DIABETES DURING AND BEYOND PREGNANCY: A LIFE COURSE PERSPECTIVE

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DIABETES DURING AND BEYOND PREGNANCY: A LIFE COURSE PERSPECTIVE

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ORIGINAL RESEARCH
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Short Body Height and Pre-pregnancy Overweight for Increased Risk of Gestational Diabetes Mellitus: A Population-Based Cohort Study

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Background: Short height is associated with gestational diabetes mellitus (GDM) but the underlying mechanism remains unknown. This study aims to explore whether short height has a synergistic effect with pre-pregnancy overweight/obesity and undue weight gain on the risk of GDM.

Methods: We recruited 19,962 singleton pregnant women from their first antenatal care visit in urban Tianjin, China, between October 2010 to August 2012. At 24–28 weeks of gestation, women underwent a 50-g 1-h glucose challenge test (GCT) followed by a 75-g 2-h oral glucose tolerance test (OGTT) if the GCT result was ≥7.8 mmol/L. GDM was defined by the International Association of Diabetes and Pregnancy Study Group's cut-points. Univariable and multivariable logistic regression analyses were performed to obtain odds ratios (ORs) and 95% confidence intervals (CIs). Restricted cubic spline (RCS) analysis nested in the logistic regression analysis was used to identify a cutoff point of height for GDM. Additive interaction was used to test interactions between short height, pregnancy overweight/obesity and undue weight gain.

Results: A total of 1,517 (or 7.6%) women developed GDM. The risk of GDM increased rapidly with a decreasing height from 158 cm and downwards. Using height ≥158 cm as the reference group, women with <158 cm of height were at increased GDM risk (adjusted OR: 1.44, 95%CI: 1.18–1.75). Maternal overweight/obesity at the first antenatal care visit greatly enhanced the OR of short height for GDM (adjusted OR: 3.78, 95%CI: 2.84–5.03) with significant additive interaction ($P < 0.05$). However, the interaction between short height and undue weight gain was non-significant ($P > 0.05$).

Conclusions: In Chinese pregnant women in urban Tianjin, height <158 cm had a synergistic effect with pre-pregnancy overweight/obesity on the risk of GDM.

Keywords: gestational diabetes mellitus, body height, pre-pregnancy overweight, synergistic effect, Chinese women

INTRODUCTION

In the past decades, the prevalence of gestational diabetes mellitus (GDM) has been increasing all over the world including China (1). For example, the prevalence of GDM in Tianjin, China, had increased from 2.3% in 1999 to 8.1% in the period from 2010 to 2012 (2, 3). It is well-established that GDM is associated with adverse pregnancy outcomes in the short run and also has adverse health impacts on both women with prior GDM and their offspring in the long run. In this regard, women with GDM are at higher risk of hypertension, preeclampsia, infection and cesarean delivery and delivery of a macrosomic infant. The neonates from mothers with GDM are at high risk of fetal hypoglycaemia, hyperinsulinemia, hypocalcaemia, and respiratory distress (4, 5). Our meta-analysis showed that early lifestyle prevention within 15th gestational week was able to reduce the occurrence of GDM (6). More importantly, the efficacy was not limited to pre-pregnancy obese women but extended to women at high risk of GDM due to presence of other GDM risk factors (7). For possible intervention in the early pregnancy, it is essential to identify women with high risk of GDM. In addition to traditional risk factors, several studies reported that there was a negative association between body height and glucose intolerance in non-pregnant and non-diabetes subjects (8) and the association existed in both lean and obese subjects (9). Short height at birth has been reported to have long-term deleterious impacts on metabolism (10) and is associated with increased risk of GDM in Caucasians (11) and Asian women (2, 12). It is also yet to establish a cutoff point of short height to identify women at high risk of GDM in Chinese population.

Biological links between short height and increased risk of GDM are complex and still unclear. Adult height is a cumulative result of nutritional environment and genetic factors over the growing period. Presumably, under-nutrition during the key periods related with short height may cause catch-up growth, especially through the acquisition of "thrifty phenotype" in adverse intrauterine milieu. Catch-up growth is recognized as a risk factor for glucose intolerance in later life due to undue weight gain and obesity increasing insulin resistance (13, 14). It is also possible that nutritional deprivation leading to short height in childhood and early-life impairs the development of beta cells and their function (15, 16). Similar to type 2 diabetes, GDM is characterized by decreased beta cell function and increased insulin resistance, the latter either stemming from persisting insulin resistance from pre-pregnancy or insulin resistance induced by pregnancy or both. If decreased beta cell function plays a dominant role in the association between short height and GDM, we can assume that short height has a synergistic effect with pre-pregnancy obesity (i.e., pre-pregnancy insulin resistance) and/or undue weight gain (i.e., pregnancy-induced insulin resistance) on the risk of GDM.

Using an established population-based cohort of Chinese pregnant women in Tianjin, China, this study aims (1) to define a cutoff point of short height for the risk of GDM and (2) to test the hypothesis that short height and pre-pregnancy overweight/obesity, or short height and undue weight gain during pregnancy have a synergistic effect toward increasing the risk of GDM in Chinese pregnant women.

Abbreviations: AP, attributable proportion due to interaction; BMI, body mass index; BP, blood pressure; CI, confidence intervals; GCT, glucose challenge test; GDM, Gestational diabetes mellitus; IADPSG, International Association of Diabetes and Pregnancy Study Group; IQR, interquartile range; OGTT, oral glucose tolerance test; OR, odds ratios; PG, plasma glucose; PG, plasma glucose; RCS, Restricted cubic spline; RERI, Relative excess risk due to interaction; SI, synergy index; SD, standard deviation; WCHC, Women and Children's Health Center.

MATERIALS AND METHODS

Study Population and Settings

Tianjin, the fourth largest city of China, is located at 137 kms southeast of Beijing and consists of six central urban districts, one new urban district, four suburban districts, and five counties. At the end of 2012, the city of Tianjin had over 14 million residents, of which about 4.3 million lived in the six central urban districts where the study was conducted.

The antenatal care in the six urban districts was shared by three levels of antenatal care institutions, i.e., primary, secondary and tertiary care hospitals. The 3-tier prenatal care system was consisted of 65 primary hospitals (tier one), six district-level Women and Children's Health Center (WCHC) and other secondary obstetric hospitals (tier two), and a city-level Tianjin WCHC and other tertiary care hospitals (tier three). Tianjin WCHC played a key role in coordinating antenatal care by these medical institutions. All pregnant women were initially registered with a primary hospital and received antenatal care there until 32nd gestational week and then referred to one of secondary or tertiary care hospital of their choice. In 1998, our team established a universal screening and management system for GDM within the 3-tiered antenatal care network (2).

From October 2010 to August 2012, we set up a cohort of pregnant women with data collected from their first antenatal care visit till delivery and early postpartum period. During this period, 22,069 singleton pregnant women registered with a primary care hospital. We sequentially excluded 1233 women who did not undergo the GCT, 870 women with positive GCT but did not undergo the OGTT and 4 women with missing information on height. A total of 19,962 pregnant women were included in the final analysis. The ethics clearance was obtained from the Ethics Committee for Clinical Research of Tianjin WCHC. The study was carried out in accordance with the Declaration of Helsinki and written informed consent was obtained from these women before data collection. In 2009, the 3-tier prenatal care system set up a computerized Maternal and Child Health Information System in Tianjin to share data of pregnant women by care-givers at different levels.

Screening and Diagnosis of GDM

GDM was identified using a two-step procedure. All the pregnant women were offered a 50-gram 1-h GCT in non-fasting status in 24th to 28th weeks of gestation at a primary care hospital. Those who had plasma glucose (PG) reading $\geq$7.8 mmol/L were referred to the GDM clinic located within TWCHC for a standard 75-gram 2-h OGTT. The OGTT was performed

after an overnight fasting of at least 8 h. The OGTT results were interpreted according to the International Association for Diabetes in Pregnancy Study Group's (IADPSG) criteria, i.e., having met any one of the cutoff points: fasting PG $\geq$ 5.1 mmol/L, 1-h PG $\geq$ 10.0 mmol/L or 2-h PG $\geq$ 8.5 mmol/L (17).

Data Collection and Definitions

We collected demographic information, lifestyle, pregnancy-related medical conditions from these women at their first antenatal care visits and 24–28 weeks of pregnancy, such as age, parity and family history of diabetes, smoking, and drinking habits in a longitudinal manner, using a set of specially designed questionnaires (3, 18). The pregnancy outcomes including gender of infants were retrieved from the Maternal and Child Health Information System.

Measurements and Clinical Definitions

Anthropometric and clinical measurements of all subjects were measured by uniformly-trained staff members with a standardized protocol and tools. Height was measured to the nearest 0.5 cm and weight was measured to the nearest 0.1 kg. Height and weight were measured in women wearing light closing and without shoes at first antenatal care visit and weight was re-measured at GCT time. Body weight at first antenatal care visit was recorded as pre-pregnancy weight. Difference in body weight from first antenatal care visit to GCT time was estimated as gestational weight gain. Undue weight gain defined as $\geq$75th percentile (i.e., 0.37 kg per week). Body mass index (BMI) was calculated to estimate adiposity as the ratio of weight in kilograms to height squared in meters and categorized for overweight and obesity according to Chinese adults' criteria (19), i.e., underweight: BMI $<$ 18.5 kg/m^2; normal body weight: BMI at 18.5–23.9 kg/m^2; overweight: BMI at 24–27.9 kg/m^2, obesity: BMI $\geq$ 28 kg/m^2. Sitting blood pressure (BP) was measured from right arm after at least 10 min rest when then underwent GCT.

Maternal age was calculated as the period in years from the date of birth to the date of first antenatal care visit. Educational attainment was divided into two categories: junior college or below, and tertiary education or above. Family history of diabetes was defined as having any first-degree relatives with diabetes. Habitual smoker was defined as continuously smoking one or more cigarettes per day for at least 6 months before or during pregnancy. Habitual drinker was defined as drinking occasionally or once or more per week before or during pregnancy.

Statistical Analysis

All analyses were performed using the Statistical Analysis System (Release 9.2) (SAS Institute Inc., Cary, USA) and all data were expressed as mean $\pm$ standard deviation (SD) or median (interquartile range, IQR) where appropriate. Student's *t*-test or Wilcoxon two-sample test was used to compare means (or median) of continuous variables. Chi-squared test (or Fisher exact test where appropriate) was used to compare categorical variables between the GDM group and the non-GDM group. Binary logistic regressions were performed to obtain odds ratios (OR) and 95% confidence intervals (CI) of height and other variables under study for GDM in univariable and multivariable analyses. In the multivariable analysis, we adjusted for traditional GDM risk factors, including age, habitual smoker, alcohol drinker, Han-ethnicity, parity, systolic/diastolic BP at GCT, education attainment, family history of diabetes on the first-degree relations as well as infant gender.

Restricted cubic spline (RCS) is piecewise cubic polynomials connected across different intervals of a continuous variable, which can fit sharply curving shapes (20). We used to employ this method in numbers of our previous studies to identify cutoff points of lipids for cancer in type 2 diabetes (21) and alanine aminotransferase for GDM (22). In this study, we used RCS nested in logistic regression analysis to examine the full range association between height and the risk of GDM and to define cutoff points of height for GDM if any. Briefly, we chose 4 knots at quintiles 0.05, 0.35, 0.65, and 0.95 as suggested by Harrell (20). ORs between two heights can be estimated by EXP (Y_2–Y_1), where Y_2 and Y_1 were the values of RCS functions at heights 2 and 1. As before, a cutoff point was selected if the risk of GDM rapidly increased since that point by visual checking of the curve's shape. Further confirmation logistic regression analysis was performed by stratifying height into a binary variable at a selected cutoff point.

Synergistic effects between short height and pre-pregnancy overweight and undue weight gain from the first antenatal care visit to GCT time were estimated using additive interaction (23). Three measures, i.e., relative excess risk due to interaction (RERI), attributable proportion due to interaction (AP), and synergy index (SI), were used to estimate additive interaction. A significant RERI $>$ 0, AP $>$ 0, or SI $>$ 1 indicates an additive interaction or synergistic effect between short stature and overweight/undue weight gain for GDM. A calculator was available at http://epinet.se/res/xls/epinetcalculation.xls. (23) Because age is among the strongest risk factors for GDM, we also performed additional analysis to test additive interaction between short weight and old age (i.e., $\geq$30 years).

Additional analysis using analysis of covariance (ANCOVA) was performed to compare adjusted means of BMI and weight gain between women with short height and their counterparts without short height. Sensitivity analysis was also performed to check consistency of the results after exclusion of 1,209 women who registered after 14th gestational week in the main analysis.

RESULTS

The 19,962 pregnant women had a mean age of 28.5 (*SD*: 2.9) years, a mean height of 163.2 (*SD*: 4.7) cm, a mean body weight of 59.5 (*SD*: 9.8) kg and a mean BMI of 22.3 (*SD*: 3.4) kg/m^2. These women gained a mean body weight of 0.29 (*SD*: 0.2) kg per week from registration to the GCT time. Of them, 7.6% (n = 1,517) developed GDM. Women with GDM had an older age, shorter height, heavier body weight, higher pre-pregnancy BMI and higher PG at GCT. They were also more likely to be multiparous, to give birth to a male infant and to have family history of diabetes in first degree relatives and higher BPs (**Table 1**).

Height was inversely associated with the risk of GDM in multivariable analysis. The OR of GDM increased with

TABLE 1 | Clinical and biochemical characteristics of subjects according to occurrence of gestational diabetes mellitus diagnosed by the IADPSG's criteria.

	Non-GDM	GDM	*P*-value
N	18,445	1,517	
AT REGISTRATION WITH PREGNANCY			
Age, year	28.4 ± 2.9	29.5 ± 3.3	<0.001*
Age group, year			
<25	1,845 (10.0%)	72 (4.7%)	<0.001**
≥25∼ <30	1,2176 (66.0%)	907 (59.8%)	
≥30	4,424 (24.0%)	538 (35.5%)	
Height. cm	163.2 ± 4.7	162.7 ± 4.8	0.001*
Height group < 158 cm	1,530 (8.3%)	162 (10.7%)	
Body weight, kg	59.1 ± 9.5	64.1 ± 11.5	<0.001*
Pre-pregnancy BMI, kg/m^2	22.2 ± 3.3	24.2 ± 3.9	<0.001*
BMI GROUP, kg/m^2			
<18.5	1,892 (10.3%)	58 (3.8%)	<0.001**
≥18.5–<24	12,044 (65.3%)	755 (49.8%)	
≥24–<28	3,423 (18.6%)	483 (31.8%)	
≥28	1,086 (5.9%)	221 (14.6%)	
Nationality			0.016**
Han	17,591 (95.4%)	1,467 (96.7%)	
Parity			0.008**
≥1	686 (3.7%)	77 (5.1%)	
Education attainment			0.845**
Junior college and below	8,404 (45.6%)	688 (45.4%)	
Tertiary education	10,041 (54.4%)	829 (54.7%)	
Family history of diabetes in first degree relatives	1,381 (8.2%)	206 (15.8%)	<0.001**
Gestation week at registration	10.9 ± 2.4	10.8 ± 2.3	0.109*
AT GCT			
Plasma glucose	6.3 ± 1.3	9.4 ± 1.5	<0.001*
Weight gain from first antenatal care visit to GCT, kg	7.4 ± 7.3	7.3 ± 3.6	0.848 *
Weight gain per week, kg/wk	0.3 ± 0.2	0.3 ± 0.1	0.760*
Weight gain group, kg/wk			0.842**
More (>0.37)	4,358 (23.6%)	355 (23.4%)	
Gestational weeks at GCT, week	25.2 ± 2.5	25.3 ± 1.8	0.181*
Systolic BP at GCT, mmHg	106.2 ± 10.5	109.6 ± 10.	<0.001*
Diastolic BP at GCT, mmHg	68.2 ± 7.4	70.3 ± 7.8	<0.001*
Smoker†	176 (1.0%)	19 (1.3%)	0.256**
Drinker‡	5,620 (30.5%)	459 (30.3%)	0.863**
AT DELIVERY			
Male infant gender	9,398 (51.8%)	846 (56.4%)	0.003**

BMI, body mass index; GCT, glucose challenge test; BP, blood pressure; Data are reported in mean ± SD or number (%).

**Derived from Student's t- test.*

***Derived from Chi-square test or Fisher's Exact Test.*

† Defined as smoking one or more cigarette per day before or during pregnancy.

‡Defined as drinking occasionally or once or more per week before or during pregnancy.

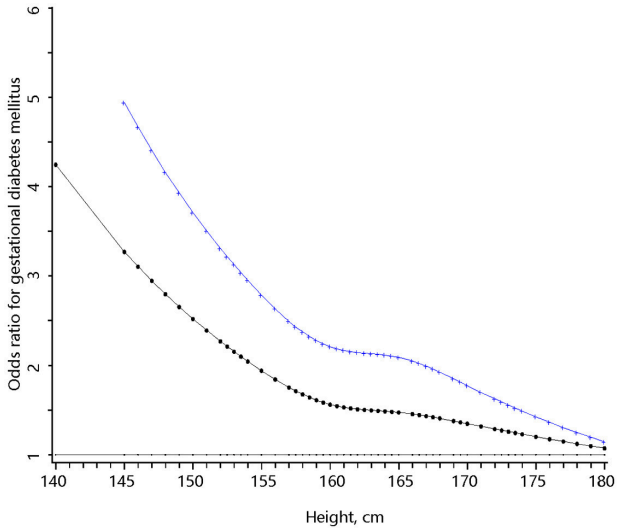

FIGURE 1 | The full range association between body height and the risk of gestational diabetes mellitus. The bottom (dotted) curve was derived from univariable analysis and the upper (cross) curve was derived from multivariable analysis that adjusted for pre-pregnancy body mass index, weight gain per week from first antenatal care visit to glucose challenge test (GCT), age, habitual smoker and drinker, Han-ethnicity, parity, systolic, and diastolic blood pressure at GCT, education, family history of diabetes, and baby gender.

decreasing height down to 158 cm and the risk of GDM rapidly increased in a linear manner with decreasing height from 158 cm downwards (**Figure 1**). If 158 cm was used as the cutoff point to define short height, 8.5% (n = 1,692) of the women had short height and 9.6% (n= 162) of the short women developed GDM. Women with short height were at higher risks of GDM in univariable analysis and multivariable analysis (**Table 2**). Compared to normal weight, overweight, or obesity (BMI ≥ 24 kg/m^2) was also associated with increased risk of GDM. In multivariable analysis, women with short height had similar pre-pregnancy BMI but gained less as compared with women with taller height (Appendix Table 1 in Supplementary Material).

In the subgroup analysis, short height was associated with increased risk of GDM among women with pre-pregnancy BMI ≥24 kg/m^2 in univariable analysis (OR: 1.46; 95%CI: 1.13–1.87) and multivariable analysis (OR: 1.68; 95%CI: 1.26–2.24). On the other hand, the OR of short height for GDM was not significant among women with pre-pregnancy BMI <24 kg/m^2 in multivariable analysis (OR: 1.27; 95%CI: 0.96–1.67). If height ≥158 cm and BMI <24 kg/m^2 were used as the reference, BMI ≥24 kg/m^2 alone but not height <158 cm alone was associated with increased risk of GDM in multivariable analysis. Co-presence of both risk factors greatly increased the OR further to 3.78 (95%CI: 2.84–5.03). All the three additive interaction measures are significant (AP: 0.33, 95%CI: 0.12–0.54; RERI: 1.26, 95%CI: 0.16–2.35 and SI: 1.83, 95%CI: 1.15–2.89) (**Table 3**).

If the 75th percentile of weight gain from registration to GCT time was used to define undue weight gain, short height was associated with increased risk of GDM among women with undue weight gain and also among women without undue weight gain. However, all the additive interaction measures between short height and undue weight gain were not significant (Appendix Tables 2, 3 in Supplementary Material).

TABLE 2 | Odds ratios (ORs) of short height and pre-pregnancy BMI for the risk of gestational diabetes mellitus.

	N (%)	Odds ratio	95% confidence intervals	*P*-value
INDEPENDENT MODELS				
Univariable analysis[†]				
Height < vs. ≥158 cm	162 (10.7%)	1.32	1.11–1.57	0.001
BMI ≥ 24 vs. < 24 kg/m^2	704 (46.4%)	2.68	2.41–2.98	<0.001
Multivariable analysis[‡]				
Height < vs. ≥ 158 cm	126 (11.2%)	1.44	1.18–1.75	<0.001
BMI ≥ 24 vs. < 24 kg/m^2	527 (46.9%)	2.33	2.04–2.65	<0.001
INDEPENDENT MODELS IN THE SUBGROUPS				
Univariable analysis among women with BMI ≥ 24 kg/m^2[†]				
Height < vs. ≥ 158 cm	83 (11.8%)	1.46	1.13–1.87	0.004
Multivariable analysis among women with BMI ≥ 24 kg/m^2[‡]				
Height < vs. ≥ 158 cm	66 (12.5%)	1.68	1.26–2.24	<0.001
Univariable analysis among women with BMI < 24 kg/m^2[†]				
Height < vs. ≥ 158 cm	79 (9.7%)	1.20	0.94–1.52	0.144
Multivariable analysis among women with BMI < 24 kg/m^2[‡]				
Height < vs. ≥ 158 cm	60 (10.1%)	1.27	0.96–1.67	0.096
ADDITIVE INTERACTION MODELS AMONG THE WHOLE COHORT				
Univariable analysis[†]				
Height <158 cm and BMI ≥ 24 kg/m^2	83 (5.5%)	3.82	2.97–4.89	<0.001
Height <158 cm and BMI < 24 kg/m^2	79 (5.2%)	1.20	0.94–1.52	0.144
Height ≥ 158 cm and BMI ≥ 24 kg/m^2	621 (40.9%)	2.62	2.34–2.93	<0.001
Height ≥ 158 cm and BMI < 24 kg/m^2	734 (48.4%)	1	Reference	
Multivariable analysis[‡]				
Height < 158 cm and BMI ≥ 24 kg/m^2	66 (5.9%)	3.78	2.84–5.03	<0.001
Height < 158 cm and BMI < 24 kg/m^2	60 (5.3%)	1.26	0.96–1.67	0.097
Height ≥ 158 cm and BMI ≥ 24 kg/m^2	461 (41.0%)	2.26	1.97–2.59	<0.001
Height ≥158 cm and BMI < 24 kg/m^2	537 (47.8%)	1	Reference	

BMI, body mass index; GCT, glucose challenge test; SBP/DBP, systolic/diastolic blood pressure.

