computing). Statistical significance was defined as $p < 0.05$.

3 Results

A total of 6499 mother-infant pairs were included in this study, and their detailed demographic characteristics are presented in Table 1. Of the participants, the average age was 30.15 ± 4.30 years, 2947 (45.3%) were nulliparous, and 2345 (36.1%) underwent cesarean section. Only one woman had a history of smoking, and none of them smoked during pregnancy. Moreover, 4376 (67.3%) had a college degree or higher. The mean gestational age at lipids testing were 18.56 ± 3.82. Additionally, 5.3% (n = 342) of mothers experienced hypertensive disorders of pregnancy, and 17.9% (n = 1163) were diagnosed with gestational diabetes mellitus. The seasonal distribution of vitamin D testing in the second trimester was nearly equal between Fall and Winter, whereas spring had the highest percentage (34.8%). Additionally, there was seasonal variation in serum 25(OH)D concentration in this study.

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TABLE 1 Clinical data of the study population.

Characteristics	Mean ± SD or n (%)
Maternal age (years)	30.15 ± 4.30
parity	
Multiparous	3552 (54.7%)
Nulliparous	2947 (45.3%)
Education level	
College	4376 (67.3%)
High School	1019 (15.7%)
< High School	1104 (17%)
Cesarean section	2345 (36.1%)
Pre-pregnancy BMI (kg/m^2)	21.04 ± 4.83
BMI status	
Underweight	4348 (66.9%)
Normalweight	1262 (19.4%)
Overweight	717 (11%)
Obesity	172 (2.6%)
GWG	13.74 ± 4.56
GDM	1163 (17.9%)
HDP	342 (5.3%)
PTB	310 (4.8%)
Season at Vitamin D testing	
Spring	2262 (34.8%)
Summer	1604 (24.7%)
Autumn	1243 (19.1%)
Winter	1390 (21.4%)
Gestational age at lipid testing	18.56 ± 3.82
Neonatal characteristics	
Boys	3429 (52.8%)
SGA	635 (9.8%)
LGA	447 (6.9%)
Birth weight (kg)	3.19 ± 0.43
Length (cm)	33.52 ± 1.35
Head (cm)	49.46 ± 1.93
Gestational age (weeks)	39.23 ± 1.42

SD, standard deviation; BMI, body mass index; GWG, Gestational weight gain; HDP, hypertensive disorders of pregnancy; GDM, gestational diabetes mellitus; SGA, small for gestational age; LGA, large for gestational age.

Maternal 25(OH)D was highest in summer (62.12 ± 22.81 nmol/L, n = 1604) followed by autumn (60.15 ± 21.23 nmol/L, n = 1243), winter (54.17 ± 21.78 nmol/L, n = 1390) and spring (54.04 ± 21.54 nmol/L, n = 2262), respectively (Supplementary Figure S1). Among the infants, 52.8% (n = 3429) were male. The mean birth weight, length, and gestational age at birth for the infants were 3.19 ± 0.43 kg, 49.46 ± 1.93 cm, and 39.23 ± 1.42 weeks, respectively.

The median (25th–75th) values of the four lipid parameters in the second trimester were as follows: 1.83 (1.32–2.15) mmol/L for TG, 5.61 (4.85–6.24) mmol/L for TC, 1.89 (1.65–2.10) mmol/L for HDL-C, and 3.09 (2.56–3.56) mmol/L for LDL-C (Table 2). The overall range of vitamin D concentrations was 10.5–159.2 nmol/L, with a mean ± SD of 57.23 ± 22.14 nmol/L. 20.7% of the patients (n=1350) were classified into the sufficient vitamin D group. Women with sufficient vitamin D in mid pregnancy have higher cholesterol levels than those with non-sufficient vitamin D (Supplementary Table S1). Similar findings were also observed in Spearman correlation analysis, it is suggested that vitamin D concentrations was correlated with cholesterol levels except for TG (Supplementary Table S2).

