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RESEARCH ARTICLE

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Excessive bile acids level predisposes to adverse perinatal outcomes in women with abnormal pre-pregnancy body mass index

Yulai Zhou[a,b#], Juan Li[c#], Jinwen Zhang[b,d#], Huan Li[e], Fuzhen Song[f], Wei Gu[a,b] and Weibin Wu[b,d]

[a]Department of Obstetrics, The International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; [b]Shanghai Key Laboratory of Embryo Original Diseases, Shanghai, China; [c]Department of Pathology, The International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; [d]Department of Biobank, The International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; [e]Department of Obstetrics and Gynecology, Songjiang Maternity and Child Health Hospital, Shanghai, China; [f]Department of Radiology, The International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

ABSTRACT

Background: Both low/high pre-pregnancy body mass index (BMI) and increased bile acid levels during pregnancy (known as gestational hypercholanemia) were risk factors for adverse pregnancy outcomes, with limited information on their joint effects.

Methods: A total of 63,066 pregnant women were involved in a large retrospective cohort study from May 2014 to December 2018 in Shanghai, China. Data of pregnancy outcomes including hypercholanemia, hypertensive disorders in pregnancy (HDP), preterm delivery, and small for gestation age (SGA), were obtained for multivariable logistic analysis.

Results: Pre-pregnancy BMI was negatively associated with serum total bile acid (TBA) concentrations during gestation and the risk of hypercholanemia ($p < 0.001$). Low pre-pregnancy BMI and hypercholanemia coexisting were related to a 2.71-fold risk (95% confidence intervals [CI], 2.10-3.50) of SGA. Whereas, overweight/obese (OWO) with hypercholanemia are associated with 5.34-fold risk (95% CI 3.93-7.25) of HDP when compared with normal weight women without hypercholanemia. Women with excessive gestational weight gain (GWG) and hypercholanemia had a higher risk of HDP (odds ratio [OR] 3.56, 95% CI 2.91-4.36), and macrosomia (OR 2.95, 95% CI 2.42-3.60), compared with non-hypercholanemia women with adequate GWG. Whereas, women with inadequate GWG and hypercholanemia had increased risks of preterm delivery (OR 1.87, 95% CI 1.44-2.43), and SGA (OR 2.32, 95% CI 1.82-2.96).

Conclusions: Low maternal BMI before pregnancy was an independent risk factor for hypercholanemia. Additionally, pre-pregnancy underweight or OWO may amplify the effect of hypercholanemia on adverse pregnancy outcomes. Thus, pre-pregnancy BMI should be considered in the management of adverse perinatal outcomes related to gestational hypercholanemia.

KEY MESSAGES

Low maternal BMI before pregnancy was a risk factor for gestational hypercholanemia. Pre-pregnancy overweight mothers with hypercholanemia had a higher risk of hypertensive disorders of pregnancy, while underweight mothers with hypercholanemia had a higher risk of delivering small for gestational age infants. These findings emphasize the importance of considering pre-pregnancy BMI as a stratification factor in the management of adverse maternal and neonatal outcomes related to hypercholanemia.

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Pre-pregnancy BMI; total bile acid; gestational hypercholanemia; adverse pregnancy outcomes; joint effect

Introduction

During normal pregnancy, the concentration of serum total bile acid (TBA) tends to exhibit a slight elevation compared to the pre-pregnancy period as a consequence of metabolic alterations and fluctuating hormone levels. Nevertheless, a minority of pregnant women surpass the normal range, a condition known as gestational hypercholanemia [1]. Disrupted TBA levels are closely linked to intrahepatic cholestasis of

CONTACT Wei Gu krisgu70@163.com; Weibin Wu wuweibin01@gmail.com Shanghai Key Laboratory of Embryo Original Diseases, Shanghai, China.

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[#]These authors contributed equally to this work.

pregnancy (ICP), hepatic injury, and preeclampsia, *etc* [2]. Acting as a crucial metabolic regulator, bile acids take an irreplaceable role in facilitating the absorption of nutrients through biliary pathways. However, despite their important role, supraphysiological concentrations during pregnancy can exert cytotoxic effects on various organs such as the liver, placenta, and fetal heart [3]. Dysregulated TBA has been found to be attributable to antenatal complications, including impaired glucose tolerance, dyslipidemia, gestational diabetes, and preeclampsia [4–6]. Moreover, Dysregulated maternal TBA level poses threats to fetal well-being, leading to preterm delivery, meconium-stained amniotic fluid, fetal hypoxia, and intrauterine fetal demise [7–11]. Our previous study has demonstrated that women with hypercholanemia were at a 29% heightened risk of delivering infants with low birth weight and more than double the incidence of intrauterine growth restriction [12].

Inconsistent evidence across different populations has suggested a possible association between abnormal pre-pregnancy BMI and gestational hypercholanemia [13,14]. Previous studies indicated a prevalent coexistence of ICP and obesity/overweight (OWO) in European and American populations [4,15]. However, recent prospective cohort and cross-sectional studies completed in China have proposed that low pre-pregnancy BMI and inadequate gestational weight gain may serve as potential risk factors for ICP [16,17]. Furthermore, both pre-pregnancy underweight and OWO have been recognized as risk factors for adverse pregnancy outcomes [18]. Our recent experimental study has demonstrated that excessive bile acid can exacerbate placental trophoblast injury under nutritional stress conditions, thereby adversely affecting fetal health [19]. Nonetheless, it is unclear whether pre-pregnancy BMI has a combined effect on the risk of adverse pregnancy outcomes in association with elevated TBA levels during pregnancy.

Therefore, this retrospective cohort study endeavored to elucidate the relationship between maternal pre-pregnancy BMI and dysregulated TBA concentrations during pregnancy. Additionally, we sought to explore the combined effect of maternal BMI before pregnancy and dysregulated TBA levels during gestation on the hazard of adverse pregnancy outcomes.