[†] *Not adjusted for any other variables.*

[‡] *The variables adjusted in the multivariable analysis included weight gain per week from first antenatal care visit to GCT, age, habitual smoker and drinker, Han-ethnicity, parity, SBP, and DBP at GCT, education, family history of diabetes, baby gender, in addition to the variables listed in the model.*

Short height was also associated with increased risk of GDM in women aged <30 years but not in women aged ≥30 years. However, the additive interaction between short height and ≥30 years of age was not significant (Appendix Tables 4, 5 in Supplementary Material).

After exclusion of women who registered after the 14th gestational week, the additive interaction between short height and overweight/obesity remained significant in multivariable analysis (AP: 0.28, 95%CI: 0.04–0.51; RERI: 1.01, 95%CI: −0.10 to 2.11; SI: 1.62, 95%CI: 1.01–2.60) (Appendix Tables 6, 7 in Supplementary Material).

DISCUSSION

This study found that 158 cm was a cutoff point to define short height for GDM in Chinese pregnant women, and height below the cutoff point was associated with markedly increased risk of GDM, and the effect was limited to women with pre-pregnancy BMI ≥ 24 kg/m^2.

Overweight/obesity and undue weight gain during pregnancy are well-established risk factors for GDM. Consistent epidemiological data also suggested that short height was associated with increased risk of GDM in pregnant women as well as abnormal carbohydrate metabolism in the general population. For example, several studies reported that height was inversely correlated with abnormal glucose tolerance and insulin resistance in non-pregnant subjects without diabetes (8, 24). In one study of 9,471 pregnant women in Tianjin, China (2), we reported that a centimeter increase in height was associated with 4% decrease in the risk of GDM. A Korean study ($n = 9,005$)(12) reported that women in the shortest quartile (≤157 cm) were two times more likely to develop GDM than women in the highest quartile (≥163 cm). Similarly, a smaller Greek study ($n = 2,772$) (9) reported an increased risk for GDM in women with a stature in the lowest quartile (<159 cm). In Brazil, short stature was also associated with GDM risk, with women who had a short stature (≤151 cm) having a 60% increase in the odds of GDM (25). Using a more sophisticated method, we further refined

TABLE 3 | Additive interaction between height < 158 cm and BMI ≥ 24 kg/m^2 for the risk of gestational diabetes mellitus.

Measures of additive interaction	Estimate	95% confidence intervals
UNIVARIABLE ANALYSIS[†]		
RERI	1.00	0.03–1.97
AP	0.26	0.06–0.46
SI	1.55	1.06–2.27
MULTIVARIABLE ANALYSIS[‡]		
RERI	1.26	0.16–2.35
AP	0.33	0.12–0.54
SI	1.83	1.15–2.89

BMI, body mass index; AP, attributable proportion due to interaction; RERI, relative excess risk due to interaction; SI, synergy index; Significant RERI > 0, AP>0 or SI>1 indicates a significant additive interaction.

[†] *Not adjusted for any other variables.*

[‡] *The variables adjusted are the same as those in* ***Table 2****.*

the cutoff point of short height for the risk of GDM in Chinese pregnant women and found that women with height <158 cm had a 1.44-fold increased risk of GDM compared with women who were taller. More importantly, our study generated novel findings that the effect of short height on GDM was limited to overweight/obese women.

GDM may develop due to beta cell dysfunction, high insulin resistance including that persisting from pre-pregnancy and undue insulin resistance induced by pregnancy, or both. It is presumable that pre-pregnancy overweight/obesity is associated with insulin resistance before pregnancy while pregnancy-induced insulin resistance is associated with undue weight gain. Increased pre-pregnancy insulin resistance and undue pregnancy-induced insulin resistance may exert extra burden on the subclinical beta cell function, i.e., increased insulin resistance and beta cell dysfunction having a synergistic effect on GDM. In this connection, short height maybe, presumably, is associated with impaired development of beta cell function or increased insulin resistance, the latter being termed as growth catch-up insulin resistance (16, 26). In our analysis, short height was not associated with higher pre-pregnancy BMI and the association between short stature and GDM was independent of pre-pregnancy BMI. This observation supports the hypothesis that co-presence of impaired beta cell function and insulin resistance greatly predisposes women to GDM but does not support a role of growth catch-up in the association between short height and GDM. In a word, our findings are consistent with the thrifty phenotype hypothesis that the adaptative alterations protecting these women from undernourishment during their early development could have led them to short body height and may also lead to glucose intolerance (27, 28). It is also possible that a genetically determined insulin effect could lead to both failure to grow and diabetes (29, 30). Therefore, the short stature may be a result of thrifty genotype (31), which might have contributed to predisposition of the women to GDM.

It is possible that the observed synergistic effect of short height and overweight/obesity may also work for type 2 diabetes. Dutch Famine study revealed that pregnant women exposed to famine in early pregnancy increased the risk of offspring to develop metabolic diseases in adulthood due to epigenetic modifications (32). Therefore, our findings suggest that it is worthwhile to investigate a possible role of epigenetic modification for the increased risk of GDM among women with short height or both short height and overweight.

Our study has strong public health implications. First, the prevalence of GDM has been increasing globally. Although lifestyle intervention in early pregnancy may reduce the risk of GDM, such intervention is, at best, able to reduce 22% risk of GDM (6) and the residue risk of GDM remains quite high. Further understanding of the etiology of GDM is urgently needed. In this regard, our findings may help better understand GDM. Second, we defined a novel risk marker for GDM, i.e., co-presence of short height and overweight/obesity. This risk marker may be useful to identify those women at high risk of GDM at early pregnancy for possible intervention that had been shown to reduce the risk of GDM (6). It is also noticed that the predictive power of height is not large (27, 33). However, women with both height<158 cm and overweight/obesity were at particular high risk of GDM and they accounted for 33% of the total GDM cases in the population (i.e., AP = 0.33). Although height is an unmodifiable risk marker, overweight prior to pregnancy is modifiable. Removal of pre-pregnancy overweight in the high-risk group is expected to reduce the excess risk of GDM and can greatly contribute to control of GDM in our population.

Our study had several limitations. First, a two-step procedure in our antenatal care system was used to detect GDM, which might lead to missing of some GDM cases. Second, some women were excluded due to failure to turn up for GCT and/or OGTT. Compared with the women included in the analysis, the excluded women were older and had higher BMI though having a similar height (data not shown). This, the observed effect sizes might underestimate the true effect sizes of risk factors under investigation for GDM. Third, weight gain was calculated as that from registration to GCT time, not that from pre-pregnancy to the GCT time. Fourth, we found body height was inversely associated with increased risk of GDM consisted with earlier studies and the cutoff point of short height (<158 cm) defined in our cohort was similar with the mean height (157.7 cm) among Asians with GDM in a meta-analysis with large cohorts (27), but given the heterogeneous populations with various ethnicities in China, the cutoff point of height for increased risk of GDM need to be tested in other Chinese populations.

CONCLUSION

We found that short height defined as <158 cm was associated with markedly increased risk of GDM, and the effect was limited to women who were overweight/obese before pregnancy in Chinese population. If further replicated in other cohorts, co-presence of short height and overweight/obesity before pregnancy may be a useful risk marker for identification of women at high risk of GDM who may benefit most from lifestyle intervention before pregnancy. Further research into a possible

role of epigenetic modifications in the association between short height and GDM is warranted.

AUTHOR CONTRIBUTIONS

XY conceived and designed the study. JL analyzed the data and wrote the first draft. PW, CZ, JhL, NL, LW, WL, and HL collected the data. CZ, JhL, NL, LW, WL, and HL collected and assembly the data, gave critical comments on the manuscript. ZY, GH, and JC gave critical comments and edited the manuscripts. All authors read and approved the final manuscript.

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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.2018.00349/full#supplementary-material

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GENERAL COMMENTARY
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Commentary: Short Body Height and Pre-pregnancy Overweight for Increased Risk of Gestational Diabetes Mellitus: A Population-Based Cohort Study

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A Commentary on

Short Body Height and Pre-pregnancy Overweight for Increased Risk of Gestational Diabetes Mellitus: A Population-Based Cohort Study.
by Li J, Wang P, Zhang C, Leng J, Li N, Wang L., et al. (2018). Front. Endocrinol. 9:349. doi: 10.3389/fendo.2018.00349

Li J et al. conduct a sufficiently large cohort study and show that the risk of gestational diabetes mellitus (GDM) is inversely correlated with the height of the pregnant women (1). This association is particularly seen among Asians and may not warrant biological plausibility for using short stature as screening criteria due to several reasons (2).

First, short stature can be associated principally through the mechanism of greater risk of obesity/fat mass (3). Co-presence of short stature and overweight in the pre-pregnant women might be more useful screening criteria (4). Second, the same adaptive alterations that protected these women from undernourishment during their early development could have led them to short stature, as well as lead to glucose intolerance (thrifty phenotype hypothesis) (5, 6). It is also possible that a genetically determined insulin effect could lead to both failure to grow and to diabetes (thrifty genotype); which might have contributed to a predisposition for GDM (7, 8).

GDM, as a form of diabetes is multifactorial disease in origin. Several factors such as greater prepregnancy BMI, age, weight gain and a parental history of diabetes mellitus are independently associated with the GDM (9). The epidemiologic studies using the selective criteria such as height as a risk factor may not mean much in a heterogeneous population with different types of genetic lineage and environmental influences. Height is merely a function of nutrition and genetic lineage; therefore, measuring the height of the women in childbearing age will not reflect undernourishment or frequent infections in their infancy and through their life-course. Future studies have to reflect height as an intermediate variable between early exposures in fetal and childhood with subsequent risk of non-communicable diseases including the GDM.

AUTHOR CONTRIBUTIONS

GB wrote the first draft and reviewed all drafts of the commentary. AN reviewed and provided inputs for finalization of the commentary. DJ reviewed and provided inputs for finalization of the commentary through all stages.

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REVIEW
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Biomarkers for Macrosomia Prediction in Pregnancies Affected by Diabetes

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Large birthweight, or macrosomia, is one of the commonest complications for pregnancies affected by diabetes. As macrosomia is associated with an increased risk of a number of adverse outcomes for both the mother and offspring, accurate antenatal prediction of fetal macrosomia could be beneficial in guiding appropriate models of care and interventions that may avoid or reduce these associated risks. However, current prediction strategies which include physical examination and ultrasound assessment, are imprecise. Biomarkers are proving useful in various specialties and may offer a new avenue for improved prediction of macrosomia. Prime biomarker candidates in pregnancies with diabetes include maternal glycaemic markers (glucose, 1,5-anhydroglucitol, glycosylated hemoglobin) and hormones proposed implicated in placental nutrient transfer (adiponectin and insulin-like growth factor-1). There is some support for an association of these biomarkers with birthweight and/or macrosomia, although current evidence in this emerging field is still limited. Thus, although biomarkers hold promise, further investigation is needed to elucidate the potential clinical utility of biomarkers for macrosomia prediction for pregnancies affected by diabetes.

Keywords: diabetes, pregnancy, macrosomia, birthweight, biomarkers

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INTRODUCTION

With the increasing prevalence amongst women of childbearing age, diabetes mellitus is one of most common pre-existing medical conditions affecting pregnancy (1). Together, type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) affect around 1% of pregnancies (2). Gestational diabetes mellitus (GDM) is also on the rise, particularly since the changes in diagnostic criteria (3), with a prevalence of around 13% (4). This is of concern as maternal diabetes increases the risks associated with pregnancy (5, 6).

While there are higher rates of many adverse pregnancy outcomes, abnormal fetal growth and birthweight is particularly important due to the substantial frequency of occurrence (7, 8). Indeed, reports estimate macrosomia occurs in up to 60% of pregnancies affected by pre-existing diabetes (9). While macrosomia is common and occurs even in otherwise uncomplicated diabetic pregnancies (10), fetal growth restriction (FGR) and small-for-gestational-age (SGA) tend to occur in diabetic pregnancies complicated by other conditions, such as underlying maternal vascular disease (11, 12). For this reason, macrosomia in pregnancies affected by diabetes will form the focus of this review.

Macrosomia is defined in various ways. One method of defining macrosomia is according to the absolute birthweight being equal to or above a certain threshold, usually 4,000 g (13). Another definition is according to a birthweight percentile that accounts for the gestational age at birth, with macrosomia commonly defined as being above the 90th centile (also called large-for-gestational-age, LGA)(13, 14). The term macrosomia used here will encompass both of these definitions. Where possible, the abbreviated definition used by a referenced study will be mentioned, with the full definition provided in the supplementary table (Supplementary Table 1).

Given the potentially serious consequences for the mother and child, there is significant interest in predicting fetal macrosomia (13). Accurate identification holds potential for guiding appropriate management and interventions, with the aim of improving outcomes (15). However, currently available methods of macrosomia prediction demonstrate only modest predictive ability, which limits their use in tailoring obstetric decisions (13, 15).

The latest area of interest for potentially improving macrosomia prediction has been in the field of biomarkers. A biomarker in this context refers to a biological molecule that can be objectively assessed as an indicator of a physiological or pathological process or state, and therefore may have potential value for predicting certain outcomes (16, 17). A number of biomarkers have been investigated to determine whether they have an association with birthweight and macrosomia. Relevant biomarkers have been purposefully selected for further discussion.

The aim of this review is to examine the available literature for a relationship between selected biomarkers and birthweight and/or macrosomia. Pregnancies without diabetes in addition to pregnancies affected by diabetes will be discussed for comparison. The review will provide a brief overview of the determinants of fetal growth, the need for macrosomia prediction, and current prediction strategies. It will then focus on the selected biomarkers and provide evaluation of their birthweight/macrosomia prediction potential.

METHODS

Medline, Embase, and PubMed databases were searched in 2017. The following subject headings (and synonyms) were combined: pregnancy, diabetes mellitus, type 1 diabetes, type 2 diabetes, gestational diabetes, birthweight, fetal weight, macrosomia, large-for-gestational-age, biomarker, predictor. Specific searches also included the terms blood glucose, glycosylated hemoglobin, 1,5-anhydroglucitol, lipids, adiponectin, and insulin-like growth factor-1, as well as a search without diabetes terms. In addition, bibliographies of collected publications were manually searched.

Articles were selected if a biomarker from a maternal or fetal/neonatal biological sample was tested for an association with any measure of birthweight or macrosomia. This search identified a list of previously studied biomarkers (**Table 1**). Exclusion criteria included samples taken from pregnancies affected by FGR/SGA, multiple pregnancy, non-human studies, conference abstracts, and non-English articles.

DISCUSSION

Determinants of Fetal Growth

Normal fetal growth relies on the complex interplay of multiple factors, including genetic and environmental influences arising from the parents, fetus, and placenta (218). A key determinant of abnormal growth is altered substrate supply to the fetus (219). In normal pregnancy, maternal insulin resistance increases across gestation, becoming most pronounced in the third trimester when the majority of fetal growth takes place (220). This adaptive change promotes diversion of glucose across the placenta down its concentration gradient to the fetus (221). However, in pregnancies affected by diabetes, such transfer is exaggerated due to maternal hyperglycaemia (221). This excess glucose supply is believed to be central to diabetes-related fetal overgrowth. Indeed, the hyperglycaemia-hyperinsulinaemia hypothesis (also known as the Pedersen hypothesis), has been the prevailing explanation for macrosomia in diabetic pregnancies (220). It proposes that maternal hyperglycaemia leads to fetal hyperglycaemia, which stimulates maturation and hypertrophy of the fetal pancreas (222). This results in hypersecretion of insulin, and as insulin is a dominant fetal growth hormone, acceleration of fetal growth occurs (219). The modified Pedersen hypothesis also includes maternal amino acids and lipids in addition to glucose, contending that these insulin-responsive maternal fuels lead to an increase in "mixed nutrients" supplied to the fetus, which in turn elevates fetal insulin and drives excessive growth (220).

Rationale for Macrosomia Prediction

Antenatal prediction of fetal macrosomia prediction is desirable for many reasons. Firstly, macrosomia is a common obstetric complication, affecting a significant number of pregnancies. According to recent Australian figures, the rate of macrosomia (birthweight $\geq$ 4,000 g) amongst pregnancies with pre-existing type 1 and type 2 diabetes was 25 and 18% for male and female offspring, respectively (2). However, as previously mentioned, other populations have reported rates up to 60% (for pre-existing diabetes), which is approximately six times the rate for women without diabetes (9).

Secondly, macrosomia carries risks for the mother and fetus (**Figure 1**). Prominent risks include obstructed labor, cesarean section, instrumental delivery, perineal trauma, and birth injuries such as shoulder dystocia, an obstetric emergency involving difficulty delivering the fetal shoulders (14, 223, 224). Moreover, the risk of shoulder dystocia is greater in pregnancies affected by diabetes for any given birthweight, perhaps due to the altered fetal body proportions (14, 232). Longer-term risks include obesity and diabetes in the offspring (225, 226), possibly reflecting fetal programming as proposed by the "developmental origins of adult disease" or Barker hypothesis (233, 234).

By identifying pregnancies at increased perinatal risk, macrosomia prediction allows for tailoring obstetric care. Appropriate management and interventions could be employed

TABLE 1 | Biomarkers investigated for an association with birthweight or macrosomia (excluding FGR/SGA).