The prevalence of LGA and SGA infants was 6.9% (n = 447) and 9.8% (n = 635), respectively. Figure 2 presents the lipid profiles and vitamin D concentrations in the AGA, SGA, and LGA groups. Women with infants born LGA exhibited higher levels of TG and LDL-C compared to women with infants born AGA. Conversely, the TG, TC, and LDL-C levels were significantly lower compared than in the control group ($p < 0.05$) in the SGA group. In addition, women with a LGA newborn had lower levels of HDL-C. Table 3 presents the association between maternal lipid parameters in the second trimester and the risk of LGA or SGA. TG levels were positively associated with the risk of LGA (OR=1.41, 95% CI 1.17–1.70, $p < 0.001$), while maternal TG, TC, and LDL-C were negatively associated with the risk of SGA. Additionally, higher HDL-C levels in mothers were associated with a lower likelihood of delivering an LGA infant (OR = 0.58, 95% CI 0.39–0.85). No significant association was found between the vitamin D status and the risk of LGA or SGA infants (all $p > 0.05$).

The effect of vitamin D on the association between lipid profiles and risk of LGA or SGA was explored by dividing the study population into two different vitamin D categories. Although no interaction effect was observed among these birth outcomes (p for interaction > 0.15), the effect of the lipid profile differed because of the vitamin D status (Table 4). For TG, mothers in the vitamin D non-sufficiency group with higher TG level was related to an increased risk (OR=1.40, 95% CI:1.13–1.74) for LGA. Regarding

TABLE 2 Distributions of maternal vitamin D and lipid profiles in the second trimester.

Analytes	Mean	GM	Percentiles		
			25	50	75
TG	1.83	1.69	1.32	1.67	2.15
TC	5.61	5.51	4.85	5.50	6.24
HDL-C	1.89	1.86	1.65	1.87	2.10
LDL-C	3.09	2.99	2.56	3.04	3.56
vitamin D	57.23	52.83	40.3	55.4	71.9

TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; GM, Geometric Mean.

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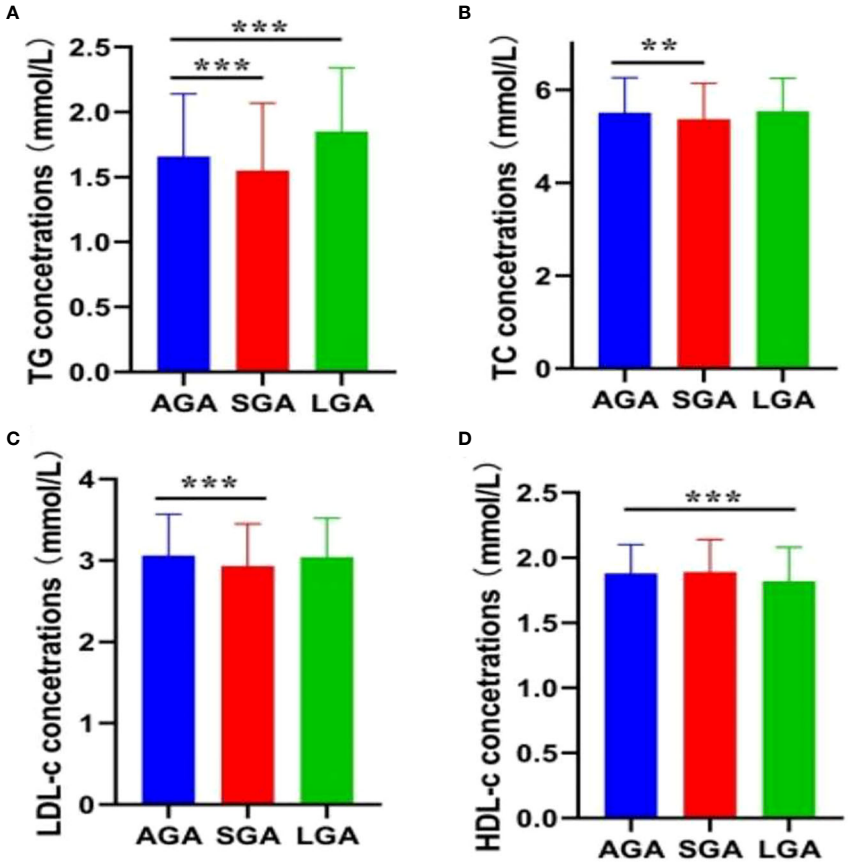