Patients and methods

Study participants

This study adopted data from a large retrospective birth cohort conducted in the International Peace Maternity and Child Health Hospital (IPMCH), Shanghai, China [12]. Similar to some large cohorts, the data of this cohort contains basic population characteristics, biochemical laboratory results, anthropometric data measured by hospital staff. Hence, various investigations within the scope of our research objectives may be carried out and evaluated individually to address distinct research inquiries. In compliance with regional regulations, the corresponding author is able to furnish the dataset for the study upon a reasonable request.

Pregnant women who had completed records of antenatal visits and delivery between May 2014 to December 2018, and had full records of pre-pregnancy BMI as well as TBA measurements during pregnancy were selected as eligible participants. Further exclusion criteria were *in vitro* fertilization (IVF), multiple pregnancies, fetal death or chromosome abnormality, pregnancies complicated with gallbladder disease, acute fatty liver disease, severe hepatic dysfunction (i.e. alanine transaminase [ALT] or aspartate aminotransferase [AST] exceeding 100 U/L) and HELLP's syndrome, with history of gallbladder diseases and hepatoma, severe hepatitis or other severe hepatic injury, and pre-existed diabetes or hypertension before pregnancy.

Blood biochemical test data

Fasting blood samples were gathered from pregnant women during their routine perinatal visits. The blood was obtained from the median cubital vein to generate serum samples. The quantification of TBA and ALT concentrations was performed per the manufacturer's instructions in the hospital laboratory on a Cobas c702 chemistry analyzer (Roche Diagnostics). The data of TBA and ALT determined at different stages of gestation: first trimester (8–14 weeks), third trimester (28–41 weeks), and the peak level throughout the entire pregnancy was retrieved.

Diagnostic criteria

Gestational hypercholanemia was defined as TBA concentration reached 10 μmol/L or higher [20]. Gestational hypercholanemia that manifests before the 28th week of gestation is categorized as early-onset hypercholanemia while hypercholanemia occurs after the 28th gestational week is defined as late-onset hypercholanemia. In addition, women with TBA levels above 40 μmol/L were diagnosed as for severe hypercholanemia while women with TBA levels ranging from 10 to 39.9 μmol/L were determined as for mild hypercholanemia.

Maternal pre-pregnancy BMI was calculated by dividing her self-reported body weight in kilograms prior to gestation by her height in square meters. The

categorization of maternal pre-pregnancy BMI followed the recommended reference for Chinese adults: underweight (BMI < 18.5 kg/m^2), normal weight (BMI between 18.5–23.9 kg/m^2), and OWO (BMI ≥ 24.0 kg/m^2) [21]. According to the recommendations of the United States Institute of Medicine (IOM), total gestational weight gain (GWG) was classified as "adequate" if falling within the range of 12.5–18 kg for women with a pre-gestational underweight, 11.5–16 kg for those with normal weight, 7–11.5 kg for those with overweight, and 5–9 kg for those with obesity [22]. GWG was deemed "inadequate" if it fell below this range, and "excessive" if it exceeded this range.

Small-for-gestational-age (SGA) and large-for-gestational-age (LGA) were determined based on birth weight percentile. SGA infants was characterized by a birth weight below the 10th percentile, while LGA pertained to neonates with a birth weight above the 90th percentile. These definitions took into account the infant's gender and gestational age, following the birth weight reference curve for Chinese neonates [23]. Macrosomia was characterized by a fetal birth weight exceeding 4000 grams, while low birth weight (LBW) was determined if the neonatal birth weight was under 2500 grams.

The diagnosis of gestational diabetes mellitus (GDM) involved a 2-hour 75 g oral glucose tolerance test conducted between the 24th and the 28th gestational week, with reference to the criteria set by the American Diabetes Association [24]. Hypertensive disorders of pregnancy (HDP) comprised gestational hypertension and pre-eclampsia. The diagnosis was made when blood pressure measurements were consistently equal to or more than 140 mmHg systolic or 90 mmHg diastolic, recorded at not less than twice within a 4-6 h period, with or without proteinuria [25]. Preterm delivery (PTD) was featured by giving birth before the 37th gestational week. The rate of neonatal intensive care unit (NICU) admission was also included as a neonatal outcome.

Statistical analyses

For the baseline characteristics of the study population the continuous and categorical variables were represented as median (interquartile range) and frequency (percentage), respectively. For better model assumptions in the subsequent analysis, the TBA level was logarithmically transformed.

The associations between maternal pre-pregnancy BMI and TBA peak concentration and hypercholanemia was examined using generalized linear models with restricted cubic splines. Multivariable linear and logistic regression models were utilized to investigate the relationships between pre-pregnancy BMI categories and TBA concentrations, as well as the risk of hypercholanemia, respectively. The multivariable analyses were adjusted for potential confounders, involving maternal educational backgrounds, age, parity, ALT level, and medical insurance. These adjustments were based on clinical significance and previous reports [12]. We also performed sensitivity analysis on the associations between gestational hypercholanemia, pre-pregnancy BMI and adverse pregnancy outcomes, by excluding women receiving ursodeoxycholic acid (UDCA) treatment.

The statistics were all conducted two-sided, and a significance level of 0.05 was considered as the threshold of statistical significance. The regression models were conducted using R software version 4.3.2 (R Project for Statistical Computing; https://www.R-project.org/), utilizing packages such as *rms, ggplot2,* and *tableone*.

Results

Population characteristics

The ultimate study population consisted of 63,066 pregnant women (Figure 1). Table 1 presents a comparison of baseline characteristics and clinical data between the hypercholanemia and non-hypercholanemia groups.