Biomarker (Alphabetical order)	Source	Significant association with birthweight/ macrosomia (Most adjusted result used. Maternal diabetes status of sample population provided; all were pregnant unless otherwise stated)	Non-significant association with birthweight/macrosomia (Most adjusted result used. Maternal diabetes status of sample population provided; all were pregnant unless otherwise stated)
1,5-Anhydroglucitol	• Maternal blood	(18) T1DM, T2DM, GDM (19) T1DM, T2DM, GDM (20) T1DM	(21) GDM, No diabetes
25-Hydroxyvitamin D (25(OH)D)	• Maternal blood		(22) T2DM, GDM, No diabetes
32-33 Split proinsulin	• Umbilical cord blood	(23) T1DM, No diabetes	
Acid-Labile Subunit (ALS)	• Umbilical cord blood	(24) Diabetes status not stated	
Acylation Stimulating Protein (ASP)	• Umbilical cord blood	(25) No diabetes (pregnant), No diabetes (non-pregnant)	
Adiponectin	• Maternal blood	(26) GDM, No diabetes (27) GDM, No diabetes (pregnant), No diabetes (non-pregnant) (28) GDM, No diabetes (29) GDM, No diabetes (30) GIGT, No diabetes (31) No diabetes (32) No diabetes (33) No diabetes (31) No diabetes (34) No diabetes (34) No diabetes (35) No diabetes	(36) GDM, No diabetes (37) GDM, No diabetes (38) No diabetes (39) No diabetes
	• Umbilical cord blood	(36) GDM, No diabetes (40) GDM, No diabetes (32) No diabetes (39) No diabetes (41) No diabetes (42) No diabetes (43) No diabetes (44) No diabetes (45) No diabetes	(23) T1DM, No diabetes (46) T2DM, GDM, No diabetes (47) No diabetes (48) No diabetes (38) No diabetes
	• Amniotic fluid		(49) Diabetes status not stated
Albumin	• Amniotic fluid	(50) GDM, No diabetes	
Alpha-Feto Protein (AFP) ratio (maternal serum AFP / amniotic fluid AFP)	• Maternal blood • Amniotic fluid	(51) Diabetes status not stated	
Alpha Human Chorionic Gonadotropin (α-hCG)	• Maternal blood		(52) IDDM, No diabetes
Amino acids	• Umbilical cord blood		(53) IDDM, No diabetes
Anti-insulin antibodies	• Maternal blood • Cord blood		(54) IDDM, GDM, No diabetes
Apelin	• Maternal blood • Cord blood	(54) GDM, No diabetes	
Apolipoprotein A1 (ApoA1)	• Maternal blood		(30) GIGT, No diabetes (55) No diabetes
Apolipoprotein A5 (APOA5) S19W polymorphism	• Umbilical cord blood	(56) Diabetes status not-stated	
Apolipoprotein B (ApoB)	• Maternal blood		(30) GIGT, No diabetes (55) No diabetes
Aspartate aminotransferase	• Maternal blood	(57) No diabetes	
Beta Human Chorionic Gonadotrophin (β-hCG)	• Maternal blood	(58) Diabetes, No diabetes	(59) No diabetes

(Continued)

TABLE 1 | Continued

Biomarker (Alphabetical order)	Source	Significant association with birthweight/ macrosomia (Most adjusted result used. Maternal diabetes status of sample population provided; all were pregnant unless otherwise stated)	Non-significant association with birthweight/macrosomia (Most adjusted result used. Maternal diabetes status of sample population provided; all were pregnant unless otherwise stated)
Beta-Hydroxybutyrate (β-OHB)	• Maternal blood	(60) Diabetes, No diabetes	
Bilirubin	• Maternal blood	(57) No diabetes	
Carcinoembryonic Antigen (CEA)	• Maternal blood • Umbilical cord blood		(61) Diabetes status not stated
Chemerin	• Umbilical cord blood	(62) GDM, No diabetes	
Coenzyme Q10 (CoQ10 or ubiquinone)	• Amniotic fluid	(63) GDM, No diabetes	
Copeptin	• Umbilical cord blood	(64) Diabetes, No diabetes	
Cortisol	• Maternal saliva	(65) No diabetes (66) Diabetes status not stated	
	• Amniotic fluid	(67) No diabetes	
C-peptide	• Umbilical cord blood	(53) IDDM, No diabetes (48) No diabetes (68) No diabetes	
	• Amniotic fluid		(69) Diabetes status not stated
C-Reactive Protein (CRP)	• Maternal blood	(30) GIGT, No diabetes (34) No diabetes	
Creatinine	• Maternal blood	(70) T1DM (57) No diabetes	
Cytokines: Interleukin (IL) IL-β, IL-6, IL-8	• Maternal blood		(71) No diabetes
Epidermal Growth Factor (EGF)	• Umbilical cord blood		(72) IDDM, GDM, No diabetes
	• Amniotic fluid		(73) Diabetes status not stated
E-selectin	• Maternal blood	(74) T1DM, T2DM	
Estriol	• Maternal blood	(38) No diabetes	
	• Umbilical cord blood		(38) No diabetes
Estradiol	• Maternal blood	(57) No diabetes	
Free thyroxine (FT4)	• Maternal blood	(75) No diabetes	
Fructosamine	• Maternal blood	(76) IDDM (77) Diabetes (type not specified) (54) IDDM, GDM, No diabetes (78) Pre-existing diabetes, GDM, No diabetes (79) GDM, No diabetes	(80) Pre-existing diabetes, GDM, No diabetes (81) T2DM, GDM (82) GDM, GIGT, No diabetes
	• Umbilical cord blood	(78) Pre-existing diabetes, GDM, No diabetes	
Fat mass- and obesity-associated (*FTO)* gene mRNA	• Placenta	(83) No diabetes	
Ghrelin	• Neonatal blood	(84) GDM, No diabetes (85) No diabetes	
Glucagon-like peptide 1 (GLP-1) - active	• Maternal blood	(86) No diabetes	
Glucose	• Maternal blood	(87) Pre-existing diabetes (88) IDDM (89) IDDM (90) T1DM (91) T1DM (92) T1DM (93) T1DM, No diabetes (94) GDM, GIGT, No diabetes (95) GDM, GIGT, No diabetes (96) GDM, No diabetes (97) GDM, No diabetes (98) GDM, No diabetes	(109) IDDM (110) GDM (111) GDM (25) No diabetes (pregnant), No diabetes (non-pregnant)

(Continued)

TABLE 1 | Continued

Biomarker (Alphabetical order)	Source	Significant association with birthweight/ macrosomia (Most adjusted result used. Maternal diabetes status of sample population provided; all were pregnant unless otherwise stated)	Non-significant association with birthweight/macrosomia (Most adjusted result used. Maternal diabetes status of sample population provided; all were pregnant unless otherwise stated)
		(99) GDM, No diabetes (100) GDM, No diabetes (101) GDM (30) GIGT, No diabetes (102) No diabetes (103) No diabetes (104) No diabetes (105) No diabetes (106) No diabetes (107) No diabetes (108) No diabetes	
	• Umbilical cord blood	(48) No diabetes	
	• Amniotic fluid		(112) No diabetes
	• Maternal urine	(113) No diabetes	
Glycated albumin	• Maternal blood	(114) GDM, No diabetes	
Glycine/valine ratio	• Amniotic fluid		
Glycosylated hemoglobin (HbA1c)	• Maternal blood	(115) Pre-existing diabetes, GDM (116) Pre-existing diabetes, No diabetes (109) Pre-existing diabetes (117) IDDM, GDM, 'Probably normal', Normal (118) IDDM, No diabetes (119) IDDM (120) IDDM, GDM, No diabetes (121) T1DM, T2DM (122) T1DM, T2DM (81) T2DM, GDM (123) T1DM (124) T1DM (20) T1DM (125) T1DM (126) T1DM (127) T1DM (91) T1DM (128) T1DM (90) T1DM (92) T1DM (129) T1DM, No diabetes (130) No diabetes (131) No diabetes (103) No diabetes (132) No diabetes	(87) Pre-existing diabetes (88) IDDM (18) T1DM, T2DM, GDM (19) T1DM, T2DM, GDM (133) T1DM (95) GDM, IGT, No diabetes (134) GDM, IGT, No diabetes (21) GDM, No diabetes (111) GDM (135) GDM (136) GIGT, No diabetes (137) No diabetes (pregnant), No diabetes (non-pregnant) (138) No diabetes (139) No diabetes
	• Umbilical cord blood		(68) No diabetes
Glycosylated proteins	• Maternal blood	(140) T1DM, No diabetes (103) No diabetes	(118) IDDM, No diabetes
	• Umbilical cord blood	(140) T1DM, No diabetes	
Growth factor receptor-bound protein 10 *(GRB10)* gene single nucleotide polymorphism rs12540874 A>G	• Placenta	(141) No diabetes	
Growth Hormone Binding Protein	• Maternal blood		(142) IDDM, NIDDM, No diabetes
HDL-Cholesterol (HDL-C)	• Maternal blood	(143) T1DM, T2DM, No diabetes (111) GDM (144) GDM	

(Continued)

TABLE 1 | Continued

Biomarker (Alphabetical order)	Source	Significant association with birthweight/ macrosomia (Most adjusted result used. Maternal diabetes status of sample population provided; all were pregnant unless otherwise stated)	Non-significant association with birthweight/macrosomia (Most adjusted result used. Maternal diabetes status of sample population provided; all were pregnant unless otherwise stated)
		(145) GDM, No diabetes (100) GDM, No diabetes (146) No diabetes (55) No diabetes (146) No diabetes (147) No diabetes	(21) GDM, No diabetes (30) GIGT, No diabetes (57) No diabetes
	• Umbilical cord blood	(43) No diabetes	(148) T1DM, No diabetes (68) No diabetes
Hepatocyte Growth Factor (HGF)	• Amniotic fluid		(149) No diabetes
Homocysteine	• Maternal blood • Umbilical cord blood	(150) Diabetes status not stated	
Insulin	• Maternal blood		(30) GIGT, No diabetes
	• Umbilical cord blood	(151) IDDM, No diabetes (23) T1DM, No diabetes (62) GDM, No diabetes (64) Diabetes, No diabetes (43) No diabetes (152) No diabetes (48) No diabetes (68) No diabetes	
	• Amniotic fluid	(153) IDDM, GDM, No diabetes (154) T1DM, T2DM, GDM, GIGT, No diabetes (155) T1DM	(69) Diabetes status not stated
Insulin-like Growth Factor-1 (IGF-1)	• Maternal blood	(156) IDDM (142) IDDM, NIDDM, No diabetes (157) T1DM, T2DM, GDM, No diabetes (158) T1DM (159) GDM, No diabetes (160) Diabetes status not stated (161) No diabetes (162) No diabetes	(163) IDDM, No diabetes (164) T1DM, No diabetes (126) T1DM (165) GDM, No diabetes (166) Diabetes status not stated (38) No diabetes (167) No diabetes (168) No diabetes
	• Umbilical cord blood	(169) Pre-existing diabetes, GDM, No diabetes (170) IDDM, NIDDM, No diabetes (171) T1DM, T2DM, GDM (172) T1DM, GDM, No diabetes (173) T1DM, No diabetes (174) GDM, No diabetes (24) Diabetes status not stated (175) Diabetes status not stated (166) Diabetes status not stated (161) No diabetes (176) No diabetes	(177) T1DM, No diabetes (164) T1DM, No diabetes (38) No diabetes (178) No diabetes (168) No diabetes
Insulin-like Growth Factor-2 (IGF-2)	• Maternal blood	(142) IDDM, NIDDM, No diabetes (156) IDDM (158) T1DM (159) GDM, No diabetes	(159) GDM, No diabetes (168) No diabetes (176) No diabetes
	• Umbilical cord blood		(38) No diabetes (169) Pre-existing diabetes, GDM, No diabetes (168) No diabetes
Insulin-like Growth Factor Binding Protein-1 (IGFBP-1)	• Maternal blood	(142) IDDM, NIDDM, No diabetes	
	• Umbilical cord blood	(179) T1DM, T2DM, GDM (178) No diabetes	
Insulin-like Growth Factor Binding Protein-2 (IGFBP-2)	• Maternal blood		(142) IDDM, NIDDM, No diabetes

(Continued)

TABLE 1 | Continued

Biomarker (Alphabetical order)	Source	Significant association with birthweight/ macrosomia (Most adjusted result used. Maternal diabetes status of sample population provided; all were pregnant unless otherwise stated)	Non-significant association with birthweight/macrosomia (Most adjusted result used. Maternal diabetes status of sample population provided; all were pregnant unless otherwise stated)
Insulin-like Growth Factor Binding Protein-3 (IGFBP-3)	• Maternal blood		(38) No diabetes (142) IDDM, NIDDM, No diabetes
	• Umbilical cord blood	(24) Diabetes status not stated	(38) No diabetes
Insulin-like Growth Factor-1 Receptor (*IGF1R)* mRNA	• Placenta	(180) GDM, No diabetes	
Interlukin-6 (IL-6)	• Umbilical cord blood		(48) No diabetes
Irisin	• Umbilical cord blood	(181) GDM, No diabetes	
LDL-Cholesterol (LDL-C)	• Maternal blood		(143) T1DM, T2DM, No diabetes (21) GDM, No diabetes (111) GDM (30) GIGT, No diabetes (55) No diabetes (57) No diabetes (146) No diabetes
	• Umbilical cord blood	(148) T1DM, No diabetes	(43) No diabetes (68) No diabetes
Leptin	• Maternal blood	(182) T1DM, No diabetes (30) GIGT, No diabetes (183) GDM, No diabetes (184) No diabetes	
	• Umbilical cord blood	(182) T1DM, No diabetes (184) No diabetes (43) No diabetes (185) No diabetes (41) No diabetes (47) No diabetes (178) No diabetes	(48) No diabetes
Lipoxin A_4 (LXA_4)	• Maternal blood	(186) GDM, No diabetes	
Metabolites: taurine, creatinine, betaine, glycine, citrate, myo-inositol	• Neonatal urine	(187) Diabetes status not stated	
MicroRNA-21 (miR-21)	• Placenta	(188) No diabetes	
MicroRNAs (miR): miR-141-3p, miR-200c-3p	• Maternal blood	(189) Diabetes status not stated	
MicroRNA-376a (miR-376a)	• Maternal blood	(190) No diabetes	
Mitochondrial DNA (mtDNA)	• Maternal blood		(191) GDM, No diabetes
Nesfatin-1	• Maternal blood		(54) GDM, No diabetes
	• Cord blood		(54) GDM, No diabetes
Obestatin	• Umbilical cord blood		(62) GDM, No diabetes
Pigment Epithelium-Derived Factor (PEDF)	• Umbilical cord blood	(46) T2DM, GDM, No diabetes	
Platelets	• Umbilical cord blood	(129) T1DM, No diabetes	
Placental Growth Factor (PlGF)	• Maternal blood	(192) Diabetes, No diabetes (193) Diabetes, No diabetes	
	• Amniotic fluid		(49) Diabetes status not stated
Placental Growth Hormone (PGH)	• Maternal blood	(142) Pre-existing IDDM, NIDDM, No diabetes (164) T1DM, No diabetes (158) T1DM (126) T1DM	(194) No diabetes (194) No diabetes

(Continued)

TABLE 1 | Continued

Biomarker (Alphabetical order)	Source	Significant association with birthweight/ macrosomia (Most adjusted result used. Maternal diabetes status of sample population provided; all were pregnant unless otherwise stated)	Non-significant association with birthweight/macrosomia (Most adjusted result used. Maternal diabetes status of sample population provided; all were pregnant unless otherwise stated)
	• Umbilical cord blood		(164) T1DM, No diabetes
Placental imprinted genes: BLCAP, DLK1, H19, IGF2, MEG3, MEST, NNAT, NDN, PLAGL1	• Placenta	(195) Diabetes status not-stated	
Placental Lactogen	• Maternal blood	(57) No diabetes	
Placental Protein 13 (PP13)	• Maternal blood	(196) Diabetes status not stated	
Plasminogen Activator Inhibitor-type 1 (PAI-1)	• Maternal blood		(34) No diabetes
Plasminogen Activator Inhibitor-2 (PAI-2)	• Maternal blood	(192) Diabetes not excluded	
Pregnancy-Associated Plasma Protein-A (PAPP-A)	• Maternal blood	(58) Diabetes, No diabetes (197) Diabetes, No diabetes (198) No diabetes (199) Diabetes status not stated	(59) No diabetes
Progesterone	• Maternal blood	(38) No diabetes	
	• Umbilical cord blood	(38) No diabetes	
Prolactin	• Maternal blood		(38) No diabetes
Regulated on Activation, Normal T cell Express and Secreted upon uptake (RANTES)	• Umbilical cord blood	(200) T2DM, GDM, No diabetes	
Retinol-Binding Protein 4 (RBP4)	• Umbilical cord blood	(201) GDM, No diabetes	
Resistin	• Maternal blood		(202) GDM, No diabetes (34) No diabetes
	• Umbilical cord blood		(202) GDM, No diabetes
RNA: *PHLDB2, CLDN1, C15orf29, LPHN3, LEP, GCH1,*	• Placenta	(203) Diabetes status not stated	
Sex Hormone Binding Globulin (SHBG)	• Maternal blood		(38) No diabetes
	• Umbilical cord blood	(174) GDM, No diabetes	(38) No diabetes
Soluble Fms-like tyrosine kinase-1 (sFlt-1)	• Maternal blood	(192) Diabetes, No diabetes	
Soluble Fms-like tyrosine kinase-1 /Placental Growth Factor ratio	• Maternal blood	(193) No diabetes	
Soluble Leptin Receptor (sOB-R)	• Umbilical cord blood	(47) No diabetes	
Soluble TNF-α receptor-2 (TNFR2)	• Maternal blood • Umbilical cord blood	(35) No diabetes	
Squalene	• Maternal blood	(204) GDM, No diabetes	
Stromal Cell-derived Factor-1a (SDF-1a)	• Amniotic fluid	(205) No diabetes	
Testosterone	• Maternal blood		(38) No diabetes
	• Umbilical cord blood		(38) No diabetes
Total cholesterol	• Maternal blood		(111) GDM (21) GDM, No diabetes (30) GIGT, No diabetes

(Continued)

TABLE 1 | Continued

Biomarker (Alphabetical order)	Source	Significant association with birthweight/ macrosomia (Most adjusted result used. Maternal diabetes status of sample population provided; all were pregnant unless otherwise stated)	Non-significant association with birthweight/macrosomia (Most adjusted result used. Maternal diabetes status of sample population provided; all were pregnant unless otherwise stated)
			(146) No diabetes (55) No diabetes
	• Umbilical cord blood	(206) GDM, No diabetes	(43) No diabetes (68) No diabetes
Total lipids	• Maternal blood		(111) GDM
Triglycerides	• Maternal blood	(143) T1DM, T2DM, No diabetes (94) GDM, GIGT, No diabetes (207) GDM, GIGT, No diabetes (208) GDM, No diabetes (209) GDM (144) GDM (210) GDM, No diabetes (146) No diabetes (211) No diabetes (212) No diabetes (213) No diabetes (146) No diabetes (214) No diabetes (25) No diabetes (pregnant), No diabetes (non-pregnant)	(148) T1DM, No diabetes (21) GDM, No diabetes (111) GDM (100) GDM, No diabetes (30) GIGT, No diabetes (215) No diabetes
	• Umbilical cord blood	(211) No diabetes (215) No diabetes	(43) No diabetes (55) No diabetes
Tumor Necrosis Factor-α (TNF-α)	• Maternal blood		(71) No diabetes
Uric acid	• Maternal blood		(55) No diabetes
Vascular Cell Adhesion Molecule-1 (sVCAM-1)	• Maternal blood	(74) T1DM, T2DM	
Vascular Endothelial Growth Factor (VEGF)	• Maternal blood	(193) No diabetes (216) Diabetes status not stated	
Very Low Density Lipoprotein (VLDL)	• Maternal blood	(57) No diabetes	
Visfatin	• Maternal blood	(48) No diabetes	
	• Umbilical cord blood		(48) No diabetes
Vitamin C	• Maternal blood • Umbilical cord blood	(217) No diabetes	

T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus; GDM, Gestational diabetes mellitus; IDDM, Insulin-dependent diabetes mellitus; NIIDM, Non-insulin dependent diabetes mellitus; GIGT, Gestational impaired glucose tolerance.

to avoid or reduce the associated risks. Induction of labor and elective cesarean section are possible options; although, definitive evidence for improved outcomes and cost-effectiveness from these strategies is lacking at present (15, 235). Thus, further development and evaluation of appropriate management options is needed, and improved prediction strategies could be instrumental in this.

Available Methods for Macrosomia Prediction

Different methods for predicting macrosomia are currently available, as outlined in **Table 2**. Risk-factor based prediction aims to assess the likelihood of macrosomia based on identified unmodifiable and modifiable risk factors (13). Diabetes is the strongest risk factor for macrosomia (13, 235), and even maternal hyperglycaemia below diagnostic thresholds for diabetes increases the risk (102). Maternal body mass index (BMI) and gestational weight gain (GWG) are also well-established risk factors (252, 253), with pre-pregnancy obesity increasing the odds of macrosomia by threefold (254). Furthermore, clinical methods of fetal size estimation include physical examination techniques, with symphysis fundal height measurement and abdominal palpation being the primary manoeuvres (255). Maternal estimation of fetal weight by parous women has also been described (247). Finally, ultrasound estimation of fetal weight is routinely used, which employs formulae incorporating fetal biometric parameters (248).

In comparing the performance of the available prediction methods, the broad conclusion is that no single method demonstrates clear superiority over the others (246, 256). Importantly, these current methods all have their limitations—a major limitation is their imprecision, displaying a sensitivity and specificity for macrosomia detection of around 55 and 90%, respectively (15, 246). The false positive and false negative

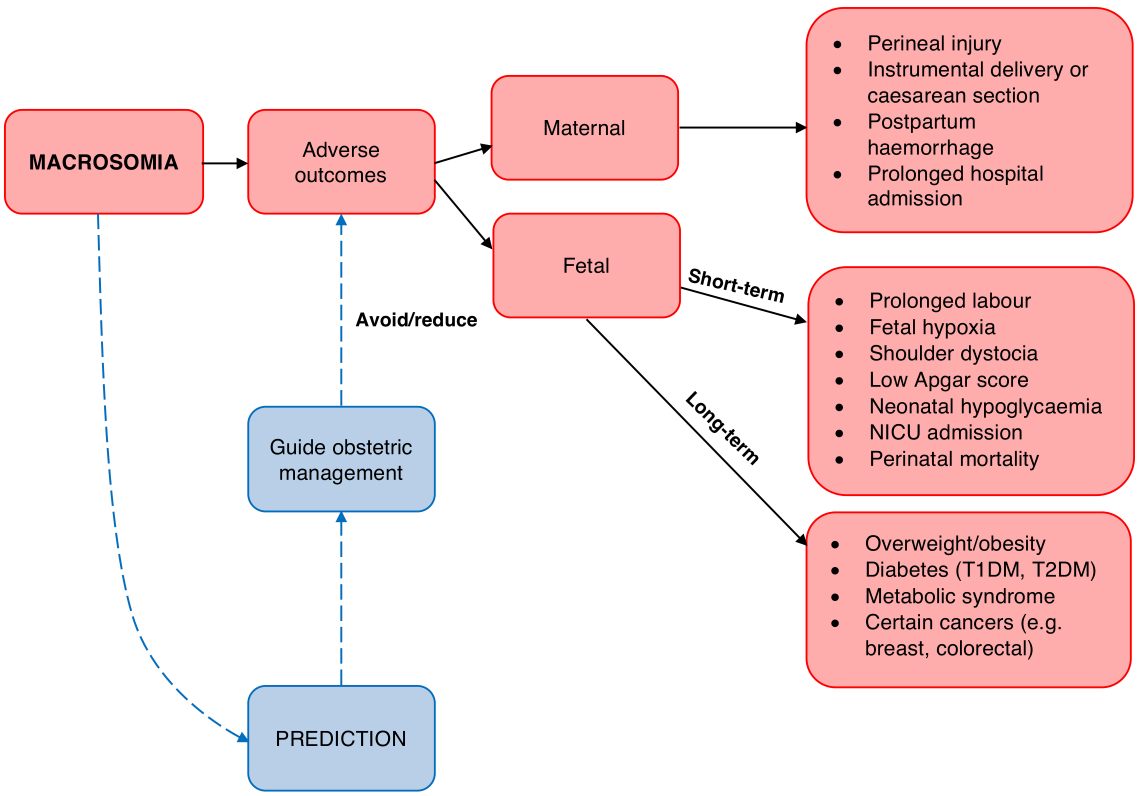

FIGURE 1 | Rationale for macrosomia prediction. Macrosomia is associated with a number of adverse outcomes for both the mother and fetus (223–231). Prediction of macrosomia may reduce or avoid these via guiding appropriate obstetric management.

rates are of concern, as inaccurate results can carry serious consequences including unnecessary intervention (14, 256, 257). Thus, these methods have limited clinical utility and caution is needed if used to guide management (15). From this it is evident there exists a need to improve macrosomia prediction beyond current capabilities, particularly in pregnancies affected by diabetes.