FIGURE 2
Maternal lipid profile in mid-pregnancy and fetal growth. **(A)** TG; **(B)** TC; **(C)** LDL-C; **(D)** HDL-C. Error bars are presented as mean (SD) for continuous variables with a normal distribution, or as median (90% range) for continuous variables with a skewed distribution. **P < 0.01; ***P < 0.001.

cholesterol, no associations were found between the HDL-C and SGA levels in this subgroup analysis. Nonetheless, HDL-C levels were negatively associated with the risk of LGA infants regardless of the vitamin D status (OR=0.65 in pregnant women with insufficient vitamin D; OR=0.42 in the sufficient vitamin D group). Furthermore, when the population was stratified by vitamin D categories, higher levels of TC and LDL-C were associated with a decreased risk of SGA (TC: OR=0.65, 95% CI: 0.46–0.94; LDL-C: OR=0.74, 95% CI: 0.57–0.95) among pregnant women in the non-sufficient vitamin D group.

TABLE 3 The association of maternal lipid profile concentrations and vitamin D categories with LGA or SGA in early pregnancy in the second trimester.

	AGA (n=5417)	SGA (n=635) OR (95% CI)	p	LGA (n=447) OR (95% CI)	p
Lipids[a]					
TG	reference	0.74 (0.62–0.88)	0.001	1.41 (1.17–1.70)	0.000
TC	reference	0.65 (0.46–0.90)	0.01	0.95 (0.65–1.39)	0.786
HDL-C	reference	1.10 (0.79–1.54)	0.563	0.58 (0.39–0.85)	0.005
LDL-C	reference	0.75(0.59–0.94)	0.013	0.98 (0.75–1.30)	0.905
Vitamin D Binary [b]					
Sufficient group		reference		reference	
Non-Sufficient group		1.20 (0.96–1.49)	0.107	1.02 (0.80–1.31)	0.847
Vitamin D [b] (10.9–159.2nmol/L)	reference	0.92 (0.80–1.07)	0.274	0.98 (0.83–1.17)	0.850

Maternal Vitamin D and lipid profiles were log2-transformed in the model
[a]The models were adjusted for education, maternal age, parity, delivery mode, infant sex, HDP, GDM, pre-BMI, and gestational age at lipid testing.
[b]The models were adjusted for education, maternal age, parity, delivery mode, infant sex, HDP, GDM, pre-BMI, and season of vitamin D testing.

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TABLE 4 Associations between maternal lipid levels in second trimester and risk of LGA or SGA in multinomial logistic regression models, stratified by vitamin D level.

	Vitamin D status		*p* for interaction
	Non-Sufficient group	Sufficient group	
TG			
AGA	reference	reference	
SGA	0.77 (0.63–0.93)**	0.66 (0.43–1.02)	0.821
LGA	1.40 (1.13–1.74)**	1.31 (0.85–2.03)	0.416
TC			
AGA	reference	reference	
SGA	0.65 (0.46–0.94)*	0.65 (0.29–1.43)	0.859
LGA	0.96 (0.62–1.48)	0.96 (0.40–2.30)	0.487
HDL-C			
AGA	reference	reference	
SGA	1.16 (0.81–1.68)	0.82 (0.37–1.80)	0.228
LGA	0.65 (0.42–0.99)*	0.42 (0.18–0.98)*	0.300
LDL-C			
AGA	reference	reference	
SGA	0.74 (0.57–0.95)*	0.82 (0.47–1.44)	0.749
LGA	0.92 (0.68–1.25)	1.36 (0.71–2.61)	0.726

Maternal Vitamin D and lipid profiles were log2-transformed in the model
The models were adjusted for education, maternal age, parity, delivery mode, infant sex, HDP, GDM, pre-BMI, Season at vitamin D testing, and gestational age at lipid testing.
P for interaction was assessed by likelihood ratio test.
*P < 0.05; **P < 0.01.