There were no significant differences observed between these two groups with regards to parity, incidence of macrosomia, LGA, and neonatal intensive care unit (NICU) admission. However, several notable differences were identified. For example, women with hypercholanemia were more likely to be above 35-year-old and with a lower pre-pregnancy BMI. Additionally, they had a higher likelihood of undergoing cesarean section (C-section), experiencing HDP, PTD, carrying a male fetus, and giving birth to LBW or SGA infants. Besides, it was observed that levels of TBA in early pregnancy, late pregnancy, and the peak level throughout the entire pregnancy were significantly higher in women with hypercholanemia in comparison with those without hypercholanemia ($p < 0.001$ for all comparisons).

Associations of pre-pregnancy BMI with maternal TBA concentrations and risk of hypercholanemia

Compared to women with a normal pre-pregnancy BMI, women with low pre-pregnancy BMI were found to have a higher risk of early-onset hypercholanemia (crude odds ratio [cOR] 1.43, 95% CI 1.24-1.65; adjusted

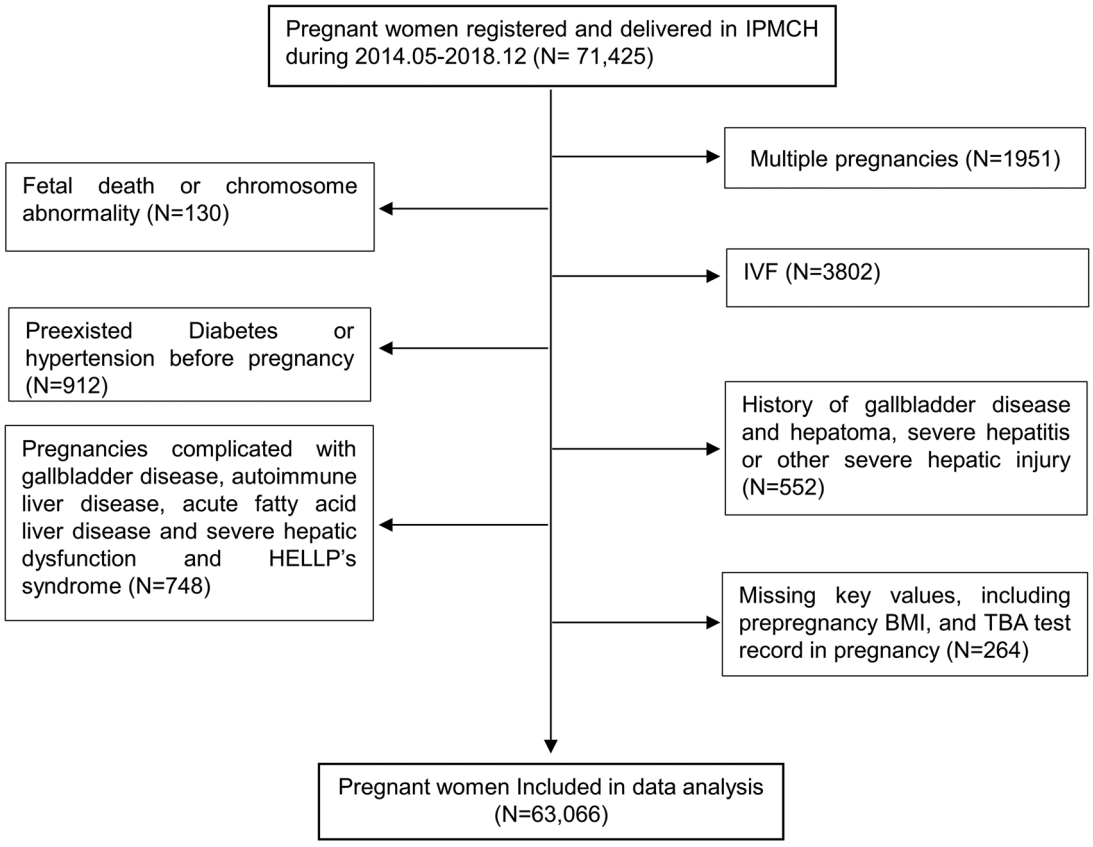

Figure 1. Flowchart illustrating the inclusion and exclusion process for study participants. This figure demonstrates the step-by-step flow of participant selection, indicating the inclusion and exclusion criteria applied during the study.
Abbreviations: IPMCH, International Peace Maternity and Child Health Hospital; IVF, in vitro fertilization; BMI, body mass index; TBA, total bile acid; HELLP's syndrome, hemolysis, elevated liver enzymes and low platelets count syndrome.

odds ratio [aOR] 1.44, 95% CI 1.24-1.66), mild hypercholanemia (cOR 1.19, 95% CI 1.09-1.30; aOR 1.25, 95% CI 1.15-1.37), and overall hypercholanemia (cOR 1.19, 95% CI 1.09-1.30; aOR 1.26, 95% CI 1.15-1.37) (Table 2). The crude model did not find a statistically significant link between pre-pregnancy underweight and late-onset hypercholanemia, but after accounting for confounders, it was found that underweight women had a higher risk of late-onset hypercholanemia (aOR 1.16, 95% CI 1.04-1.29; Table 2).

Combined effect of pre-pregnancy BMI and hypercholanemia on adverse maternal and fetal outcomes

We further investigated the combined effect of pre-pregnancy BMI and hypercholanemia on adverse maternal and fetal outcome. Among women with normal pre-pregnancy BMI (Table 3), women with hypercholanemia were at an elevated risk of HDP (aOR 1.80, 95%CI 1.53-2.11), PTD (aOR 1.29, 95%CI 1.10-1.51), LBW (aOR 1.39, 95%CI 1.13-1.73), SGA (aOR 1.31, 95%CI 1.10-1.57), and LGA (aOR 1.15, 95%CI 1.02-1.29). Comparing with women with normal pre-pregnancy BMI and normal TBA concentrations, women with hypercholanemia and low pre-pregnancy BMI had a greater chance of delivery LBW (aOR 1.93, 95%CI 1.34-2.79), and SGA infants (aOR 2.71, 95%CI 2.10-3.50). Additionally, women with hypercholanemia and pre-pregnancy OWO were at an increased risk of HDP, GDM, PTD, LGA, and macrosomia. Specifically, they had the highest risk of developing HDP (aOR 5.34, 95% CI 3.93-7.25). However, there were no apparent discrepancies observed among the groups regarding NICU admission rates. To examine the potential impact of medication treatment for gestational hypercholanemia, we further analyzed the combined effect of pre-pregnancy BMI and hypercholanemia on pregnancy outcomes by excluding women who received the first-line drug (ursodeoxycholic acid) and observed similar results (Table S1 *in the* supplementary material).