Biomarkers for Macrosomia Prediction

Biomarkers may hold potential for enhancing macrosomia prediction. Biomarkers represent a biological source of information, revealing unique insight into the in-utero environment that may be leading to accelerated fetal growth. Hence by reflecting the possible proximal determinants of excessive growth, biomarkers could provide predictive capacity for macrosomia.

A number of fetal and maternal biomarkers have been previously assessed for an association with birthweight or macrosomia in pregnancies with and without diabetes (**Table 1**;**Figure 2**). An approach to selecting biomarkers for further evaluation was informed by the known risk factors for macrosomia. The risk factors for which a detectable biological correlate (biomarker) may be present and therefore may reflect "proximal macrosomia determinants" are maternal glucose metabolism/diabetes and maternal weight (pre-pregnancy obesity and GWG). Although the underlying mechanisms by which these risk factors mediate their influence on fetal growth have not yet been definitively determined, a theory linking these two with fetal macrosomia considers "direct" and "indirect" pathways (249, 258) **(Figure 3)**.

The direct pathway relates to insulin effectiveness and action (221, 249). Maternal diabetes and/or obesity affects this pathway via exaggerating the physiological insulin resistance that develops during pregnancy, which in-turn contributes to maternal hyperglycaemia and dyslipidaemia (249). This then leads to increased nutrient delivery to the fetus, subsequently resulting in fetal hyperinsulinaemia and macrosomia as per the modified Pedersen hypothesis (249, 258). Thus, biomarkers of maternal glycaemic control that may provide indication of the glycaemia-related risk of the direct pathway include blood glucose, glycosylated hemoglobin (HbA1c), and 1,5-anhydroglucitol (1,5-AG). While maternal triglycerides and cholesterol may be markers of the dyslipidaemia-related effects on growth. On the other hand, the indirect pathway centers on placental function (249). Placental changes that have been linked to maternal diabetes and obesity, such as alterations in structure, utero-placental blood flow, and placental transporters, may lead to altered feto-placental nutrient transport (249, 259). This likewise increases nutrient delivery to the fetus and stimulates fetal hyperinsulinaeamia. As adiponectin and insulin-like growth

TABLE 2 | Evaluation of available methods for macrosomia prediction.

Method	Description	Performance	Advantages	Disadvantages
Risk-factor assessment	• Assesses the likelihood of macrosomia based on factors known to increase macrosomia risk. • Unmodifiable risk factors include maternal age, parity, parental height, ethnicity, fetal sex (male), and previous macrosomic delivery (224, 236–238). • Modifiable risk factors include pre-pregnancy weight, GWG, gestational age, impaired glucose tolerance/diabetes (224, 236, 238, 239).	• Accuracy varies with the risk factors assessed and population studied. • One prediction equation demonstrated 57% sensitivity, 90% specificity, PPV 47%, NPV 93% (cut off value 3,750 g); although this excluded women with complications including diabetes (240).	• Risk factors can be readily assessed with history and examination • No cost • Non-invasive	• A validated, accessible, user-friendly predictive tool using risk factors is lacking • A notable proportion of macrosomia occurs in pregnancies that have no or low identifiable risk
Symphysis fundal height (SFH) measurement	• The SFH is a measurement of the maternal abdomen from the superior margin of the symphysis pubis to the highest point of the uterine fundus using a tape measure (241). • Measurements greater than the normal range for gestational age as per fundal height curves may indicate a large fetus (241).	• Estimates of the predictive performance for macrosomia vary widely, with reported sensitivity ranging from 16–98% and specificity of 88-95% (242–244).	• Available at the bedside • No cost • Non-invasive	• Accuracy problems relating to the measurement technique, inter-observer variability, gestational age dating or use of different fundal height curves. • Maternal diabetes and obesity may also affect accuracy (245)
Abdominal palpation	• Abdominal palpation using Leopold manoeuvres estimate fetal size by tactile assessment of fetal parts (246).	• When performed by experienced clinicians, abdominal palpation can predict 70% of birthweights to within 10% of the actual value (246).	• Available at the bedside • No cost • Non-invasive	• Accuracy influenced by the subjective nature of the assessment and operator-dependence
Maternal estimation	• A parous women is asked to estimate the birthweight of her child prior to delivery (247).	• A study in post-term pregnancies demonstrated prediction of macrosomia with 56% sensitivity, 94% specificity, PPV 77%, NPV 86%(247).	• Available at the bedside • No cost • Non-invasive • Involves the mother	• Limited to women with a previous pregnancy
Ultrasound assessment	• Ultrasound assessment of fetal size involves determining the gestational age of the pregnancy, measurement of fetal biometry (e.g., abdominal circumference), use of various formulae to estimate fetal weight, and comparing fetal size with population standard charts for gestational age to obtain the corresponding percentile (248).	• Masurement error of ultrasound fetal weight estimation has been reported as ± 15–20% (241, 249). • Poorer accuracy at the extremes of fetal weight (250). • Mean detection rate of macrosomia is 29% in the general obstetric population (250). • Margin of error in pregnancies with diabetes is ±20–25% (251).	• Wide availability • Rapidly produces results • Perceived objectivity	• Requires trained operators • Resource requirements & costs • Inconvenience of extra appointments

PPV, positive predictive value; NPV, negative predictive value.

factor-1 (IGF-1) are proposed to be involved in these placental alterations, they therefore represent potential biomarkers of this indirect pathway (249). Hence these biomarkers are prime candidates to be assessed for associations with fetal weight. Maternal rather than fetal (cord blood) biomarkers will be the focus due to being the source relevant for antenatal predictive testing. Pre-existing diabetes, GDM, and pregnancies without diabetes will be examined in turn (results summarized in **Table 3**).

Blood Glucose

In women with pre-existing diabetes, various parameters of blood glucose have been assessed for associations with birthweight. The Diabetes in Early Pregnancy (DIEP) study focused on elucidating the contribution of fasting verses postprandial glucose to infant birthweight, comparing women with T1DM and controls across pregnancy (93). The findings indicated that postprandial glucose was more important for macrosomia (birthweight $\geq$ 90th percentile) risk, with the third trimester postprandial glucose levels the strongest predictor. Other studies also support the importance of postprandial blood glucose; with postprandial glucose levels in the third trimester predicting macrosomia (birthweight >90th percentile) (87), and mean postprandial glucose in the second trimester associated with birthweight (91). However, these studies have often analyzed the fasting and postprandial glucose measurements as the average across a whole trimester, hindering more specific timing effects of glycaemia on macrosomia risk to be determined. Addressing this by calculating the mean fasting and post-prandial glucose measurements over 3 week blocks in women with pre-existing diabetes, Persson et al. found the mean fasting glucose levels between 27 and 29 weeks' gestation were independently associated with macrosomia

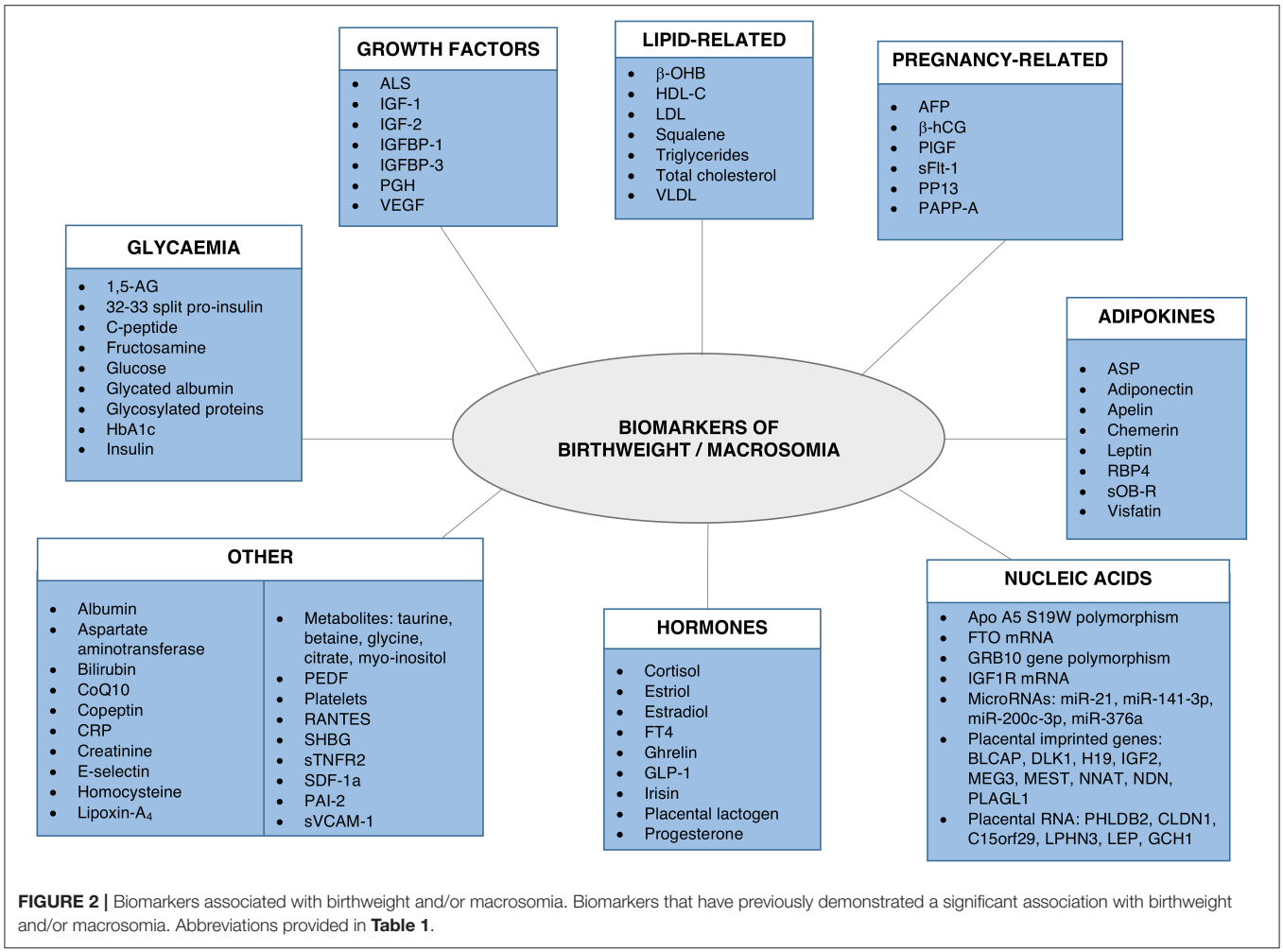

FIGURE 2 | Biomarkers associated with birthweight and/or macrosomia. Biomarkers that have previously demonstrated a significant association with birthweight and/or macrosomia. Abbreviations provided in **Table 1**.

(birthweight >2 standard deviations), whereas postprandial levels were not (88). This fasting glucose measurement and pre-pregnancy weight together accounted for 12% of the variance in birthweight. In addition to the different time periods over which averages were calculated, variations in the measurement methods including the use of patient self-monitoring of blood glucose (SMBG) via a glucometer compared to laboratory testing, could have contributed to the conflicting findings. Nonetheless, they substantiate the contribution of second and third trimester maternal glycaemia to fetal growth in pregnancies complicated by pre-existing diabetes (89, 260, 261).

Furthermore, as postprandial hyperglycaemia involves transitory glycaemic excursions, this supports the notion that glucose fluctuations in addition to chronic hyperglycaemia, are important in influencing excessive fetal growth (92, 123, 124). To comprehensively assess such temporal patterns in glucose control, continuous glucose monitoring systems (CGMS) are needed, particularly in women with T1DM, as fluctuations are often missed by SMBG (262, 263). Indeed, initial studies using CGMS have shown maternal glucose excursion profiles are related to macrosomia (90, 264–266). For example, a multi-center study involving women with T1DM and T2DM using CGMS, identified that glucose excursions at specific time periods throughout the day were associated with macrosomia (birthweight >90th percentile) in each trimester (264). For the second and third trimesters, the macrosomia-related glucose levels were higher and showed greater variability.

Meanwhile, studies in the setting of GDM have mostly used measures of maternal glucose obtained from the oral glucose tolerance test (OGTT). These measures, particularly the fasting values, have often been associated with macrosomia (95–99). Although, a Canadian study found glucose levels (fasting or post-glucose load) only independently accounted for 3–5% of the variance in birthweight (98).

Importantly, maternal glycaemia has been shown to be related to macrosomia risk amongst healthy women in the absence of overt diabetes (102, 103). The landmark study in the area is the Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study. This multinational investigation assessed outcomes associated with glucose parameters from the OGTT at 24–32 weeks' gestation in healthy pregnant women (102). The blinded data from ∼23,000 participants demonstrated linear associations between increasing maternal glucose levels

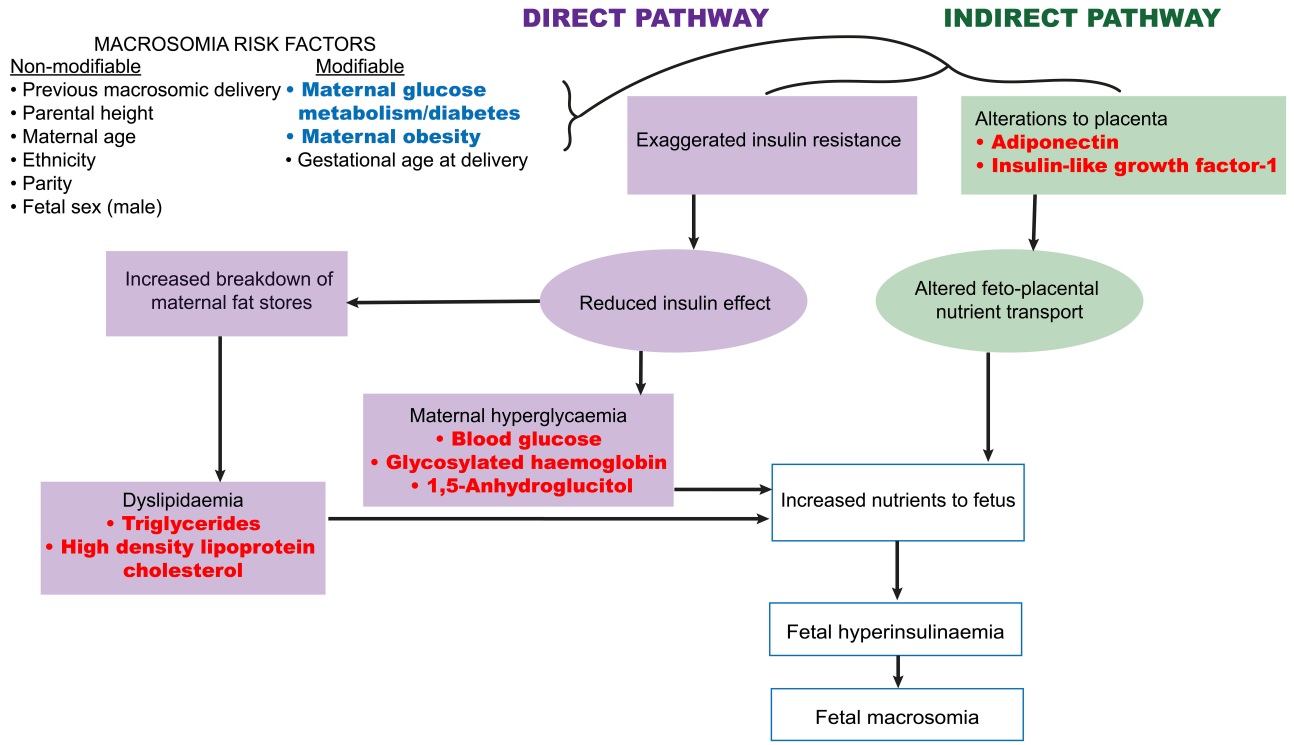

FIGURE 3 | Proposed link between macrosomia risk factors and the selected biomarkers. Maternal diabetes and obesity have proposed links to fetal macrosomia via direct and indirect effects on fetal growth (249, 258). Biomarkers (red) possibly related to these pathways may therefore capture information that has predictive capacity for macrosomia.

below diagnostic thresholds for diabetes with both birthweight above the 90th percentile and umbilical cord blood C-peptide above the 90th percentile (indicative of fetal hyperinsulinaemia). The findings support the Pedersen hypothesis and indicated even mild maternal hyperglycaemia without diabetes increases macrosomia risk, which has had subsequent implications for GDM diagnostic thresholds.

However, other studies with participants of varying diabetes status have not found blood glucose to be associated with birthweight (25, 109–111). Variations in glucose testing protocols and treatment regimens may provide some explanation for this. Thus, uncertainties remain regarding the utility of blood glucose for birthweight/macrosomia prediction (8).

Glycosylated Hemoglobin (HbA1c)

HbA1c is produced by non-enzymatic glycosylation of hemoglobin. It is a long-term marker of glycaemic control, reflecting the average glucose concentration over the previous 2–3 months (267, 268).

In women with pre-existing diabetes, macrosomia and birthweight have been significantly associated with HbA1c measured at different time points. In multiple studies, third trimester values have demonstrated positive associations with macrosomia (90, 92, 109, 123, 126, 127) and birthweight (91, 128). A notable study is a prospective nation-wide investigation of 289 women with T1DM in The Netherlands (123). Amongst this cohort with acceptable glycaemic control, the third trimester HbA1c measurement was the strongest predictor of macrosomia (birthweight >90th percentile), accounting for 4.7% of the variance of macrosomia. Furthermore, HbA1c measured in trimester 1 (119, 121), or trimester 2 and 3 (116, 124, 125, 129) have also been associated with birthweight or macrosomia. Across all of these studies, HbA1c has been reported to explain ∼5–23% of variance in birthweight (119, 123).

Contrastingly, other research groups have not found a significant association between HbA1c and birthweight (19, 87, 88, 269). Contributing factors to these inconsistencies may include the sample collection time-points and the use of averages of HbA1c across varying periods. This could be influential as HbA1c normally declines during pregnancy and is a retrospective weighted average marker (267). It may also relate to the study protocol reducing glycaemic variability (87, 88) or to differences in analytical assays (270).

In addition, a prominent issue is the persistence of high macrosomia rates in women with pre-existing diabetes even when HbA1c values indicate "good" glycaemic control (87, 92, 123, 133). This "macrosomia despite normoglycaemia" may be linked to HbA1c-determined normoglycaemia not revealing glycaemic variability. As previously mentioned, postprandial hyperglycaemia and glycaemic fluctuations are considered important in accelerating growth (8, 271). This is consistent with

TABLE 3 | Summary of evidence in support of an association between the selected biomarkers and birthweight/macrosomia.

Biomarker (Maternal source unless otherwise stated)	T1DM	T2DM	GDM	No diabetes
Blood glucose	• Strongest evidence for second & third trimester measurements. • Postprandial over fasting measurements.	• Strongest evidence for second & third trimester measurements. • Postprandial over fasting measurements.	• Support for glucose parameters in the oral glucose tolerance test.	• Support for glucose parameters in the oral glucose tolerance test.
Glycosylated hemoglobin	• Strongest evidence for third trimester measurements.	• Strongest evidence for third trimester measurements.	• Limited supportive evidence.	• Limited supportive evidence.
1,5-Anhydroglucitol	• Significant association in all available studies.	• Significant association in all available studies.	• Mixed results.	• No supportive evidence.
Lipids	• Triglycerides and HDL-C with most support.	• Triglycerides and HDL-C with most support.	• Triglycerides and HDL-C with most support.	• Triglycerides and HDL-C with most support.
Adiponectin	• Lack of maternal adiponectin studies. • Fetal adiponectin not significant (limited studies).	• Lack of maternal adiponectin studies. • Fetal adiponectin not significant (limited studies).	• Some support for maternal and fetal adiponectin.	• Some support for maternal and fetal adiponectin.
Insulin-like growth factor-1	• Mixed results for maternal IGF-1. • Stronger support for fetal IGF-1.	• Mixed results for maternal IGF-1. • Stronger support for fetal IGF-1.	• Some support for maternal and fetal IGF-1.	• Some support for maternal and fetal IGF-1.

some studies indicating tighter blood glucose control and thus reduced glycaemic excursions, can reduce macrosomia incidence (260, 272, 273).