4 Discussion

In this retrospective study, the prevalence rates of SGA and LGA in pregnant Chinese women were 9.8% and 6.9%, respectively. Only 20.5% of the participants (n=1350) demonstrated sufficient vitamin D levels during their second trimester. The TG levels during mid-pregnancy were positively associated with an increased risk of LGA infants, whereas HDL-C levels were negatively correlated with LGA risk. Maternal TG, TC, and LDL-C levels were associated with a decreased risk of being SGA; however, no significant association was observed for HDL-C. Although no significant interaction effects were identified, notable differences were observed in the subgroup analysis. Our findings suggest that TG, TC, and LDL-C levels are positively correlated with decreased odds of being SGA among pregnant women with insufficient vitamin D levels. Notably, mothers with sufficient vitamin D levels had a significantly lower risk of LGA infants than those with insufficient vitamin D levels.

Risk of LGA or SGA are associated with maternal conditions, such as maternal dietary intake, obesity, metabolic changes, genetic polymorphisms, environmental factors, and gestational weight gain. For example, we have reported that pregnant women with lower gestational weight gain and MTHFR A1298C AA genotype were more likely to experience SGA (21). A prospective multi-racial/ethnic cohort study suggested that pregnant women with poorer maternal diet in early pregnancy were more likely to have an LGA infant, even after adjustment for maternal obesity (22). As an important indicator for lipid metabolism, maternal lipid profiles are related to overnutrition and increased throughout pregnancy. This suggests that lipid profiles have play an important role in fetal growth. It was reported that higher TG levels in early pregnancy are associated with increased embryonic size, fetal head circumference, and overall growth rates (8). The pathway of TG from maternal circulation into the placenta to support fetal growth is complex because it cannot cross the placenta. Fatty acid hydrolyzed from TG can enter fetal circulation through placental trophoblasts and provided energy for the growth of fetus (23). However, hyperlipidemia can lead to adverse pregnancy complications and perinatal outcomes, potentially affecting offspring development (24, 25). In this study, we suggested a positive association between the maternal TG levels in the second trimester and the risk of LGA (OR=1.41, 95% CI=1.17–1.70), as well as a negative association between maternal TG levels and the risk of SGA (OR=0.74, 95% CI=0.62–0.88). The differential TG levels observed in our study may explain these results, as TG levels were higher in mothers of LGA infants and lower in mothers of SGA infants. Compared with normal-weight controls, we found that TG concentrations were higher in women born to LGA infants and lower in mothers with SGA infants. Maternal cholesterol is important for membrane function and development of the fetus. Recent studies have suggested that maternal TC and LDL-C levels are valuable markers of abnormal fetal development. Serizawa et al. demonstrated that lower maternal LDL-C levels in the second trimester were associated with an increased risk of delivering an SGA infant at term (26). Chen et al. reported a negative association between second trimester TC and LDL-C levels and SGA (27). Consistent with these results, our analysis showed a negative association between TC or LDL-C concentrations and the risk of SGA infants (OR=0.65 TC, OR=0.75 LDL-C). HDL-C plays an important role in cholesterol homeostasis by maintaining a favorable sterol balance in extraembryonic fetal tissues to support fetal growth and development (28). For instance, an increase of 10 mg/dL in HDL-C from preconception to 28 weeks was associated with decreased odds of LGA (OR = 0.63, 95% CI: 0.46–0.86), with a stronger association observed in women with a pre-pregnancy BMI over 25 (29). In our study, pregnant women who delivered LGA newborns had lower HDL-C levels than those who delivered AGA newborns, which is consistent with the findings of a study involving 549 pregnant Chinese women (30). Furthermore, our results indicated that higher HDL-C levels in mothers were associated with a reduced risk of LGA infants (OR=0.58), even after adjusting for pre-BMI and GDM. However, a prospective study proposed a negative association between HDL-C levels in early pregnancy and LGA, and these effects may become non-significant after adjusting for pre-pregnancy BMI and early pregnancy maternal glucose levels (8). The inconsistent results observed across studies may be attributed to differences in population settings, confounding variables, and timing of measurements. In addition, the ethnic differences and genetic factors might also modify the associations