In Figure 2, we illustrated the dose-response associations between gestational TBA levels, hypercholanemia, and the risk of SGA and HDP, stratified by maternal pre-pregnancy BMI categories. Interestingly, SGA was more common in women with hypercholanemia and pre-pregnancy underweight than in non-hypercholanemia

Table 1. Basic characteristics of study population.

	Non- hypercholanemia (*N* = 59,132)	Hypercholanemia (*N* = 3934)	p Values
Maternal characteristics			
Age, years, Mean (SD), n (%)	30.5 (3.8)	30.7 (3.9)	0.027
<25	1756 (3.0)	119 (3.0)	0.005
25–35	48,053 (81.3)	3119 (79.3)	
≥ 35	9323 (15.8)	696 (17.7)	
Pre-pregnancy BMI, kg/m^2, n (%)	21.0 (2.7)	20.6 (2.4)	<0.001
<18.5	8634 (14.6)	696 (17.7)	<0.001
18.5–23.9	43,224 (73.1)	2923 (74.3)	
≥24	7274 (12.3)	315 (8.0)	
Parity			
1	42,958 (72.6)	2837 (72.1)	0.472
≥ 2	16,174 (27.4)	1097 (27.9)	
Education degree, n (%)			
High school and below	4330 (7.3)	305 (7.8)	0.001
College or equivalent	43,120 (72.9)	2759 (70.1)	
Graduate	11,682 (19.8)	870 (22.1)	
Delivery method, n (%)			
Vaginal	34,644 (58.6)	2153 (54.7)	<0.001
C-section	24,488 (41.4)	1781 (45.3)	
HDP, n (%)	2479 (4.2)	266 (6.8)	<0.001
GDM, n (%)	7282 (12.3)	436 (11.1)	0.024
PTD, n (%)	2798 (4.7)	238 (6.0)	<0.001
TBA concentrations, Median (IQR), μmol/L			
early pregnancy	1.8 [1.2, 2.7]	2.5 [1.6, 3.9]	<0.001
late pregnancy	4.2 [3.1, 5.6]	12.3 [10.8, 15.6]	<0.001
peak during whole pregnancy	4.3 [3.2, 5.7]	12.5 [10.9, 15.8]	<0.001
Fetal characteristics			
Sex, n (%)			
Male	30,421 (51.4)	2120 (53.9)	0.003
Female	28,711 (48.6)	1814 (46.1)	
Birthweight, g	3336.0 (428.7)	3310.9 (462.6)	0.001
LBW, n (%)	1533 (2.6)	145 (3.7)	<0.001
Macrosomia, n (%)	3303 (5.6)	228 (5.8)	0.567
SGA, n (%)	2343 (4.0)	221 (5.6)	<0.001
LGA, n (%)	5835 (9.9)	413 (10.5)	0.205
NICU admission	507 (0.9)	35 (0.9)	0.789

Abbreviations: TBA, serum total bile acids; BMI, body mass index; LBW, low birth weight; SGA, small for gestation age; LGA, large for gestation age; PTD, preterm delivery; SD, standard deviation; IQR, interquartile ranges; C-section, Cesarean section; HDP, Hypertensive disorder in pregnancy; GDM, Gestational diabetes mellitus; NICU, neonatal intensive care unit.

women with a normal pre-pregnancy BMI, whereas no such trend was identified in women with pre-pregnancy OWO (Figure 2A, B). Furthermore, women with hypercholanemia and pre-pregnancy OWO had more than a 5-fold increased risk of HDP compared to non-hypercholanemia women with a normal pre-pregnancy BMI, whereas no significant increase in HDP risk was observed among underweight women complicated with hypercholanemia (Figure 2C, D). Moreover, it was shown that the risk of macrosomia between women complicated with or without hypercholanemia was similar across different pre-pregnancy BMI categories (Figure S1 *in the* supplementary material).

Differential effects of early- and late-onset hypercholanaemia on adverse maternal and fetal outcomes

In comparison with women without hypercholanemia, those with early-onset hypercholanemia demonstrated increased risks of HDP (aOR 1.38, 95%CI 1.08-1.76), PTD (aOR 2.66, 95%CI 2.23-3.19), LBW (aOR 2.71, 95%CI 2.15-3.41), SGA (aOR 1.30, 95%CI 1.00-1.68), and NICU admission (aOR 2.98, 95%CI 2.05-4.33). Additionally, elevated risks of HDP (aOR 1.67, 95%CI 1.44-1.95) and SGA (aOR 1.52, 95%CI 1. 95%CI 1.29-1.80) were observed among women with late-onset hypercholanemia (Table 4).