There is weaker evidence for a significant association between HbA1c and birthweight in women with GDM (21, 95, 111, 134, 135). This may related to the reduced aberrations in HbA1c in GDM compared with T1DM and T2DM (274). Also, many studies have measured HbA1c at the same time as GDM diagnostic testing (around 28 weeks' gestation) for convenience. However, when HbA1c was measured at delivery in women with GDM, HbA1c >6.8% was associated with a fivefold increased risk of macrosomia (birthweight ≥4,000 g) compared with HbA1c<6.0% (81). Later HbA1c testing may therefore be more useful for predicting macrosomia.

Meanwhile, for women without diabetes, a correlation between HbA1c at various times with birthweight has been identified by some (103, 130, 131, 139), but not other researchers (137, 138). In the HAPO cohort, glucose measures had a significantly stronger association with birthweight than HbA1c (132). Furthermore, another study assessed ultrasound and HbA1c prediction of macrosomia (birthweight ≥4,000 g) within 1 week prior to delivery (138). It found HbA1c measurements were not useful and thus could not improve ultrasound prediction accuracy. However, HbA1c levels were low in this cohort without diabetes. Thus overall, HbA1c may be more useful in women with pre-existing diabetes and later in pregnancy.

1,5-Anhydroglucitol (1,5-AG)

1,5-AG is the 1-deoxy form of glucose and is a short-term glycaemic marker (275). During normoglycaemia, serum 1,5-AG is in a steady-state, with >99% renal reabsorption (275). However in hyperglycaemic conditions, glucose competitively inhibits renal reabsorption of 1,5-AG, thereby increasing 1,5-AG excretion and reducing the serum 1,5-AG concentration (268). It reflects the glycaemic control over the preceding 24 h to 2 weeks, and importantly, it can capture glycaemic fluctuations (268, 275).

With these benefits in detecting glycaemic excursions, Nowak et al. compared 1,5-AG and HbA1c in pregnant women with T1DM and found 1,5-AG was the stronger predictor of macrosomia (birthweight >90th percentile) (20). The receiver operator characteristic (ROC) area under the curve (AUC) for third trimester 1,5-AG macrosomia prediction was 0.81. This improved to 0.84 with the addition of HbA1c, and could achieve sensitivity and specificity of 86 and 71%, respectively. As 80% of the cases of macrosomia occurred in women that met HbA1c targets, it suggested that glucose excursions that were not reflected in the HbA1c level but were captured by the 1,5-AG values may have contributed to fetal overgrowth. This assertion was supported by CGMS records which showed 1,5-AG was strongly correlated with CGMS indices including a measure of glucose variability, but HbA1c was not.

Building on this, a study involving women with T1DM, T2DM, and GDM found that there was a significant inverse association between 1,5-AG and birthweight *z*-score across the groups (19). Of note, HbA1c was not associated with birthweight, possibly due to the participants having overall low HbA1c measurements. The authors contend however, that as 1,5-AG was significantly associated with birthweight even amongst a population with good glycaemic control according to HbA1c, it could be used to identify the subset of pregnancies that are at risk of macrosomia, despite HbA1c within target ranges.

Wright et al. similarly found an inverse linear association between mean 1,5-AG and birthweight *z*-scores in a cohort of T1DM, T2DM, and GDM pregnancies (18). The association for mean HbA1c was not significant. The lack of blood glucose

data (SMBG/CGMS) is a limitation, as conclusions regarding glycaemic control and fluctuations require consideration of these immediate measures of glycaemia.

In contrast, a study comparing women with GDM and pregnant women without diabetes did not find 1,5-AG to be a significant predictor of birthweight (21). Although, serum 1,5-AG concentration was significantly lower in the women with GDM compared to controls and there was a trend for an inverse association between 1,5-AG and birthweight in the GDM group.

There have been concerns that the reduction in renal glucose threshold during normal pregnancy may affect renal excretion of 1,5-AG and thus serum levels, thereby possibly limiting the utility of 1,5-AG in reflecting glycaemic changes while pregnant (275, 276). However, the few available evaluations of 1,5-AG as a marker of glycaemic control in pregnancies complicated by diabetes have shown that it performs well (20, 277).

Lipids

Lipid metabolism is altered during normal pregnancy. Increased fat storage occurs initially in the "anabolic phase" of pregnancy (278). The switch to the "catabolic phase" in the third trimester involves prominent lipolysis promoted by insulin resistance (279, 280). This is paralleled with an increase in the major lipid fractions, predominantly triglycerides (220, 278); which is seen to a greater extent in women with diabetes (278, 281). In accordance with the modified Pedersen hypothesis, maternal lipids may be an important fuel in fetal overgrowth (220).

Of all lipids, triglycerides have been most consistently related to birthweight in pregnancies with diabetes. A study comparing women with T1DM, T2DM, and controls found both third trimester triglycerides and high-density lipoprotein cholesterol (HDL-C) were significantly associated with macrosomia (birthweight >90th percentile), independent of maternal glycaemic control (143). In GDM pregnancies, maternal triglycerides have also been identified as a predictor of macrosomia independent of maternal BMI and glycaemic control (144, 209). Moreover, the ratio of triglycerides to HDL-C has also been examined in women with well-controlled GDM and women without diabetes at 24–28 weeks' gestation (282). The ROC AUC for macrosomia (birthweight >90th percentile) prediction was 0.668, which increased to 0.806 when combined with HbA1c and pre-pregnancy BMI. However, the overall prevalence of macrosomia was low in this population. Together these results indicate lipid alterations may play a distinct role in macrosomia development amongst women with diabetes. Indeed, maternal lipids have been proposed as a potential key factor in "macrosomia despite normoglycaemia" (10, 283).

In women without diabetes, second or third trimester maternal triglycerides have been found to be positively associated with birthweight (25, 211), as well as an independent predictor of macrosomia (94, 146, 207, 212, 213). In a Japanese cohort, maternal fasting hypertrigylceridaemia significantly independently predicted macrosomia (birthweight >90th percentile), with an odds ratio of 11.6 (214). Notably, triglycerides were more strongly associated with fetal growth than maternal glycaemia; although, the small sample size (146 people) may have limited analysis. It is supported however by a similar finding in a cohort that included women with GDM (208). In this study, the triglyceride concentrations after an OGTT were independently associated with birthweight and also predicted glucose intolerance.

Furthermore, macrosomia risk, and birthweight has been inversely associated with second and third trimester maternal HDL-C concentrations in women with pre-existing diabetes (143) and GDM or healthy pregnancies (55, 100, 111, 146, 147). In Zhou et al.'s study which included GDM pregnancies, low HDL-C (<2.2 mmol/L) at 20 weeks' gestation predicted macrosomia (birthweight >4,000 g) with 65% sensitivity and 48% specificity (55).

In contrast, other lipid parameters have less supportive evidence. In women without diabetes, very-low density lipoprotein cholesterol (VLDL-C) has been negatively associated with birthweight (57). While low-density lipoprotein cholesterol (LDL-C) has mostly demonstrated non-significant results (21, 30, 55, 143).

Overall, these studies suggest differential importance of maternal lipid fractions for fetal growth. Again, some studies have not found an association between lipids and birthweight or macrosomia in diabetic or healthy pregnancies (21, 30). Measurement timing may be relevant to this due to the changes in lipid profile and hence possibly their role across pregnancy.

Adiponectin

Adiponectin is an adipokine—a bioactive peptide derived from adipose tissue (284). It has important roles in regulating insulin sensitivity and metabolism, and is inversely related to adipose mass and insulin resistance (284, 285). Given this, adiponectin is a possible mediator in the link between maternal adiposity, insulin resistance, and excessive fetal growth (284, 286). Maternal adiponectin may influence fetal growth via altering placental substrate transport as it does not traverse the placenta (286).

There is a notable lack of investigation of maternal adiponectin in women with pre-existing diabetes. Fetal adiponectin however, has received some attention. A comparison of neonates from mothers with T1DM with healthy controls found umbilical cord blood adiponectin collected at birth was not associated with birthweight (23). Similarly, cord blood adiponectin was not associated with birthweight in a study examining offspring of women with T2DM, GDM, and controls (46).

Amongst GDM pregnancies there have been variable results. Tsai and associates compared maternal adiponectin levels collected between 24 and 31 weeks' gestation in women with GDM and controls (26). Adiponectin was significantly lower in the GDM women, and a negative association between maternal adiponectin and birthweight was evident but only significant for the GDM group. Pre-pregnancy BMI ≥27 was also associated with lower adiponectin levels. Cseh et al. corroborated that maternal plasma adiponectin is significantly lower in women with GDM compared to non-diabetic pregnant women and age-matched non-diabetic non-pregnant women (27). Contrastingly though, a significant positive linear correlation was demonstrated between maternal plasma adiponectin and birthweight corrected for gestational age in both the GDM and non-diabetic groups.

Meanwhile, others have not found an association between adiponectin and birthweight (36, 37). These conflicting findings may be related to differences between the populations, including BMI, ethnicity, and GDM management, as well as timing of the samples. The adiponectin fractions assessed may also be relevant, as one research group found that only the middle molecular weight isoforms were significantly negatively associated with birthweight in GDM (28).

Verhaeghe et al. also evaluated the value of metabolic biomarkers for birthweight prediction in GDM and control women (29). Maternal adiponectin combined with four other metabolic markers together added 2% to the ∼10% explained birthweight variance from maternal body size parameters alone. However, only a single measurement was taken at 24–29 weeks' gestation and given insulin resistance is maximal in the third trimester, greater utility may be provided with later testing.

Findings from healthy pregnancies also provide insight. In two case-control studies involving women without diabetes, macrosomia groups had significantly lower maternal adiponectin concentrations compared with the controls (31, 32). In one of these, maternal adiponectin measured between 11 and 13 weeks' gestation improved macrosomia (birthweight >95th percentile) detection to 38.2% when added to maternal characteristics and obstetric history (compared with 34.6% without adiponectin) (31). Moreover, an independent inverse relationship was identified between maternal adiponectin and birthweight in a subset of the HAPO cohort (34). Although, other studies have not found a significant association with maternal adiponectin (38, 39).

Thus, despite inconsistencies there is indication adiponectin may be related to birthweight. Pre-existing diabetes is an area that particularly requires further investigation.

Insulin-Like Growth Factor-1 (IGF-1)

IGF-1 is a peptide hormone principally produced in the liver (287). Normal pregnancy involves changes in the maternal IGF axis, including variations to IGF-binding proteins (IGFBPs) (287). Consequently, this results in increased free IGF-1, the biologically active form. With mitogenic and metabolic actions, maternal and fetal IGF-1 are believed to be important mediators in fetal growth (285, 287). For maternal IGF-1, this may be via its role in regulating transplacental nutrient transport (288, 289).

However, the literature on maternal IGF-1 in pre-existing diabetes is conflicting. In a prospective study involving serial maternal IGF-1 measurements in women with pre-existing diabetes, IGF-1 was significantly positively associated with macrosomia (156). Yet in a similar study involving only women with T1DM, there were no differences in maternal IGF-1 across pregnancy in diabetic women delivering a macrosomic neonate compared with appropriate birthweight neonate (126). A notable difference between the studies were the methods of macrosomia assessment. The first study created *post-hoc* groupings according to birthweight ratio whereas the second used the definition of birthweight >90th percentile. Likewise, another research group found macrosomia according to this latter definition was not significantly associated with maternal IGF-1 (142). Although, they did show maternal IGF-1 measured at 36 weeks' gestation was significantly associated with birthweight *z*-score (142). Thus, the assessment measure of birthweight/macrosomia may be relevant in identifying an association with a biomarker.

GDM pregnancies have also been investigated. In a study of GDM and control women, elevated IGF-1 in maternal blood in mid- and late- gestation and fetal cord blood at birth predicted macrosomia (birthweight >90th percentile) (159). This is consistent with a case-control study in which serum IGF-1 levels were significantly higher in the GDM women and their macrosomic neonates (birthweight >2 standard deviations) compared to matched controls with appropriate birthweight neonates (290). Other studies have also found IGF-1 related to birthweight (174, 291).

The relationship of IGF-1 to fetal growth across the different types of diabetes requires clarification. An investigation comparing women with T1DM, T2DM, GDM, and controls and found the third trimester median maternal IGF-1 values were not significantly different between the groups (157). Considering all the women with diabetes together, third trimester IGF-1 was positively associated with birthweight percentile, explaining 24% of the variation in birthweight. However, other reports suggest the changes across pregnancy in IGF-1 for pre-existing diabetes and GDM are different, with lower levels in pre-existing diabetes (163, 164, 177) and higher levels in GDM (258) compared with controls.

For pregnancies without diabetes, maternal IGF-1 (161, 162, 167) and fetal IGF-1 (38, 161, 166, 175, 176) have been associated with birthweight and macrosomia. These studies have been conducted in populations of various ethnicities. Non-significant associations have also been reported (165, 168, 178, 292). Such discrepancies may relate to factors such as the method of IGF-1 analysis, timing of measurements, or the sample size (287).

Altogether the evidence is conflicting. Adequate assessment has not been made of the predictive utility of IGF-1 for birthweight and macrosomia in women with diabetes.

Evaluation of Macrosomia Biomarkers

Biomarkers face many challenges in becoming adopted into routine clinical practice. One of the most important requirements is biomarker validation (16), which is particularly an issue in the field of macrosomia biomarkers. In this area, substantial variability exists amongst published results, with the heterogeneity of the studies a prominent contributor. Indeed, there is considerable variation in study designs, populations, measurement timing, outcome variables (including macrosomia definitions), and analytical methods. This limits the conclusions that can be made at this time and highlights the need for rigorous validation protocols to comprehensively evaluate macrosomia biomarker predictive performance. Future work in this field must also assess the cost-effectiveness of biomarker use. Biomarker adoption may become more feasible with improved accessibility of commercial biomarker kits and multiparametric biomarker testing for multiple pregnancy disorders together (e.g., with preeclampsia biomarkers as they also become validated).

Furthermore, as biomarkers may be useful as part of a combination approach, whereby biomarkers are incorporated into a prediction algorithm with other elements such as

macrosomia risk factors, physical examination and ultrasound measurements, further investigation is also needed that compares biomarkers to and in combination with the other methods of macrosomia prediction. Of the limited such assessments available, biomarkers have improved predictive performance when combined with risk factors (29, 31, 282) but not ultrasound (138). A further step is to determine if improved macrosomia predictive accuracy can improve clinical outcomes.

CONCLUSION

Accurately predicting fetal macrosomia remains a desirable but challenging goal. While biomarkers hold promise for assisting in this plight, the current state of knowledge for macrosomia biomarkers is limited. The selected biomarkers in this review each have a theoretical link with macrosomia with some supportive evidence for an association with birthweight/macrosomia. However, due to the limitations of the literature, the true value of biomarkers is not yet clear. Further research is needed to address this, particularly in pregnancies affected by diabetes. A focus on this area is warranted as there is great potential and much to be gained by further exploration. Indeed, broader implications of this research includes providing greater insight into the pathophysiological processes of excessive growth; which is of special interest due to the links with later development of chronic disease (Barker hypothesis). Ultimately, improving outcomes for pregnant women and their babies is the driving force for this research.

AUTHOR CONTRIBUTIONS

SN was the primary author. EE, JS, and AS edited the manuscript. CH reviewed the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2018.00407/full#supplementary-material

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Improved Glucose and Lipid Metabolism in the Early Life of Female Offspring by Maternal Dietary Genistein Is Associated With Alterations in the Gut Microbiota

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Maternal over-nutrition can lead to metabolic disorders in offspring, whereas maternal dietary genistein may have beneficial effects on the metabolic health of offspring. Our objective was to determine whether maternal dietary genistein could attenuate the detrimental effects of a maternal high-fat diet on their offspring's metabolism and to explore the role of the gut microbiota on their offspring's glucose and lipid metabolism. C57BL/6 female mice were fed either a high-fat diet without genistein (HF), high-fat diet with low-dose genistein (0.25 g/kg diet) (HF.LG), high-fat diet with high-dose genistein (0.6 g/kg diet) (HF.HG) or normal control diet (Control) for 3 weeks prior to breeding and throughout gestation and lactation. The female offspring in the HF group had lower birth weights and glucose intolerance and higher serum insulin, triacylglycerol (TG) and total cholesterol (TC) levels at weaning compared with the Control group. Offspring from HF.LG dams had increased birth weight, improved glucose tolerance, and decreased fasting insulin, whereas the serum TG and TC levels were decreased in HF.HG offspring in comparison with HF offspring. The significant enrichment of *Bacteroides* and *Akkermansia* in offspring from genistein-fed dams might play vital roles in improving glucose homeostasis and insulin sensitivity, and the significantly increased abundance of *Rikenella* and *Rikenellaceae_RC9_ gut_group* in the HF.HG group may be associated with the decreased serum levels of TG and TC. In conclusion, maternal dietary genistein negates the harmful effects of a maternal high-fat diet on glucose and lipid metabolism in female offspring, in which the altered gut microbiota plays crucial roles. The ability of maternal genistein intake to improve offspring metabolism is important since this intervention could fight the transmission of diabetes to subsequent generations.

Keywords: dietary genistein, glucose and lipid metabolism, gut microbiota, maternal high-fat diet, female offspring

INTRODUCTION

Obesity and type 2 diabetes mellitus (T2DM) are highly prevalent and lead to tremendous health and economic burdens. However, the etiology and pathogenesis of obesity and diabetes are still unclear. Since the developmental origins of health and disease (DOHaD) hypothesis was first put forward in the early 1990s (1), a large number of epidemiological investigations (2–4) and animal studies (5, 6) have highlighted the importance of the developmental environment in early life in determining the trajectories of chronic disease in later life, including obesity and T2DM. Numerous recent studies (7, 8) and our previous research (9) have shown that a maternal high-fat diet during pregnancy and lactation can significantly increase the susceptibility of offspring to obesity, glucose intolerance and insulin resistance. Thus, interventions during early life may reset the disease trajectories and prevent the onset and development of diabetes.

Several large epidemiological studies have shown that intake of soy foods and isoflavones are associated with a lower risk of T2DM (10–12). Soy isoflavones have a weak estrogen-like effect, and the main components of soy isoflavones include genistein, diadzein, and glycitein. Genistein has been widely used as a dietary supplement in the United States and has been explored for the potential effects in cognitive function, cancer therapy, and bone and cardiovascular health (13). In recent years, a growing number of studies have shown that genistein improves glucose and lipid metabolism and have demonstrated that genistein intake reduces the levels of blood glucose, triglycerides (TG) and total cholesterol (TC) as well as prevents weight gain, without side adverse effects (14–16). Modulating the hepatic glucose output, enhancing β-cell proliferation, reducing apoptosis, activating the cAMP/PKA signaling pathway and antioxidant effects are all potential mechanisms for the anti-diabetic functions of genistein (13). However, currently, studies exploring the effects of genistein intervention in early life on glucose and lipid metabolism are rare.

During the last few decades, the gut microbiota has become a focus of medical research. Numerous lines of evidence (17–19) have suggested that the gut microbiota plays an important role in glucose and lipid metabolism. Recently, a growing number of human (20) and animal studies (1, 21, 22) have indicated that the gut microbiota is disordered in offspring from obese mothers and high-fat fed dams. Thus, the gut microbiota may play a pivotal role in a poor maternal intrauterine growth environment, programming the offspring to develop metabolic disturbances. Furthermore, an association between the genistein improvement of glucose tolerance and alterations of the gut microbiota has been shown (23). However, investigations into the effects of maternal genistein intervention on the gut microbiota in offspring are limited.

In the current study, we aimed to research the effects of maternal dietary genistein on metabolic health in the early life of female offspring and determine whether maternal genistein intake could reverse the detrimental metabolic effects of a maternal high-fat diet in female offspring. In addition, we explored the role of the gut microbiota on offspring glucose and lipid metabolism.

MATERIALS AND METHODS

Animals and Study Design

Four-week-old C57BL/6 female mice were obtained from the National Institutes for Food and Drug Control (Beijing, China; SCXK-2014-0013). Animals were maintained in controlled animal facilities at a room temperature of 22 ± 2°C with a 12 h light/dark cycle and were fed a normal control diet (AIN-93G diet) with corn oil substituted for soybean oil. After 1 week of environmental acclimatization, dams were randomly divided into four groups and were fed a high-fat diet without genistein (HF, $n = 6$), high-fat diet with low-dose genistein (CAS: 466-72-0, G0272, TCI Development Co., Ltd.) (0.25 g/kg diet) (HF.LG, $n = 6$), high-fat diet with high-dose genistein (0.6 g/kg diet) (HF.HG, $n = 8$) or normal control diet (Control, $n = 8$) for 3 weeks. The soybean oil in the high-fat diet was also substituted by corn oil. The ingredients are shown in **Table S1**. The high-fat diet included (kcal %): fat, 60%; carbohydrate, 20%; and protein, 20%, with a 5.24 kcal/g energy supply, whereas the control diet contained (kcal %): fat, 15.8%; carbohydrate, 63.9%; and protein, 20%, with a 3.9 kcal/g energy supply.

Female mice were mated to 8-week-old C57BL/6 males and fed a normal diet. The dams were checked for postcopulatory plugs every morning after mating, and the appearance of a plug was recorded as d 0.5 of pregnancy. Females were fed their assigned diet during pregnancy and lactation and had access to food and water *ad libitum*. The litters were all culled to five pups to ensure that there was no nutritional bias between litters. Offspring were weaned at 3 weeks of age. At weaning, all female offspring ($n = 6$–8 per group) were sacrificed. Blood samples were collected from the intraorbital retrobulbar plexus after 10 h of fasting from anesthetized mice, and the uterus and ovaries were removed and weighed; the cecal contents were quickly removed, snap frozen in dry ice, and then stored at −80°C for further analysis. All operations were conducted under chloral hydrate anesthesia, and best efforts were done to minimize suffering. All of the procedures were approved by the animal care and use committee of the Peking Union Medical College Hospital (Beijing, China, SYXC-2014-0029). All of the animal operations were conducted in compliance with the Guide for the Care and Use of Laboratory Animals.