between maternal cholesterol and birth weight (31, 32). Future multiple-ethnic studies must investigate the effect of genetic differences on the relationship between maternal lipid metabolism and fetal development.

Vitamin D, a fat-soluble vitamin biosynthesized via an ultraviolet radiation-mediated process or absorbed from dietary sources, plays a crucial role in the calcium phosphate metabolism and bone construction. In this analysis, we found that the maternal 25(OH)D concentration in second trimester were highest in summer (62.12 ± 22.81 nmol/L) and lowest in spring (54.04 ± 21.78 nmol/L). During pregnancy, low vitamin D concentrations are commonly observed in pregnant women due to the increased physiological demand for vitamin D. In this study, the mean vitamin D concentration was 57.23 nmol/L, which is similar with a retrospective cohort study conducted in Guangzhou. They reported that pregnant women exhibited an average vitamin D level of 59.3 nmol/L (33). The prevalence of insufficient vitamin D (< 75 nmol/L) was 79.2% (n = 5149), which was nearly four times higher than that in the sufficient vitamin D group. These findings are consistent with a prospective observational study conducted in Guangzhou, which reported a 67.5% prevalence of insufficient vitamin D among pregnant women (34). Similar results were observed in pregnant women from Brazil (69%), Kenya (74.4%), and rural Bangladesh (64.5%) (35–37). Although increased studies have shown that vitamin D deficiency in serum during pregnancy is closely related to a series of adverse pregnancy outcomes (38, 39), our study suggests that the vitamin D status at mid-pregnancy, even in the vitamin D-deficient group, was not associated with LGA or SGA.

Several studies have shown that the vitamin D status may be related to improvements in lipid profiles. For example, a prospective birth cohort study of 6714 pregnant women in Hefei (another city in China) suggested that increased serum vitamin D levels were significantly associated with decreased maternal TC, TG, HDL-C, and LDL-C levels in the second trimester (14). Sharif-Askari et al. found that vitamin D deficiency was associated with HDL-C dyslipidemia in insulin-resistant individuals (40). Pregnant women with sufficient vitamin D have higher cholesterol levels (TC, HDL-C, and LDL-C) than those with non-sufficient vitamin D in our study population. This may be because vitamin D and cholesterol metabolism share a similar biosynthetic pathway. Additionally, vitamin D exerts a potent anti-lipolytic action, increases the intracellular calcium levels, regulates the renin-angiotensin system, and suppresses lipolysis in human adipocytes (41). Vitamin D can directly and indirectly influence lipid levels through its effects on serum parathyroid hormone (PTH) and calcium balance, thereby regulating lipid metabolism (42). However, there is no consensus on the association between vitamin D and lipid metabolism. In zebrafish model, vitamin D was reported to reduce the deposition of lipid via regulation of mitochondrial biogenesis (11). Considering the effect of vitamin D on fat storage and lipid metabolism, we hypothesized that vitamin D may have a modifying effect on the association between lipid levels and LGA and SGA. In the subgroup analysis, the effects of TG, TC, and LDL-C on LGA or SGA risks were only observed in the vitamin D insufficient group. Furthermore, a higher HDL-C level was associated with a lower likelihood of giving birth to an LGA infant among pregnant women with sufficient vitamin D levels in the second trimester (OR = 0.42) than among those with insufficient vitamin D levels (OR = 0.65). The vitamin D status in a sufficient status appears to have a beneficial effect in reducing the serum TC, LDL-C, and TG levels (43). Although the effect of dietary intake did not evaluate on the level of vitamin D and lipid profile in the present study, our results suggest that the vitamin D status at mid-pregnancy may modify the association between the lipid profile and risk of LGA or SGA.