Furthermore, we explored the effects of early- and late-onset hypercholanaemia on pregnancy outcomes among women in differed pre-pregnancy BMI

Table 2. Risk of gestational hypercholanemia in women with different pre-pregnancy BMI categories.

hypercholanemia and its subtypes	BMI < 18.5 (*N* = 9330)			BMI 18.5 ~ 23.9 (*N* = 46,147)			BMI ≥24 (*N* = 7589)		
	N (%)	cOR 95% CI	aOR 95% CI [a]	N (%)	cOR 95% CI	aOR 95% CI[a]	N (%)	cOR 95% CI	aOR 95% CI [a]
Overall hypercholanemia	696 (7.46)	**1.19 (1.09-1.30)**	**1.26 (1.15-1.37)**	2923 (6.33)	Ref.	Ref.	315 (4.15)	**0.64 (0.57-0.72)**	**0.60 (0.53-0.67)**
Early-onset hypercholanemia	246 (2.64)	**1.43 (1.24-1.65)**	**1.44 (1.24-1.66)**	860 (1.86)	Ref.	Ref.	107 (1.41)	**0.75 (0.62-0.92)**	**0.73 (0.60-0.90)**
Late-onset hypercholanemia	450 (4.82)	1.08 (0.98-1.20)	**1.16 (1.04-1.29)**	2063 (4.47)	Ref.	Ref.	208 (2.74)	**0.60 (0.52-0.70)**	**0.55 (0.48-0.64)**
Severe hypercholanemia	22 (0.24)	1.24 (0.77-1.97)	1.39 (0.87-2.24)	88 (0.19)	Ref.	Ref.	7 (0.09)	0.48 (0.22-1.04)	**0.38 (0.17-0.82)**
Mild hypercholanemia	674 (7.22)	**1.19 (1.09-1.30)**	**1.25 (1.15-1.37)**	2835 (6.14)	Ref.	Ref.	308 (4.06)	**0.65 (0.57-0.73)**	**0.61 (0.54-0.69)**

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); cOR, crude odds ratio; aOR, adjusted odds ratio.
[a]Adjusted for maternal age, education level, parity, insurance status, and alanine aminotransferase level.

Table 3. The combined effect of pre-pregnancy BMI and hypercholanemia on adverse maternal and fetal outcomes.

Adverse maternal and fetal outcomes	BMI < 18.5		BMI 18.5 ~ 23.9		BMI ≥24	
	Non-hypercholanemia (*N* = 8634)	hypercholanemia (*N* = 696)	Non-hypercholanemia (*N* = 43,224)	hypercholanemia (*N* = 2,923)	Non-hypercholanemia (*N* = 7274)	hypercholanemia (*N* = 315)
	aOR 95% CI [a]	aOR 95% CI [a]	aOR 95% CI [a]	aOR 95% CI [a]	aOR 95% CI [a]	aOR 95% CI [a]
HDP	**0.52 (0.45-0.62)**	1.13 (0.77-1.64)	*Ref.*	**1.8 (1.53-2.11)**	**3.26 (2.98-3.57)**	**5.34 (3.93-7.25)**
GDM	**0.69 (0.64-0.76)**	**0.63 (0.47-0.84)**	*Ref.*	**0.86 (0.76-0.97)**	**2.01 (1.89-2.15)**	**2.18 (1.68-2.84)**
PTD	**1.12 (1.00-1.25)**	1.31 (0.95-1.82)	*Ref.*	**1.29 (1.10-1.51)**	**1.3 (1.17-1.45)**	**1.66 (1.10-2.51)**
LBW	**1.59 (1.39-1.81)**	**1.93 (1.34-2.79)**	*Ref.*	**1.39 (1.13-1.73)**	**1.23 (1.05-1.42)**	**2.23 (1.36-3.66)**
Macrosomia	**0.37 (0.31-0.43)**	**0.33 (0.19-0.58)**	*Ref.*	1.11 (0.95-1.3)	**2.06 (1.89-2.25)**	**2.39 (1.70-3.35)**
SGA	**1.85 (1.67-2.04)**	**2.71 (2.10-3.50)**	*Ref.*	**1.31 (1.10-1.57)**	**0.6 (0.51-0.71)**	1.21(0.69-2.12)
LGA	**0.41 (0.36-0.46)**	**0.4 (0.28-0.59)**	*Ref.*	**1.15 (1.02-1.29)**	**1.96 (1.83-2.09)**	**2.08 (1.57-2.76)**
NICU	1.25 (0.98-1.6)	0.58 (0.19-1.82)	*Ref.*	1.25 (0.85-1.85)	**1.63 (1.30-2.06)**	1.6 (0.59-4.31)

Abbreviations: TBA serum total bile acids; BMI, body mass index; LBW, low birth weight; SGA, small for gestation age; LGA, large for gestation age; PTD, preterm delivery; SD, standard deviation; IQR, interquartile ranges; C-section, Cesarean section; HDP, Hypertensive disorder in pregnancy; GDM, Gestational diabetes mellitus; NICU, neonatal intensive care unit; cOR, crude odds ratio; aOR, adjusted odds ratio.
[a]adjusted for maternal pre-pregnancy BMI, age, education level, parity, insurance status, and ALT level.