Measurement of Body Weight and Food Intake

The body weights of both the mother and offspring were measured once per week. We measured the 3-day food intake each week by the mother, and their food consumption was estimated by weighing the remaining food.

Glucose Tolerance Tests

Oral glucose tolerance tests (OGTT) were performed on both dams and their female offspring at weaning. Mice were fasted for 6 h. Then, a glucose load (2.0 g/kg body weight) was given by gavage. Before (0 min) and at 30, 60, and 120 min after the gavage, the blood glucose (BG) concentration was measured in blood collected from a tail bleed using a Contour TS glucometer

(ACCU-CHEK Mobile, Beijing, China). The area under the curve (AUC) of the OGTT was calculated as previously described (9).

Measurement of Serum Insulin, Triacylglycerol, and Total Cholesterol Levels

The blood samples collected from female offspring at weaning were centrifuged at 3,000× g for 10 min at 4°C, and the serum was stored in aliquots at −80°C. The serum insulin concentrations were measured using an ELISA kit (80-INSMSU-E01, Salem, NH, USA). Insulin sensitivity was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR). The HOMA-IR was calculated as previously described (9). Serum total cholesterol (TC) (K603-100, kits were from BioVision, Inc., Mountain View, CA, USA) and triacylglycerol (TG) (K622-100, kits were from BioVision Inc., Mountain View, CA, USA) were measured by colorimetric methods.

Gut Microbiota Analysis

The gut microbiota was analyzed according to the methods described in our previous publication (24). Microbial DNA was extracted from the cecal content using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). The V3-V4 regions of the 16S rRNA gene were amplified using the primers 341F 5′-CCTAYGGGRBGCASCAG-3′ and 806R, 5′-GGACTACNNGG GTATCTAAT-3′. Amplicons were purified using a quick PCR purification kit (Qiagen, Hilden, Germany). Microbial 16S rDNA was sequenced on the Illumina HiSeq 2500 platform (Norcross, GA, USA).

After merging paired-end reads, reads were performed by quality filtering. High quality reads were assigned to operational taxonomic units (OTUs) at the 97% similarity level using UPARSE software (version 7.0.1001) (25), and representative sequences for each OTU were screened using QIIME software (version 1.7.0, Quantitative Insights into Microbial Ecology) (26). Then, the GreenGene Database (27) was used to annotate taxonomic information based on the RDP classifier version 2.2 algorithm (28). The relative abundance of each OTU was analyzed at the phylum, class, order, family, genus and species levels. Alpha and beta diversity were examined using QIIME software (Version 1.7.0) and calculated with R software (Version 2.15.3). For alpha diversity, Chao1, Simpson and the Shannon index were analyzed. For beta diversity, principal component analysis (PCA) plots were performed using both weighted and unweighted UniFrac. In addition, linear discriminant analysis (LDA) of the effect size (LEfSe) and MetaStat were used to determine differences among the groups.

Statistical Analysis

The results are expressed as the mean ± standard error of the mean (S.E.M). The statistics were analyzed by one-way ANOVA and two-way ANOVA, with Tukey and Bonferroni *post-hoc* analyses. Correlations between the relative abundance of bacterial taxa at different taxonomic levels and metabolic parameters were performed by Spearman correlation coefficient test. Correction for correlation analysis by false discovery rate (FDR) with the Benjamini-Hochberg procedure were displayed by R software (Version 2.15.3) with values of <0.05 considered statistical significance. Analysis of similarities (ANOSIM) was used to test the statistical significance for β diversity. A $p < 0.05$ was considered statistically significant. For the MetaStat analysis, a $q < 0.05$ was considered statistical significance. Prism version 7.0 (GraphPad Software Inc., San Diego, CA, USA) was used for statistical analysis.

RESULTS

Characterization of Dams

During the 9-week of dietary intervention, the energy intake of dams among the four groups was not significantly different. At weaning, the body weights of high-fat fed dams (HF) were higher than that of dams in the control group ($p < 0.0001$; **Table 1**). The AUC of the OGTT was significantly larger in dams fed a high-fat diet compared to that of dams in the control group ($p < 0.05$). However, in contrast to the HF group, the body weights and glucose tolerance were not significantly different in dams fed either a high-fat diet with low-dose genistein (HF.LG) or high-fat diet with high-dose genistein (HF.HG). There was no difference among the four groups in regard to litter size, uterus index (weight ratio of uterus to body weight) or ovary index (weight ratio of ovaries to body weight) of dams.

Birth Weight and Body Weight of Offspring

The birth weights of offspring of high-fat fed dams (HF) were lower than those of offspring of control group dams ($p < 0.05$, **Figure 1A**). In offspring of high-fat diet with low-dose genistein dams, the birth weights improved and were higher than those of offspring of HF group dams ($p < 0.05$, **Figure 1A**). However, there was no difference among the four groups in regard to the body weight of female offspring at weaning (**Figure 1B**).

TABLE 1 | Dam and liter characteristics.

Parameters	HF	HF.LG	HF.HG	Control
	(*n* = 6)	(*n* = 6)	(*n* = 8)	(*n* = 8)
Body weight(g)	31.5 ± 0.9	30.7 ± 0.6	30.2 ± 0.6	24.1 ± 0.6*
AUC of OGTT(mmol/l•h)	21.8 ± 1.2	19.7 ± 0.9	21.4 ± 1.2	17.5 ± 0.8*
Pups/litter	8.0 ± 0.6	6.8 ± 1.0	7.2 ± 0.5	7.0 ± 0.6
Uterus index (%) to body weight(%)	0.33 ± 0.1	0.42 ± 0.0	0.29 ± 0.1	0.39 ± 0.0
Ovary index (%) to body weight(%)	0.11 ± 0.0	0.13 ± 0.0	0.10 ± 0.0	0.12 ± 0.0

Data are expressed as means ± S.E.M (n = 6–8/group).
**p < 0.05 vs. HF group. HF, high-fat diet without genistein; HF. LG, high-fat diet with low-dose genistein; HF. HG, high-fat diet with high-dose genistein; Control, normal control diet. Uterus index: weight ratio of uterus to body weight; Ovary index: weight ratio of ovaries to body weight.*

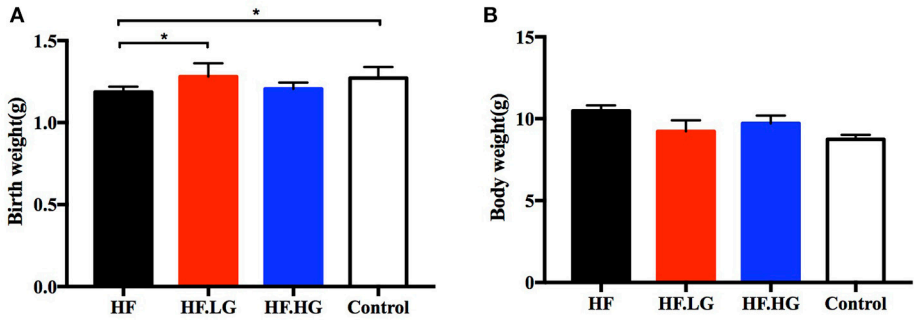

FIGURE 1 | Birth weight and body weight at weaning in offspring. **(A)** Birth weight, and **(B)** Body weight of female offspring at weaning. HF, high-fat diet without genistein; HF. LG, high-fat diet with low-dose genistein; HF. HG, high-fat diet with high-dose genistein; Control, normal control diet. Data are expressed as means ± S.E.M. (n = 6–8/group). Mean values were significantly different between other group and the HF group: *$p < 0.05$.

Dietary Low-Dose Genistein Prevents the Deleterious Effects of a Maternal High-Fat Diet on Glucose Metabolism and Insulin Sensitivity of the Offspring

At weaning, offspring from high-fat diet fed dams (HF) had impaired glucose tolerance as measured by OGTT compared to offspring from normal control diet fed dams (Control). The blood glucose levels were higher at 30 min ($p < 0.0001$) and the AUC was significantly larger ($p < 0.0001$) for offspring of HF group (**Figures 2A,B**). To determine whether maternal dietary genistein affected the glucose metabolism of the female offspring, we compared offspring from dams that were fed a high-fat diet with or without genistein. As shown in **Figures 2A,B**, female offspring of low-dose genistein fed dams (HF.LG) demonstrated a marked improvement in glucose tolerance. The blood glucose levels at 30 min ($p < 0.0001$) and AUC of these female offspring were significantly lower ($p < 0.0001$). Furthermore, to determine whether insulin sensitivity was influenced in female offspring, the level of fasting serum insulin was detected. High-fat fed dams resulted in a significantly higher insulin concentration ($p < 0.001$) and HOMA-IR index ($p < 0.0001$) in offspring at weaning. Low-dose genistein fed dams (HF.LG) led to improved insulin sensitivity in their offspring (**Figures 2C,D**). However, no significant difference was detected in regard to glucose tolerance and insulin sensitivity between female offspring of HF.HG group and HF group (**Figures 2A–D**). In addition, no significant differences were identified between offspring of HF.HG and HF.LG group dams in regard to blood glucose levels at different times during the glucose tolerance test, serum insulin levels and HOMA-IR, other than the significantly lower AUC in offspring of HF.LG group ($p < 0.001$).

Maternal Dietary High-Dose Genistein Improves Lipid Metabolism in the Early Life of Offspring

In addition to glucose metabolism, we detected the levels of serum lipids to evaluate the differences between groups regarding lipid metabolism of female offspring at weaning. The levels of serum TG ($p < 0.05$, **Figure 3A**) and TC ($p < 0.01$, **Figure 3B**) in the offspring of high-fat fed dams (HF) were higher than those in the offspring of Control group dams. High-dose genistein feeding of dams (HF.HG) resulted in a significant improvement in the serum TG ($p < 0.01$, **Figure 3A**) and TC levels ($p < 0.01$, **Figure 3B**) in female offspring. In contrast to the changes in glucose tolerance and insulin sensitivity, there was no significant difference in the serum lipid levels between the offspring of HF.LG group and HF group.

Effects of Maternal Dietary Genistein on Gut Microbiota in Offspring

To explore the mechanisms of maternal dietary genistein improvement of glucose and lipid metabolism in offspring, we analyzed the gut microbiota changes in offspring using 16s rDNA gene sequences. The sequence data in this study has been submitted to the Sequence Read Archive (SRA) database (accession number SRP156380). A total of 1836395 high quality reads were obtained from 28 samples, with an average of 65586 sequences per sample. After clustering at the 97% similarity level, 653 operational taxonomic units (OTU) were identified among the four groups. Alpha diversity analysis showed that there was a parallel community richness (Chao 1) and diversity (Simpson and Shannon index) among groups (**Table S2**, **Figure S1**). To compare the overall structure of the gut microbial community, principal component analysis (PCA) was performed to determine differences among groups. As shown in **Figure 4**, the gut microbial communities were well separated in the HF group compared with both the Control group ($p < 0.01$) and HF.HG group ($p < 0.05$), with 55.76 and 22.51% variations explained by principal component (PC) 1 and PC2, respectively. The results were supported by ANOSIM and showed that there significant differences in the microbial structure were caused by the maternal diets during pre-pregnancy, pregnancy and lactation. This result was also verified by a heatmap according to the bacterial genus level among the four groups (**Figure 5**).

There were significant alterations in the composition of the intestinal microbial community among the four groups. MetaStat analysis showed that the phylum Proteobacteria,

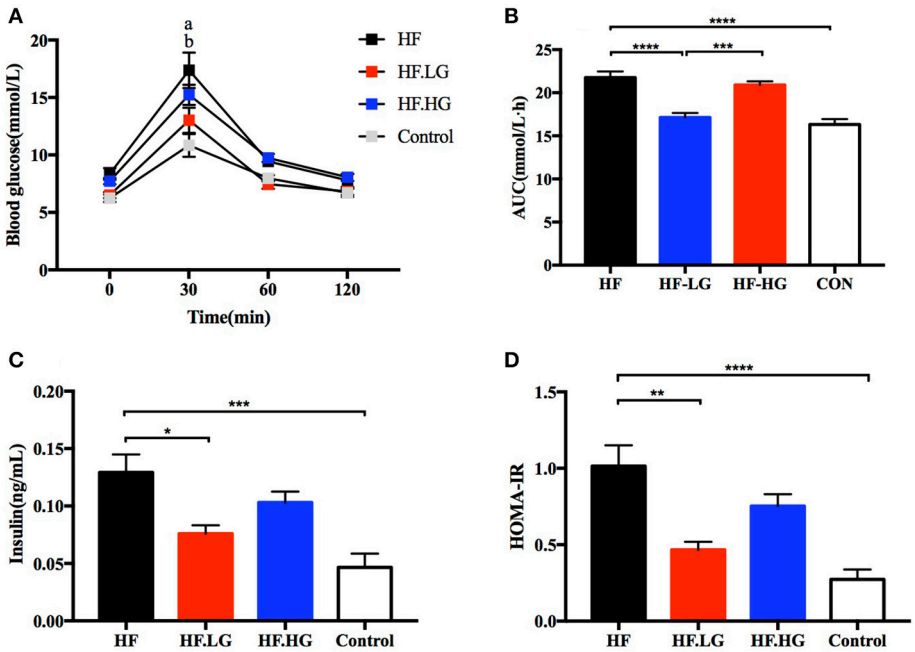

FIGURE 2 | Glucose metabolism of the female offspring at weaning. **(A)** OGTT; **(B)** AUC; **(C)** Serum insulin levels; **(D)** HOMA-IR. HF, high-fat diet without genistein; HF. LG, high-fat diet with low-dose genistein; HF. HG, high-fat diet with high-dose genistein; Control, normal control diet; OGTT, oral glucose tolerance test; AUC, area under the curve; HOMA-IR, the homeostasis model assessment of insulin resistance. Data are expressed as means ± S.E.M. (n = 6-8/group). Mean values were significantly different between other group and the HF group: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; Mean values were significantly different between Control group and the HF group: 'a' $p<0.0001$; Mean values were significantly different between HF.LG group and the HF group: 'b' $p < 0.001$.

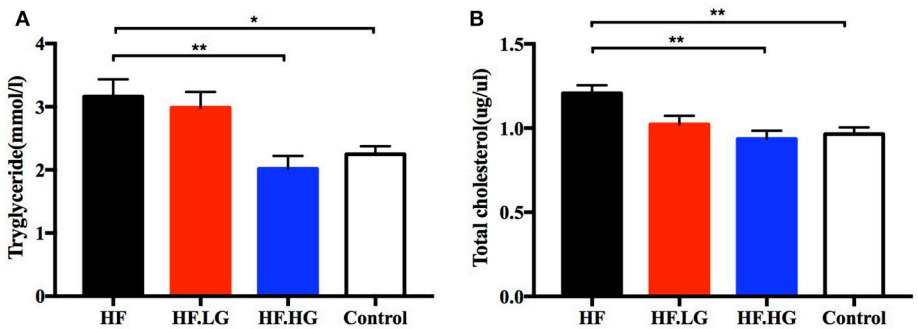

FIGURE 3 | Lipid metabolism of the female offspring at weaning. **(A)** Serum triacyglycerol; and **(B)** serum total cholesterol. HF, high-fat diet without genistein; HF. LG, high-fat diet with low-dose genistein; HF. HG, high-fat diet with high-dose genistein; Control, normal control diet. Data are expressed as means ± S.E.M (n = 6-8/group). Mean values were significantly different between other group and the HF group: *$p < 0.05$, **$p < 0.01$.

class Deltaproteobacteria, family *Desulfovibrionaceae* and genus *Desulfovibrio* were all significantly increased in the HF group compared with the Control group ($q < 0.05$) and were also relatively lower in the HF.LG group and HF.HG group compared to the HF group. However, there was a lower relative abundance of the family *Porphyromonadaceae*, genus *Ruminoccaceae_UCG-004*, genus *[Eubacterium]_brachy_group*, genus *Rikenella* and genus *Rikenellaceae_RC9_gut_group* in the HF group compared to the Control group ($q < 0.05$), all of which also tended to increase in female offspring at weaning after a maternal dietary genistein treatment in comparison with offspring from dams fed a high-fat diet without genistein. The genera *Rikenella* and *Rikenellaceae_RC9_gut_group*, both from the family *Rikenellaceae*, were significantly increased in the HF.HG group compared to the HF group and may play important roles in lipid metabolism (**Figures 6A–I**). **Figure 7** lists significant microbiota changes from the phylum level to the species level, as performed by LEfSe. The phylum Firmucutes, class Clostridia, family *Rikenellaceae*, family *Porphyromonadaceae*, genus *Alistipes* and genus *Anaerotruncus* were significantly enriched in the control group. The phylum Proteobacteria, class Deltaproteobacteria, order Desulfovibrionales, family

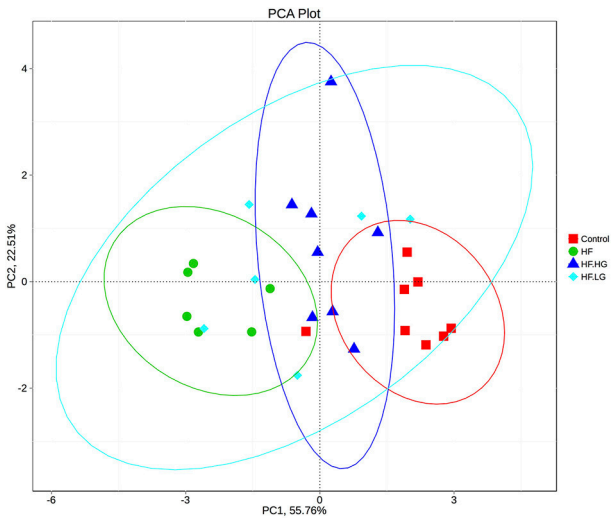

FIGURE 4 | PCA plots of gut communities in the female offspring at weaning (n = 6–8/group). HF, high-fat diet without genistein; HF. LG, high-fat diet with low-dose genistein; HF. HG, high-fat diet with high-dose genistein; Control, normal control diet.

Desulfovibrionaceae and genus *Desulfovibrio* exhibited higher abundances in the HF group. Low-dose and high-dose genistein treatment both increased *Bacteroides* and the *Akkermansia* at the genus level (**Figures 7A,B**). By contrast, the species Bacteroides_acidifaciens was uniquely enriched in the HF.HG group (**Figure 7B**).

Correlation Analyses of the Gut Microbiota and Glucose and Lipid Metabolic Parameters

To assess associations between glucose and lipid metabolism and the gut microbiota in offspring, the AUC of the OGTT, insulin, HOMA-IR, TC, and TG levels were correlated with the intestinal bacterial relative abundance (**Table 2**). The AUC of the OGTT, fasting insulin levels, HOMA-IR and serum TC levels were positively correlated with the relative abundance of Proteobacteria at the phylum level and the class Deltaproteobacteria from the phylum Proteobacteria. However, the fasting serum insulin concentration and HOMA-IR were negatively correlated with the class Clostridia (phylum Firmicutes). At the family level, the AUC of the OGTT, insulin levels, HOMA-IR and serum TC levels were positively correlated with the abundance of *Desulfovibrionaceae* from the class Deltaproteobacteria. By contrast, the AUC of the OGTT, insulin levels, and HOMA-IR were all negatively correlated with the families *Porphyromonadaceae* and insulin levels, and HOMA-IR were both negatively correlated with *Rikenellaceae,* both of which belong to the phylum Bacteroidetes. At the genus level, the AUC of the OGTT, insulin and HOMA-IR were positively correlated with the genus *Desulfovibrio* from the phylum Firmicutes, but insulin and HOMA-IR were negatively correlated with the *Alistipes* and *Rikenella* from the phylum Bacteroidetes. In addition, HOMA-IR were also positively correlated with the species *Bacteroides_acidifaciens.* In regard to lipid metabolism, TC was positively correlated with the genus *Desulfovibrio.*

DISCUSSION

It is well established that a poor maternal diet is an important factor in the development of metabolic disorders in offspring (29–32), which may have contributed to the current rapid increase in the prevalence of obesity and diabetes. Similarly, the present study also demonstrated that a maternal high-fat diet before pregnancy and during pregnancy and lactation could result in glucose intolerance, insulin resistance and higher serum levels of TC and TG in the early life of female offspring. It has been reported that genistein has anti-diabetic (13) and lipid metabolism improvement (33) functions, but the effects of genistein intake during pregnancy and lactation on glucose and lipid metabolism in offspring are poorly understood. In the present study, we explored the effects of maternal dietary genistein on glucose and lipid metabolism in female offspring at weaning. We found that maternal dietary low-dose genistein (0.25 g/kg diet) fully counteracted the detrimental effects of the maternal high-fat diet on glucose tolerance, circulating insulin, HOMA-IR and birth weight in female offspring. Moreover, disorders of the serum lipid profiles in offspring due to a maternal high-fat diet were prevented if dams were fed high-dose genistein (0.6 g/kg diet). The uterus and ovary index showed that the genistein intervention had no adverse effects on dams. These data indicate that maternal dietary genistein is pivotal for improving metabolic health in the early life of female offspring in a dose-dependent manner.