In this study, we conducted a comprehensive investigation involving 6499 mother-infant pairs to assess the association between vitamin D levels, lipid profiles in the second trimester, and the occurrence of SGA or LGA. Additionally, we explored the potential effect of vitamin D status on the association between maternal lipid metabolism and risk of SGA or LGA. Our findings suggest that pregnant women with abnormal lipid profiles should be monitored for their vitamin D status to mitigate the risk of SGA or LGA. However, it is important to acknowledge the limitations of this study. First, we collected serum samples during the second trimester, although it is recommended to collect maternal lipid concentrations throughout pregnancy and before conception. Second, our analysis did not account for various potential confounding factors, such as eating patterns, vitamin D supplementary, physical activity, and other environmental exposures, which may have influenced the reliability of our results. Further investigations with larger sample sizes, diverse populations, and prospective study designs are necessary to validate the association between maternal vitamin D levels and subsequent delivery outcomes.

5 Conclusion

In summary, our retrospective study, based on a Chinese population encompassing 6499 mother-infant pairs, examined the relationship between vitamin D levels, lipid profiles, and the risk of SGA or LGA. We observed a significant association between vitamin D and cholesterol levels during mid-pregnancy. Moreover, our findings provide evidence that the vitamin D status may modify the association between HDL-C levels and the risk of LGA. These results could serve as guidelines for managing lipid profiles and nutritional interventions during pregnancy to improve birth outcomes in Chinese populations. However, further investigations with larger sample sizes, diverse populations, and prospective or multicenter designs are warranted to confirm and expand upon our findings.

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 the Ethics Committee of the Guangdong Women and Children's Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

XHZ: Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – original draft. KL: Data curation, Investigation, Software, Writing – original draft. CL: Investigation, Methodology, Project administration, Writing – original draft. YC: Conceptualization, Data curation, Methodology, Writing – original draft. XDZ: Data curation, Investigation, Methodology, Validation, Writing – original draft. WW: Formal analysis, Software, Supervision, Writing – original draft. ML: Conceptualization, Supervision, Writing – review & editing. CG: Conceptualization, 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1297373/full#supplementary-material

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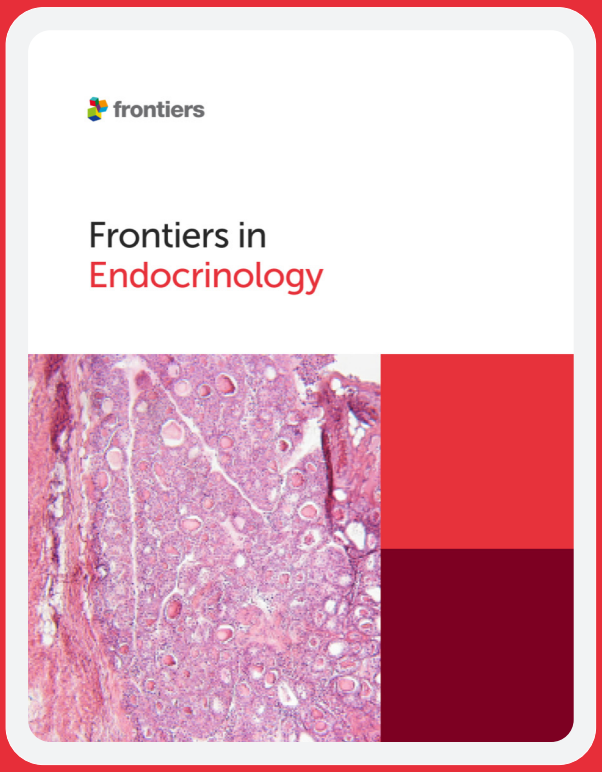

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