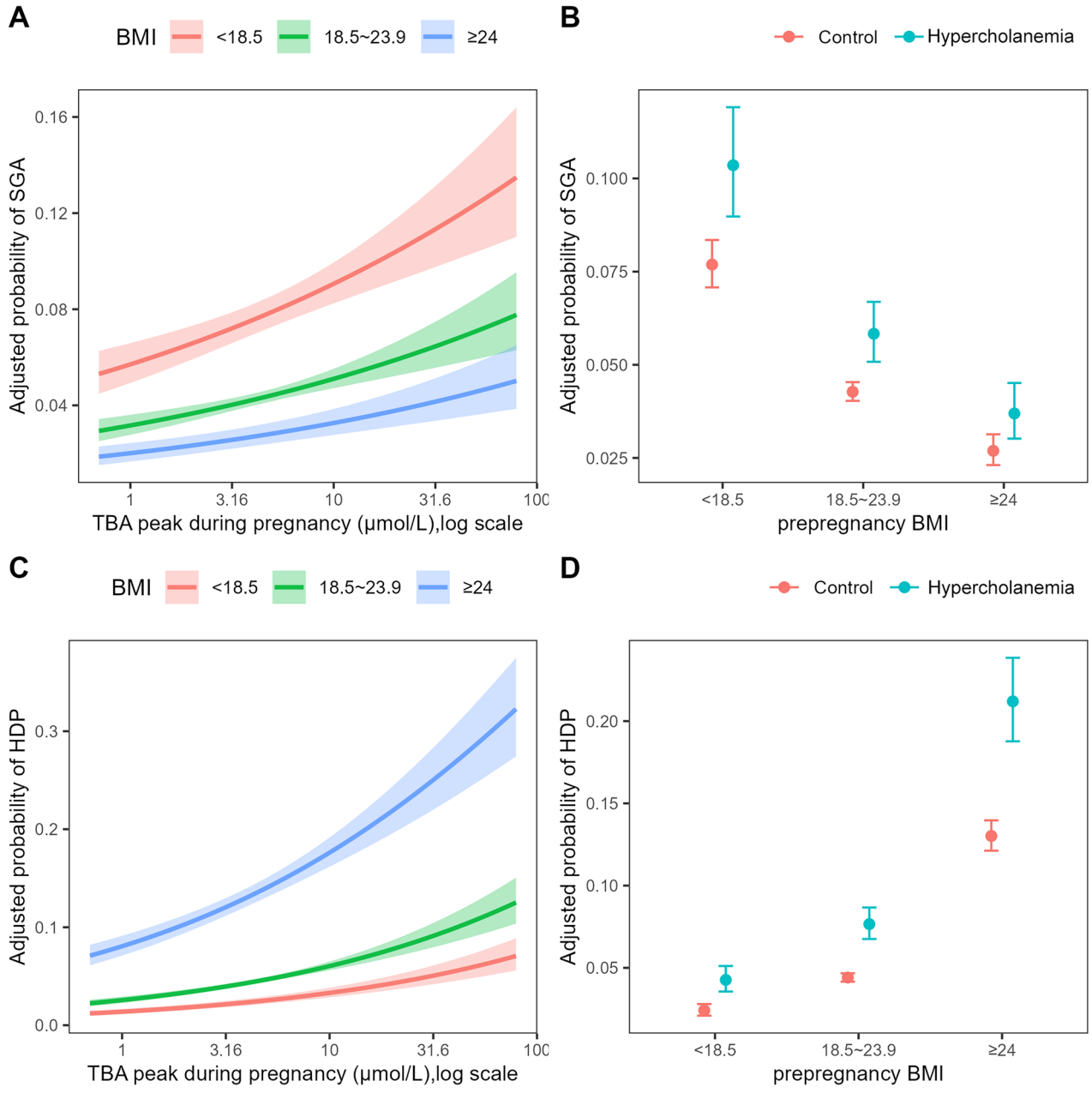

Figure 2. Association between TBA concentration in pregnancy, gestational hypercholanemia, and the risk of adverse pregnancy outcomes stratified by maternal pre-pregnancy BMI categories. (A) The dose-response relationship between maternal peak TBA concentration during pregnancy and the risk of SGA, stratified by maternal pre-pregnancy BMI categories, adjusting for maternal age, education, parity, insurance status, and ALT level. (B) The adjusted probabilities and 95% confidence intervals of SGA across different pre-pregnancy BMI categories, stratified by hypercholanemia status, with adjustment for maternal age, education, parity, insurance status, and ALT level. (C) The dose-response relationship between maternal peak TBA concentration during pregnancy and the risk of HDP, stratified by maternal pre-pregnancy BMI categories, adjusting for maternal age, education, parity, insurance status, and ALT level. (D) The adjusted probabilities and 95% confidence intervals of HDP across different pre-pregnancy BMI categories, stratified by hypercholanemia status, with adjustment for maternal age, education level, parity, insurance status, and ALT level.

Abbreviations: SGA, small for gestation age; HDP, hypertensive disorders in pregnancy; BMI, body mass index.

categories (Table S2 *in the* supplementary material). Compared to women with normal pre-pregnancy BMI and gestational sTBA levels, women with early-onset hypercholanemia and pre-pregnancy OWO were at an elevated risk of HDP (aOR 4.10, 95%CI 2.63-7.41), PTD (aOR 3.47, 95%CI 2.03-5.93), LBW (aOR 4.43, 95%CI 2.36-8.32), LGA (aOR 1.70, 95%CI 1.01-2.87, and NICU admission (aOR 4.76, 95%CI 1.74-13.03). Increased risk of HDP (aOR 6.07, 95%CI 4.21-8.74), macrosomia (aOR 2.50, 95% CI 1.66-3.76) and LGA (aOR 2.28, 95%CI

Table 4. Association between early-onset or late-onset hypercholanemia and adverse maternal and fetal outcomes.

Adverse maternal and fetal outcomes	Non-hypercholanemia (*N* = 59,132)	Early-onset hypercholanemia (*N* = 1213)	Late-onset hypercholanemia (*N* = 2721)
	aOR (95% CI) [a]	aOR (95% CI) [a]	aOR (95% CI) [a]
HDP	Ref.	**1.38 (1.08-1.76)**	**1.67 (1.44-1.95)**
GDM	Ref.	0.63 (0.51-0.77)	0.94 (0.84-1.06)
PTD	Ref.	**2.66 (2.23-3.19)**	0.69 (0.56-0.85)
LBW	Ref.	**2.71 (2.15-3.41)**	0.86 (0.66-1.11)
Macrosomia	Ref.	0.83 (0.63-1.08)	1.11 (0.95-1.30)
SGA	Ref.	**1.30 (1.00-1.68)**	**1.52 (1.29-1.80)**
LGA	Ref.	0.93 (0.77-1.13)	1.10 (0.97-1.24)
NICU	Ref.	**2.98 (2.05-4.33)**	0.21 (0.09-0.51)

Abbreviations: BMI, body mass index; HDP, Hypertensive disorder in pregnancy; GDM, Gestational diabetes mellitus; PTD, preterm delivery; LBW, low birth weight; SGA, small for gestation age; LGA, large for gestation age; NICU, neonatal intensive care unit; aOR, adjusted odds ratio; CI, confidential interval.
[a]adjusted for maternal age, pre-pregnancy BMI, education levels, parity, insurance status, ALT level.