There might be changes in many organizations of the offspring that play important roles in the beneficial effects of maternal dietary genistein and the deleterious effects of a maternal high-fat diet on metabolic disorders in offspring. Given the vital role that the intestinal microbial community play in metabolic health, we hypothesized that the intestinal microbiota of the offspring changed. Indeed, our results showed that maternal high-fat feeding leaded to significant alterations in the overall structure and composition of the intestinal microbial community and that maternal genistein intake reversed these detrimental effects. The present study showed that Proteobacteria, Deltaproteobacteria, Desulfovibrionales, *Desulfovibrionaceae* and *Desulfovibrio* significantly increased and were positively correlated with glucose and lipid metabolic parameters, whereas *Porphyromonadaceae*, *Ruminoccaceae_UCG-004*, *[Eubacterium]_brachy_group*, *Rikenella* and *Rikenellaceae_RC9_gut_group* significantly decreased and were negatively correlated with glucose and lipid metabolic parameters in female offspring from high-fat fed dams compared to those in female offspring of Control group dams. As previously shown, high-fat feeding resulted in significantly increased abundance of Proteobacteria and *Desulfovibrionaceae.* Tomas et al. (34)

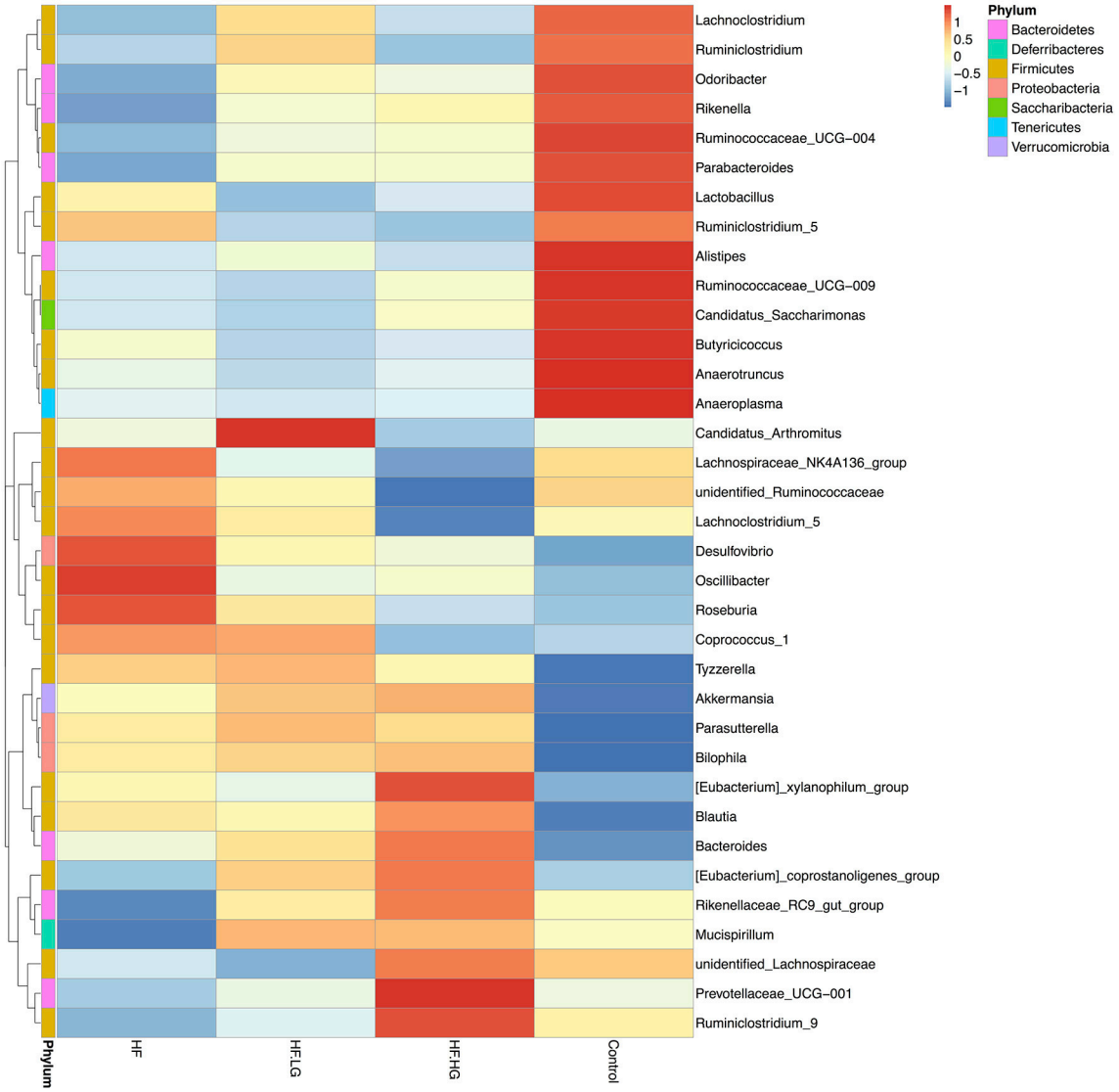

FIGURE 5 | Heat map analyses of abundant genera in each group (n = 6–8/group). HF, high-fat diet without genistein; HF. LG, high-fat diet with low-dose genistein; HF. HG, high-fat diet with high-dose genistein; Control, normal control diet.

found that a high-fat diet altered the composition of the fecal and cecal microbial community even after 30 d of consumption and that the relative abundance of Proteobacteria significantly increased. In Proteobacteria, the main increase was in class Deltaproteobacteria (Desulfovibrionales order, *Desulfovibrionaceae* family). Meanwhile, the physical integrity of the epithelial barrier was disrupted and intestinal permeability was increased. Similarly, another previous study showed that the intestinal microbiota of wild-type mice switched to high-fat diets has changed a lot, including an increase in Proteobacteria. The main group of Proteobacteria increased in relative abundance, as did the class DeltaProteobacteria and genus *Desulfovibrio* (35). Several genera belonging to the *Desulfovibrionaceae* family are considered to be opportunistic pathogens and have been linked to some inflammatory diseases (36, 37). These genera produce endotoxins and have the capacity to reduce sulfate to H_2S (38), thereby damaging the intestinal barrier (39). Maternal dietary genistein decreases the abundance of these bacteria and reverses their detrimental effects on metabolism. In addition, Li et al. (40) analyzed the effect of the antibiotic azithromycin on the gut microbiota and adipogenesis in mice and found that the abundance of *Rikenella* was significantly lower in the azithromycin group and was associated with a higher body weight and larger percentage of body fat. Another human study also indicated that the abundance of *Rikenellaceae*, along with other bacterial components, contributed to a lean body type (41). In the present study, maternal dietary high-dose genistein significantly increased the abundance of *Rikenella*

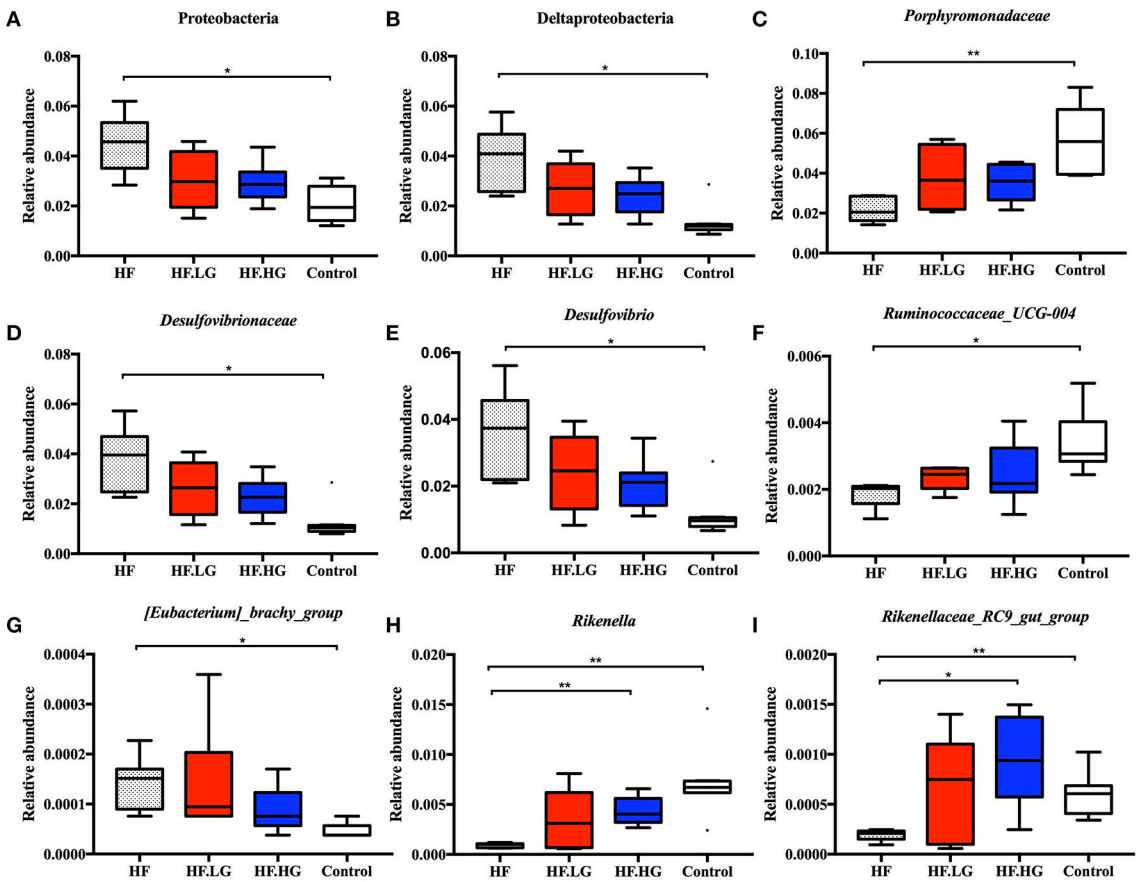

FIGURE 6 | Relative abundance of bacterial taxa at different taxonomic levels in each group. (n = 6–8/group). **(A)** Proteobacteria; **(B)** Deltaproteobacteria; **(C)** *Porphyromonadaceae*; **(D)** *Desulfovibrionaceae*; **(E)** *Desulfovibrio*; **(F)** *Ruminococcaceae_UCG-004*; **(G)** *[Eubacterium]_brachy_group*; **(H)** *Rikenella*; and **(I)** *Rikenellaceae_RC9_gut_group*. HF, high-fat diet without genistein; HF. LG, high-fat diet with low-dose genistein; HF. HG, high-fat diet with high-dose genistein; Control, normal control diet. Data was analyzed by MetaStat. Mean values were significantly different between other group and the HF group: $^{*}q < 0.05$, $^{**}q < 0.01$.

and *Rikenellaceae_ RC9_ gut_group* and improved the levels of TG and TC in female offspring at weaning. Thus, the genus of *Rikenella* and *Rikenellaceae_ RC9_ gut_group* might play crucial roles in the improved lipid metabolism by high-dose genistien.

In the current study, at the genus level, maternal dietary genistein (including HF.LG and HF.HG) significantly enriched *Bacteroides* and *Akkermansia*. Several human studies have shown that the relative abundance of *Bacteroides* was decreased in type 2 diabetes patients in comparison to normal control subjects (42, 43). A fiber-rich macrobiotic Ma-Pi 2 diet increased the abundance of propionate producers (*Bacteroides*) (42). In addition, another obese mice study showed that resveratrol improved glucose tolerance while increasing the relative abundance of *Bacteroides* (44). Our study found that *Bacteroides* was enriched in female offspring from genistein fed dams and might play crucial roles in negating the deleterious effects of a poor maternal diet on the metabolism of offspring. The beneficial effects of maternal genistein intake on the metabolism of offspring were also associated with a significant enrichment in the relative abundance of *Akkermansia*, which could maintain the mucus layer thickness and reduce leakage of LPS and intestinal permeability (45).

To our knowledge, this is the first study that showed that the phytoestrogen genistein exerts a significant effect on the abundance of *Akkermansia* in the gut microbial commuity of an animal model of maternal high-fat diet-induced offspring metabolic disorders. Recently, it has been reported that administration of polyphenols was also associated with an increased abundance of *Akkermansia* in both human and animal studies (46, 47). Moreover, an increase in the gut proportion of this bacterium has also been associated with the beneficial effects of the anti-diabetic drug metformin and gastric bypass surgery on metabolism (48, 49). Although we have not directly determined the causality between the increased proportion of *Akkermansia* and the improvement of glucose and lipid metabolism in offspring from genistein intake high-fat fed dams, it has been reported that oral administration of *Akkermansia* reverses the metabolic abnormalities induced by a high-fat diet (45) and also mimics the antidiabetic effects of metformin in diabetic mice (48). More importantly, our results indicated that the increase

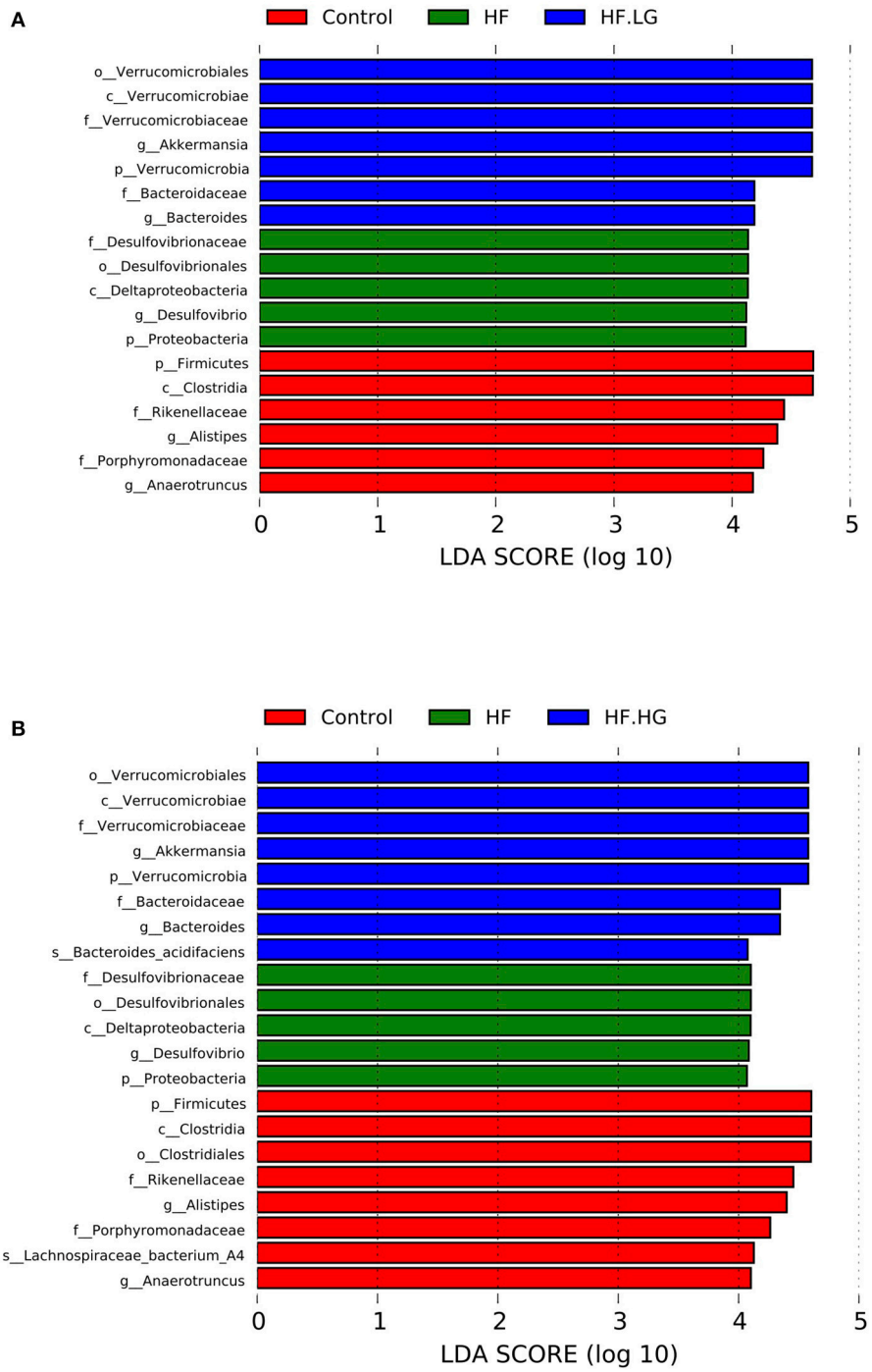

FIGURE 7 | The LEfSe analysis of the different gut microbiota from the phylum level down to the species level (n = 6–8/group). **(A)** Differently enriched bacteria among the HF-HF.LG-Control group; and **(B)** Differently enriched bacteria among the HF-HF.HG-Control group. HF, high-fat diet without genistein; HF. LG, high-fat diet with low-dose genistein; HF. HG, high-fat diet with high-dose genistein; Control, normal control diet.

in *Akkermansia* associated with genistein might be sufficient to improve metabolic disorders in offspring induced by a maternal high-fat diet without significant alterations in the proportions of Firmicutes and Bacteroidetes.

In addition, LEfSe analysis showed that the species *Bacteroides_acidifaciens* was uniquely enriched in the HF.HG group. Renouf et al. (50) first identified fecal microbes that were responsible for the degradation of isoflavone

TABLE 2 | Correlation analyses between relative abundance of bacterial taxa at different taxonomic levels and glucose and lipid metabolism parameters (n = 6–8/group).

Metabolic index	Taxonomic level	Specific Taxon	*r*	*p*-value	FDR
AUC	phylum	Proteobacteria	0.59	0.0009	0.0033
	class	Deltaproteobacteria	0.59	0.0010	0.0028
	family	*Desulfovibrionaceae*	0.60	0.0008	0.0044
	family	*Porphyromonadaceae*	−0.52	0.0043	0.0095
	genus	*Desulfovibrio*	0.61	0.0005	0.0055
Insulin	phylum	Proteobacteria	0.50	0.0067	0.0082
	class	Clostridia	−0.52	0.0044	0.0081
	class	Deltaproteobacteria	0.51	0.0057	0.0090
	family	*Desulfovibrionaceae*	0.53	0.0041	0.0090
	family	*Porphyromonadaceae*	−0.58	0.0013	0.0143
	family	*Rikenellaceae*	−0.53	0.0036	0.0099
	genus	*Alistipes*	−0.53	0.0034	0.0125
	genus	*Rikenella*	−0.54	0.0029	0.0160
	genus	*Desulfovibrio*	0.51	0.0061	0.0084
	species	*Bacteroides_acidifaciens*	0.38	0.0460	0.0506
HOMA-IR	phylum	Proteobacteria	0.58	0.0013	0.0024
	class	Clostridia	−0.47	0.0108	0.0132
	class	Deltaproteobacteria	0.60	0.0007	0.0026
	family	*Desulfovibrionaceae*	0.62	0.0005	0.0055
	family	*Porphyromonadaceae*	−0.62	0.0005	0.0028
	family	*Rikenellaceae*	−0.50	0.0071	0.0111
	genus	*Alistipes*	−0.49	0.0083	0.0114
	genus	*Rikenella*	−0.59	0.0010	0.0022
	genus	*Desulfovibrio*	0.59	0.0009	0.0025
	species	*Bacteroides_acidifaciens*	0.46	0.0130	0.0143
TC	phylum	Proteobacteria	0.45	0.0161	0.0443
	class	Deltaproteobacteria	0.53	0.0037	0.0407
	family	*Desulfovibrionaceae*	0.51	0.0059	0.0325
	genus	*Rikenellaceae_RC9_gut_group*	−0.39	0.0382	0.0840
	genus	*Desulfovibrio*	0.46	0.0136	0.0499

Spearman's correlations coefficients are listed for each taxonomy level. AUC, area under the curve; HOMA-IR, the homeostasis model assessment of insulin resistance; TC, total cholesterol; TG, triacylglycerol.

using molecular genetic techniques and demonstrated that *Bacteroides_acidifaciens* increased the disappearance of isoflavone genistein in human fecal incubating under anaerobic and nutrient-rich conditions, which indicated that *Bacteroides_acidifaciens* played a role in the metabolism of genistein in the intestine. Thus, the enrichment of *Bacteroides_acidifaciens* in the HF.HG group increased the degradation of isoflavones genistein. Correlation analysis showed that the relative abundance of *Bacteroides_acidifaciens* was positively related with HOMA-IR, which clarified the differences of the effects of maternal low-dose genistein and high-dose genistein on the glucose metabolism and insulin sensitivity in the offspring.

In summary, maternal dietary genistein provided before and during pregnancy and lactation significantly improved the metabolism of female offspring in early life and compensated for the detrimental effects of a maternal high-fat diet. The improvement in glucose and lipid metabolism is associated with the alterations in the gut microbiota of offspring. This is the first study to report the role that the gut microbiota plays in the effects of maternal dietary genistein on glucose and lipid metabolism in female offspring. However, our study only analyzed the relationship between gut microbiota and metabolism in offspring and the causality is still needed to be further explored. Furthermore, only female offspring was studied in this study. The effects of maternal dietary genistein on metabolic health of male offspring is worth to be studied in the future. Our results suggest that the provision of maternal dietary genistein before pregnancy and during pregnancy and lactation may be an important tool for combating obesity and diabetes in offspring.