Table 5. The combined effect of gestation weight again and gestational hypercholanemia on adverse maternal and fetal outcomes.

Adverse maternal and fetal outcomes	Inadequate GWG		Adequate GWG		Excessive GWG	
	Non-hypercholanemia (*N* = 11,773)	Hypercholanemia (*N* = 881)	Non-hypercholanemia (*N* = 21,414)	Hypercholanemia (*N* = 1463)	Non-hypercholanemia (*N* = 21,404)	Hypercholanemia (*N* = 1267)
	aOR 95% CI [a]	aOR 95% CI [a]	aOR 95% CI [a]	aOR 95% CI [a]	aOR 95% CI [a]	aOR 95% CI [a]
HDP	0.84 (0.73-0.97)	**1.44 (1.03-2.02)**	*Ref.*	**1.84 (1.44-2.35)**	**2.28 (2.06-2.51)**	**3.56 (2.91-4.36)**
GDM	**1.99 (1.87-2.12)**	**1.75 (1.47-2.09)**	*Ref.*	0.76 (0.63-0.91)	0.79 (0.74-0.84)	0.63 (0.51-0.78)
PTD	**1.75 (1.58-1.94)**	**1.87 (1.44-2.43)**	*Ref.*	**1.28 (1.01-1.64)**	0.78 (0.70-0.86)	0.98 (0.74-1.31)
LBW	**2.03 (1.79-2.31)**	**2.21 (1.61-3.04)**	*Ref.*	**1.40 (1.03-1.90)**	0.57 (0.49-0.66)	1.11 (0.78-1.59)
Macrosomia	0.55 (0.48-0.64)	0.64 (0.41-0.99)	*Ref.*	**1.29 (1.01-1.67)**	**2.87 (2.63-3.12)**	**2.95 (2.42-3.60)**
SGA	**1.71 (1.55-1.89)**	**2.32 (1.82-2.96)**	*Ref.*	**1.45 (1.15-1.82)**	0.56 (0.50-0.62)	0.82 (0.60-1.12)
LGA	0.55 (0.49-0.61)	0.66 (0.49-0.89)	*Ref.*	**1.22 (1.01-1.47)**	**2.36 (2.22-2.52)**	**2.45 (2.09-2.87)**
NICU	**1.70 (1.35-2.14)**	1.41 (0.72-2.78)	*Ref.*	1.06 (0.58-1.97)	0.63 (0.49-0.81)	0.86 (0.42-1.76)

Abbreviations: BMI, body mass index; HDP, Hypertensive disorder in pregnancy; GDM, Gestational diabetes mellitus; PTD, preterm delivery; LBW, low birth weight; SGA, small for gestation age; LGA, large for gestation age; NICU, neonatal intensive care unit; aOR, adjusted odds ratio; CI, confidential interval.
[a]Adjusted for maternal age, education levels, parity, insurance status, ALT level.

1.63-3.20) were identified among women with late-onset hypercholanemia and pre-pregnancy OWO. For women with pre-pregnancy underweight, early-onset hypercholanemia increased the risk of PTD (aOR 2.72, 95%CI 1.82-4.08), LBW (aOR 4.12, 95%CI 2.65-6.43), and SGA (aOR 2.62, 95%CI 1.71-4.00), while late-onset hypercholanemia was associated with an incremental risk of SGA (aOR 2.77, 95%CI 2.02-3.78).

Combined effect of gestational weight gain and hypercholanemia on adverse maternal and fetal outcomes

As shown in Table 5, women with excessive GWG and hypercholanemia were found to have a higher risk of HDP (aOR 3.56, 95% CI 2.91-4.36), macrosomia (aOR 2.95, 95% CI 2.42-3.60), and LGA (aOR 2.45, 95% CI 2.09-2.87), compared with women with adequate GWG and non-hypercholanemia. In addition, it was also indicated that an increased risk of PTD (aOR 1.87, 95% CI 1.44-2.43), LBW (aOR 2.21, 95% CI 1.61-3.04), and SGA (aOR 2.32, 95% CI 1.82-2.96) was presented in women with inadequate GWG and hypercholanemia. Whereas, gestational hypercholanemia was also associated with moderately incremental risk of HDP (aOR 1.84, 95% CI 1.44-2.35), PTD (aOR 1.28, 95% CI 1.01-1.64), LBW (aOR 1.40, 95% CI 1.03-1.90), macrosomia (aOR 1.29, 95% CI 1.01-1.67), SGA (aOR 1.45, 95% CI 1.15-1.82), and LGA (aOR 1.22, 95% CI 1.01-1.47) among women with adequate GWG.

Discussion

The current study offered a thorough examination of the associations among pre-pregnancy BMI, GWG, TBA levels, and adverse pregnancy outcomes. A dose-response correlation was observed between maternal BMI prior to pregnancy and TBA concentration, with lower maternal BMI identified as an independent risk factor for hypercholanemia. Additionally, compared to non-hypercholanemia women with a normal pre-pregnancy BMI, hypercholanemia women with pre-pregnancy OWO had a heightened risk of HDP. Furthermore, elevated TBA levels were found to be associated with an increased risk of SGA infants and

LBW infants, especially among mothers with pre-pregnancy underweight. Besides, both inadequate and excessive GWG may increase risks of adverse pregnancy outcomes in women complicated with hypercholanemia

The relationship between pre-pregnancy BMI and TBA concentration during pregnancy is still not well understood. Previous studies conducted in developed countries have indicated a higher prevalence of ICP in women with pre-pregnancy OWO [4,15]. However, the incidence of ICP is relatively low, and the prevalence of underweight is high in these populations. There is a lack of reliable evidence regarding the association between pre-pregnancy BMI and gestational TBA concentration in China or other developing countries, where the prevalence of OWO is increasing but the prevalence of underweight remains high. Consistent with recent observational studies in China [16,17], our data suggest that maternal pre-pregnancy underweight might be a potential risk factor for hypercholanemia. Additionally, we found an inverse correlation between maternal pre-pregnancy BMI and peak maternal TBA concentrations during gestation. One possible explanation for this association is that a state of supraphysiologic hypercholanemia may result from hormonal alterations and maladaptation to metabolic remodeling that occurs in early pregnancy [1,26]. During the anabolic period of early pregnancy, there is enhanced lipogenesis and fat accumulation to prepare for rapid neonatal growth in late gestation. Therefore, women with pre-pregnancy underweight might be more susceptible to nutritional deficiencies, contributing to the development of hypercholanemia.

Our findings also revealed a combined effect of hypercholanemia and abnormal maternal BMI prior to pregnancy on adverse pregnancy outcomes. Maternal pre-pregnancy underweight is known to be linked to maternal malnutrition and increased susceptibility to prematurity and SGA infants [27–29]. In our previous publication, we demonstrated that elevated TBA levels during gestation could independently contribute to the likelihood of LBW and intrauterine growth restriction [12]. In the current study, we observed that the joint effect of pre-pregnancy underweight and gestational hypercholanemia on the risk of SGA was amplified. Women with hypercholanemia and pre-pregnancy underweight had an increased risk of delivering SGA infants. Our previous research suggested that elevated concentrations of bile acids could inhibit autophagic flux and enhance apoptosis in placental trophoblasts, particularly in the context of nutritional deprivation [19]. Furthermore, mitochondrial dysfunction may be involved in the pro-apoptotic mechanisms through which excessive bile acids induce trophoblast cell death under conditions of nutritional deprivation [19]. Therefore, we proposed that maternal pre-pregnancy underweight and hypercholanemia might synergistically contribute to the pathogenesis of SGA possibly through these mechanisms.

Maternal pre-pregnancy obesity is known to be an essential risk factor for adverse maternal and fetal outcomes due to factors such as genetic susceptibility, nutritional status, fat accumulation, and low-grade inflammation [30–33]. Maternal OWO in pregnancy have been associated with alterations in angiogenic biomarkers during the first and second trimesters, which may partially explain the underlying mechanism of placenta-mediated adverse pregnancy outcomes, including preeclampsia [34]. A recent meta-analysis has reported that elevated TBA levels in pregnant women also increased the risk of preeclampsia [35]. Zhang et al. observed a potential link between elevated levels of S1PR2 agonists (such as conjugated bile acids) and activation of S1R2 signals in endothelial dysfunction, providing a possible explanation for the increased risk of preeclampsia among women with elevated sTBA levels [35,36]. Additionally, the accumulation of bile acids can directly affect the placenta, leading to oxidative damage of the placental unit [37,38]. Our data indicated an amplified joint effect of maternal OWO and high maternal TBA concentrations on the pathogenesis of HDP. Considering that both factors independently increase the risk of preeclampsia, the joint effect of this "double hit" may be amplified. Therefore, it is important to pay close attention to pregnant women with hypercholanemia and pre-pregnancy OWO in terms of their risk for developing HDP.

This large retrospective cohort study provided valuable insights into the relationship between maternal pre-pregnancy BMI, TBA levels in pregnancy, and the risk of adverse pregnancy outcomes. However, there are some limitations to consider. Firstly, our findings are based on retrospective observational data obtained from a single maternal and child health center, which limits the ability to establish causality. Further studies involving multiple obstetrics centers would be beneficial to validate these results. Secondly, pre-pregnancy BMI was calculated based on a self-reported maternal weight before pregnancy and maternal height measured at the hospital. This method may introduce underestimation and potential misclassification bias, so caution should be exercised when interpreting these results [39]. Lastly, although we had access to maternal pre-pregnancy BMI and TBA concentrations measured during pregnancy, we did not investigate the potential effects of dynamic changes in gestational weight gain or other biomarkers related to maternal nutritional status throughout pregnancy.

Conclusions

In summary, this large retrospective cohort study revealed that low pre-pregnancy BMI was associated with elevated TBA levels and an increased risk of hypercholanemia. We also observed that mothers with both pre-pregnancy overweight and hypercholanemia had an increased risk of HDP. Additionally, the association between high TBA levels and the risks of SGA infants was more prominent among hypercholanemia mothers with pre-pregnancy underweight. Moreover, Excessive GWG or inadequate GWG also increased risks of adverse maternal and fetal outcomes in women with hypercholanemia. These findings provide new insights into the potential differential effect of gestational hypercholanemia in the management of adverse outcome with emphasizing the importance of considering BMI as the stratification factors.

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Author contributions statement

Y.Z, J.Z., and W.W. designed the study; W.W. and G.W. supervised data collection and conducted statistical analyses; J.L., H.L., and F.S. involved in data acquisition and interpretation; Y.Z. and J.Z. drafted the original version of manuscript. All authors contributed to revision and approved the final version for publication. W.W. is the guarantor for this work, had full access to the data, and controlled the decision to publish.

Consent to participate

The requirement for informed consent was waived for using anonymized and de-identified data as approved by the Ethics Committee.

Disclosure statement

No potential conflict of interest was reported by the authors.

Ethical statement

This study adhered to the Declaration of Helsinki. The study protocol was endorsed by the IPMCH Medical Ethics Committee (GKLW2019-43, the date of approval was 30 December 2019). Analysis of data in this study were conducted from 2022 to 2023.

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ORCID

Weibin Wu http://orcid.org/0000-0003-3637-754X

Data availability statement

The data supporting the findings of the study are available on reasonable request to the corresponding author.

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