DATA AVAILABILITY

The datasets supporting the conclusions of this manuscript are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

XX and JZ designed the experiments. LZ, XW, MD, RL, and XZ performed the experiments. LZ analyzed the data and wrote the original draft. XX, JZ, QZ, ML, and MY reviewed the manuscript. All of the authors had final approval of the submitted version.

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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.2018.00516/full#supplementary-material

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Excessive Neutrophil Activity in Gestational Diabetes Mellitus: Could It Contribute to the Development of Preeclampsia?

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Gestational diabetes mellitus is a transient form of glucose intolerance occurring during pregnancy. Pregnancies affected by gestational diabetes mellitus are at risk for the development of preeclampsia, a severe life threatening condition, associated with significant feto-maternal morbidity and mortality. It is a risk factor for long-term health in women and their offspring. Pregnancy has been shown to be associated with a subliminal degree of neutrophil activation and tightly regulated generation of neutrophil extracellular traps (NETs). This response is excessive in cases with preeclampsia, leading to the presence of large numbers of NETs in affected placentae. We have recently observed that circulatory neutrophils in cases with gestational diabetes mellitus similarly exhibit an excessive pro-NETotic phenotype, and pronounced placental presence, as detected by expression of neutrophil elastase. Furthermore, exogenous neutrophil elastase liberated by degranulating neutrophils was demonstrated to alter trophoblast physiology and glucose metabolism by interfering with key signal transduction components. In this review we examine whether additional evidence exists suggesting that altered neutrophil activity in gestational diabetes mellitus may contribute to the development of preeclampsia.

Keywords: pregnancy, neutrophils extracellular traps, gestational diabetes, preeclampsia, TNFalpha, leptin, A1AT, elastase

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as glucose intolerance manifesting during pregnancy in women with no prior history of diabetes (1–4). It is, thus, by definition unlike either Type 1 or Type 2 diabetes mellitus (T1DM and T2DM, respectively), in that it is of a transient nature and gender specific. Furthermore, it only occurs during a unique physiological condition, namely pregnancy (1–4).

GDM does, however, share a number of traits with T2DM. These include insulin resistance or aspects of the metabolic syndrome (1–4). Due to its transient appearance in pregnancy, GDM has been suggested to be a pre-diabetic state or a momentary unmasking of a T2DM-like condition triggered by gestation (1–4). Unlike T1DM, there is no auto-immune component in GDM.

Although a number of strategies exist to manage pregnancies affected by GDM, these are nevertheless associated with several fetal and maternal complications (2, 5). These comprise fetal macrosomia, necessitating cesarean delivery, as well as an increased risk for severe complications such as preeclampsia (PE) (6). Post-partum complications can include neonatal hypoglycaemia, jaundice or respiratory distress syndrome. The condition triggers as to yet to be defined epigenetic alterations in mother and child, resulting in an increased risk for development of T2DM in both in the post-partum period (7, 8).

Current screening protocols rely on a glucose challenge test (GCT), also termed oral glucose tolerance test (OGTT), generally performed in the 2nd trimester of pregnancy (9). This test can also be used to stratify the degree of severity of GDM, and thereby provide a guide for the route of therapy to be taken. In severe cases this may include treatment with insulin or metformin, whilst less severe forms are usually managed by a regimen of diet and exercise (2, 3, 10, 11).

The underlying etiology of GDM has yet to be determined, but previous studies suggest that it may involve a contra insulin effect mediated by placentally produced hormones and cytokines (12, 13). It is further proposed that this condition may be exacerbated by high calorie diet, obesity, or genetic predisposition prevalent in certain ethnicities (3).

Since obesity and the associated metabolic syndrome have a dramatic effect on pregnancy (14), the significant rise in the prevalence of this condition can be expected to lead to an increase in the number of pregnancies affected by GDM. Consequently this will lead to an increase in associated complications, such as PE (15, 16). This demographic change could have a seismic shift-like impact on health care systems, both in terms of a cost explosion and additionally straining already short stretched staff resources (16).

PE AND DIABETES—A COMPLEX RELATIONSHIP

PE is a severe disorder of pregnancy, characterized by hypertension, proteinuria, oedema and multiple organ distress, in previously normotensive women (17–19). PE typically affects between 3 and 8% of all pregnancies, having a higher prevalence in certain ethnicities (6).

Despite a relatively high prevalence in pregnancy, PE has recently been classified as an "orphan disease" on account of its low incidence in the entire population; a feature which will hopefully enable the accruement of additional research funding (20).

PE typically arises in the 2nd or 3rd trimester of pregnancy, but can also occur post-partum (21). If left untreated, PE advances to eclampsia, a condition characterized by epilepsy-like seizures and associated with high rates of maternal mortality (22). PE is also a significant risk factor for subsequent pregnancies, as well as the long-term health in women and their offspring.

The underlying etiology of PE is complex and multifactorial, more akin to a syndrome than a singular disease (17–19, 23). Evidence suggests that the lesion initiating the cascade of events leading to the manifestation of clinical symptoms occurs early in pregnancy (17–19, 24). PE therefore seems to involve a long asymptomatic phase, the appearance of symptoms depending on the accrual of secondary or tertiary hits (17–19).

On account of its complex underlying etiology, it has been suggested that PE can be stratified into an early onset (eoPE; <34 weeks) and late onset form (loPE; ≥ 34 weeks), according to the time of onset during gestation, and that these two forms can be viewed as disparate entities, akin to the segregation of diabetes mellitus into Type 1 and T2 forms (25).

EoPE is associated with more severe symptoms and poorer feto-maternal outcome, particularly due to premature delivery of fetuses, many of which are growth restricted (25). In general, the early form of PE is less prevalent than the late form (loPE), by a proportion of approximately 1:10 (6, 22). Both forms of PE have made the quest to develop suitable biomarkers to detect at risk pregnancies a challenging task (26).

Key features of PE include abnormal placentation, particularly aberrant modification of the maternal spiral arteries, altered expression of key angiogenic and anti-angiogenic factors, elevated feto-maternal cell trafficking, release of placentally-derived cell-free DNA and occurrence of neutrophil extracellular traps (NETS) in the intervillous space of affected placentae (18, 26–29). These features vary between early and late onset forms, with placental deficiencies, such an inadequate modification of the spiral arteries and infarction, being more pronounced in cases with eoPE than in the late onset form (18, 26). On the other hand, maternal inflammation appears to be a key contributor to the development of loPE, because it can be triggered by extraneous influences such as air pollution or obesity (15, 26, 30).

Although diabetes in pregnancy is frequently associated with poor outcome, it poses a significant increased risk for the development of PE (6, 31, 32). This is particularly high in instances with pre-existing T1DM, where 20% or more of such pregnancies can develop PE (31). The presence of T2DM prior to conception is also associated with a significant increase (2–4 fold) in the development of PE (31).

Although less dramatically than in cases with T1DM, pregnancies affected by GDM incur a higher risk of developing PE. The aetiological trigger leading to the onset of PE by either pre-existing diabetes or GDM is currently unclear (31).

To date few studies have addressed the association of diabetic conditions with the development of either early or late-onset forms of PE. Probably the most salient study was performed by Lisonkova and Joseph, who analyzed risk factors associated with the development of either eoPE or loPE in a very large cohort ($n = 456668$) (6).

In this study, the overall incidence of PE was 3.1%, of which 0.38% was affected by eoPE and 2.72% by loPE (6). Risk factors for eoPE were determined to include ethnicity (African-American), chronic hypertension and congenital anomalies. On the other hand, young maternal age, nulliparity and diabetes prior to conception were determined to pose a significant risk for the manifestation of loPE. In this study the issue of GDM and the incidence of PE was unfortunately not addressed (6).

The role of GDM in the development of PE was recently examined in 120 pregnant women, of whom 60 each had

early-onset and late-onset GDM (33). The results indicated that early onset GDM was associated with a significantly higher incidence of PE (23.3%) compared with 10% in the late-onset group. Early onset GDM was also determined to be more severe, requiring a greater amount of therapeutic intervention with agents such as insulin. Unfortunately, no clear indication was given regarding the stratification of eoPE and loPE (33).

The interplay between PE, diabetes, or GDM is however not restricted to the current pregnancy, but may influence the course of subsequent pregnancies. In this manner, recent data indicated that PE in a previous pregnancy increases the risk of GDM in a subsequent pregnancy (34). This was even more pronounced in cases when the previous pregnancy was affected by both GDM and PE (34).

PE and GDM may also contribute to other pathologies post-partum. It has been demonstrated that PE leads to an increased risk of developing T2DM in previously non-diabetic women; a feature also evident post-partum in women affected by GDM (35, 36). The incidence of post-partum diabetes, however, appears to be significantly higher in cases affected by GDM (19%) than those by PE (2%), as suggested by a large-scale epidemiological study (36).

In cases with a pre-existing diabetic condition, the effects of ensuing PE can be far reaching by contributing to the post-partum development of retinopathy or nephropathy (31). It is also well established that both GDM and PE are associated with an increased risk for the post-partum development of cardiovascular pathologies (22, 37, 38).

GDM AND PE—THE CONTRIBUTION OF OBESITY

A causal relationship between obesity and insulin resistance in T2DM is historically well established, and includes pioneering observations that insulin levels were greater in obese diabetic individuals than lean healthy counterparts (39, 40). A key contribution into understanding this interaction was by uncovering a low-grade chronic inflammation mediated by metabolic cells in response to nutrient overload (39, 40). Hence, the so-called metabolic syndrome provided an important link between obesity, sedentary lifestyle, stress, and onset of T2DM.

The initial observation of an inflammatory event triggered by diet was made in adipose cells, where obesity was determined to trigger TNFα production (41, 42). This observation was soon extended to show that a host of tissues were affected by nutrient overload including liver, pancreas, brain and muscle, involving the pro-inflammatory cytokines including IL-6, IL-1β, and CCL2 (39, 40). The inflammatory cascade appears to involve the infiltration of affected tissues, such as adipose tissue, by macrophages and other regulatory immune cells in obesity. The underlying molecular process is suggested to involve the activation of Toll-like receptors (TLRs) and the inflammasome by circulating free fatty acids (FFAs), resulting in the production of IL-1β or TNFα, which promote tissue infiltration and immune cell activation. Furthermore, in the context of T2DM, these highly pro-inflammatory cytokines have the ability to dampen or hinder insulin signaling, hence their original description as *insulin antagonists* (39, 40).

As mentioned above, obesity has been suggested to fuel the increase in both GDM and PE (3, 15). It is noteworthy that GDM is not restricted to women with an elevated BMI, while not all overtly obese women develop PE. This is most evident when comparing the incidence of GDM in various population groups, for instance in the Indian women from the Indian sub-continent who have an 11 fold greater risk for glucose intolerance during pregnancy than their Caucasian counterparts (43). The aspect of race or ethnicity was more extensively examined by Hedderson et al. in a cohort of 216 089 pregnant women (44). Their results indicated that the incidence of GDM was lowest in Caucasian women (4.1%) and highest in Asian Indians (11.1%) (43). Obesity was determined not to correlate significantly with the incidence of GDM in Asian Indian migrant populations (45, 46).

On the other hand, obesity has profound effects during pregnancy, and has been shown to affect fetal development, enhancing the risk for macrosomia, fetal defects and preterm labor, independent of the occurrence of either GDM or PE (3, 16).

That obesity can directly contribute to the onset of PE was strikingly shown by Mbah et al., in a study where they examined the outcome of over one million live births (15). They noted that an increase in BMI was associated with an almost exponential increase in the incidence of PE. This was most striking when examining cases with super obesity with a BMI > 50, where an almost 4 fold increase in PE was noted (15).

A key feature of this analysis was the stratification of PE into early and late onset forms, which clearly indicated that increased BMI lead to a significant increase in late-onset PE, whereas only a modest increase in eoPE was noted (15).

This would appear to support the tenet that loPE is promoted by maternal inflammation, while eoPE appears to result from underlying placental dysfunction (17, 26). It would thus seem that an underlying inflammation in loPE triggered by obesity, such as is evident in the metabolic syndrome, may be a causative feature promoting the occurrence of loPE associated with elevated BMI (26).

These datasets suggest that the overall scenario is quite complex, and that underlying genetic propensities in combination with environmental factors, interact in rendering pregnant women susceptible to the advent of GDM, PE or both, by obesity (17, 26).

GDM AND PE: UNDERLYING PLACENTAL CHANGES

Numerous reports indicate that the placenta is adversely affected by GDM (12, 47). Poorly controlled diabetes can result in gross morphological aberrancies such as an enlarged, thickened plethoric placenta, with reduced fetal-placental weight ratio. In addition, calcification and other features associated with GDM can be detected by sophisticated ultrasound examinations (12, 47).

GDM can also influence villous development, resulting in villous immaturity, aberrant villous branching and hyper-vascularization of the villous tissue. In addition, turnover of the villous tissue is altered resulting in an abnormally thin syncytiotrophoblast layer, which is nearly devoid of nuclei. This may be due to reduced apoptosis in the underlying villous trophoblast tissue (12, 47).

These alterations could have a pronounced effect of the release of placental micro-debris by the trophoblast. Commonly these syncytiotrophoblast—derived microparticles are referred to as STBM (48). As we will see below, STBM have long been implicated in the etiology of PE, and may hence provide for a link between GDM and the enhanced occurrence of PE in affected pregnancies. Additionally, increased expression of IL-1β and TNFα may occur in GDM placentae, which could profoundly influence the behavior of maternal immune effector/regulator cells in this milieu (49, 50). These cytokines could also promote insulin resistance by antagonizing insulin signaling (41).

A generally accepted canonical view is that the placenta plays a key role in the development of PE, since most cases of PE are resolved following delivery and removal of the placenta (18). Furthermore, PE can occur in hydatidiform molar pregnancies that consist exclusively of trophoblast tissue. An important placental anomaly associated with PE, especially eoPE, is the inadequate modification of maternal spiral arteries by invasive cytotrophoblast cells (18, 51).

This defect appears to be a consequence of placental development during human pregnancy (52), which is unique in having a very deep form of placentation. Additionally, the placenta is subject to significant changes in oxygen tension during the course of gestation. This is especially evident during early embryo development, where there is limited contact of the placenta with the maternal circulation, leaving most of the fetal tissues in a hypoxic state (52). The reason for limited feto-maternal contact is due to the blockage of maternal spiral arteries by fetal cytotrophoblast cells. This blockage is suggested to protect the fetus from the teratogenic action of toxic reactive oxygen species during this crucial stage of development. By the end of the first trimester these plugs are gradually removed and subsequently the spiral arteries are widened by the action of invasive cytotrophoblast cells, permitting an even high capacity flow of maternal blood to the underlying fetal tissues (52).

Failure of this modification is frequently high-lighted as being a key placental anomaly occurring in PE (18, 51). Such an anomaly is however not restricted to PE and is frequently evident in cases affected by IUGR (intra-uterine growth restriction) and to a lesser extent in cases with idiopathic preterm delivery or premature preterm rupture of membranes (51). Further, it is evident that these placental defects are less common in cases with loPE than eoPE (18).

PE is sometimes referred to as pregnancy toxemia, in the context of which pioneering studies by the Oxford group of Redmond and Sargent, identified a candidate for such a noxin in the form of placental micro-debris, specifically the STBM (syncytiotrophoblast microparticles) alluded to above (48). These STBM are released by the turn-over of the syncytiotrophoblast, the large multinucleate single cell layer covering the entire villous tree. During their studies they noted that STBM release into the maternal circulation was elevated in cases with manifest preeclampsia (48). Subsequent studies indicated that STBM were highly pro-inflammatory, capable of activating maternal immune cells or having a deleterious effect on maternal endothelium (48, 53).

GDM AND PE: THE ISSUE OF PLACENTAL MASS

It is currently not clear how pregnancy triggers the onset or development of GDM. It does, however, appear that multiple fetuses, and therefore placental mass, could be a key component. This is evident from studies on multi-fetal pregnancies, where a significant increase in glucose intolerance and GDM was noted compared to singleton pregnancies. The degree of GCT discordance was greatest in pregnancies with triplet fetuses, whilst it was still significantly altered in those with twins (54). An independent examination of pregnancies with triplet fetuses indicated that 10% were affected by GDM (55).

In a similar manner, PE is elevated in multi-fetal pregnancies and may be influenced by placental chorionicity (6). A recent report examining birth outcome in approximately 100,000 pregnancies, indicated that the incidence of PE in singleton pregnancies was 2.3%, 6% in monochorionic twin and 8.1% in dichorionic twin pregnancies (56). In triplet pregnancies the rate of PE can approach 20% (55). While not clear, there is some evidence that the incidence of PE may be higher in multi-fetal pregnancies conceived via assisted reproductive technologies (ART) than spontaneously (32). This could be related to major histocompatibility complex (MHC) differences between mother and fetus and lack of previous exposure to paternal antigens, which have been implicated in the development of PE (57, 58).

PREGNANCY IS ASSOCIATED WITH A MATERNAL INFLAMMATORY RESPONSE: NEUTROPHILS ENTER THE CENTRE COURT

Neutrophils, also termed polymorphonuclear neutrophil granulocytes, are the most prevalent leucocytes in human circulation (59). They are an essential component of the primary immune response to microbial infection largely by phagocytic activity or by the release of cytotoxic granular enzymes such as myeloperoxidase (MPO) or neutrophil elastase (NE). The action of these is frequently enhanced by the concomitant release of reactive oxygen species (ROS) (59).

During their investigations into the action of STBM in normal pregnancy and those affected by PE, the Oxford group made the notable observation that normal pregnancy was associated with a subliminal inflammatory response (60). This was especially evident when examining maternal innate immune cells such as neutrophils. A pertinent and often overlooked aspect of these

studies is that the activation status of neutrophils, as assessed by expression of CD11b and ROS production, was significantly greater in cases with PE than those with sepsis used as an inflammatory disease control (60). Therefore, PE was clearly associated with a highly inflammatory state, possibly initiated by the release of placental micro-debris.

Although there is still a paucity of data on the role of neutrophils in reproduction, they have been implicated in spiral artery remodeling via the action of Placental Protein 13 (PP13), as well as in recurrent fetal loss associated with anti-phospholipid antibodies [recently reviewed in (61)]. There is, however, no doubt that the key finding leading to a renewed interest in neutrophil biology is their ability to form neutrophil extracellular traps (NETs).

NEUTROPHILS IN PE: LEAVING THE BASELINE TO STORM THE NET

As mentioned, a noteworthy aspect of neutrophil physiology is their ability to form extracellular traps (NETs), a process whereby they extrude their nuclear chromatin into the surrounding environment, via a process termed NETosis (62, 63). This process relies on a series of discrete events that include calcium mobilization, ROS production, nuclear translocation of granular enzymes (MPO and NE), and histone citrullination by peptidylarginine deaminase 4 (PAD4) (64, 65). NETs were originally described as a novel tool to ensnare and kill invasive pathogens, a process facilitated by the adhesion of bacteriocidal granular proteins to the DNA lattice structures (63). They have, however, in the interim become implicated in the underlying etiology of a number of human pathologies, including PE (29, 66).

Our interest in NETs stems from our studies into the use of cell-free DNA in maternal blood for non-invasive prenatal diagnosis of fetal aneuploidies and Mendelian disorders (67). During the course of these analyses we had observed that the levels of cell-free DNA were significantly elevated in cases with manifest PE (68). A unique aspect of our analyses was that we examined for the quantity of both placentally-derived fetal cell-free DNA, as well as that of maternal origin (68). This examination indicated that there was a reciprocal elevation in the quantity of both cell-free DNA entities, which corresponded to severity of PE symptoms (68).

By examining samples collected early in pregnancy prior to onset of PE symptoms, we furthermore observed that in asymptomatic conditions, only the levels of placentally-derived fetal cell-free DNA were elevated in those pregnancies which subsequently developed PE (69). This provided yet further evidence of an initiating placental lesion occurring early in gestation, weeks prior to manifestation of maternal symptoms (70).

As the origin of maternal cell-free DNA was unclear, particularly the elevated quantities in PE, we were intrigued by the discovery of NETs and questioned whether these new entities could be a potential source (71). It should be noted there was no indication that NETs could be involved in human pathology at the time.

Prompted by the findings of the Oxford group, we examined the activity of STBM on isolated neutrophils, wherein we confirmed that these were activated by STBM, as assessed by increased expression of CD11b and generation of ROS (29). To our amazement, we clearly observed the generation of NETs following STBM treatment. We were furthermore able to readily detect the abundant presence of NETs directly in the intervillous space of affected placentae (29); thereby underscoring the possible involvement of such a process in the underlying etiology of PE. Based on the overt presence of these lattice structures in the intervillous space we hypothesized that they could facilitate placental hypoxia or ischemia, features associated with PE (52, 72). This presented the first report indicating that NETs could contribute to a human pathology, a feature that has since been reported in a wide host of disorders including rheumatoid arthritis, systemic lupus erythematosus, small vessel vasculitis, coagulopathies, diabetes, and possibly tumor growth (66).

We were subsequently able to show that NETs could induce apoptosis of adjacent cells, such as endothelial cells, indicating that they lead to considerable damage of surrounding placenta tissue (73). Using specific immuno-assays for NETs-derived products, we subsequently demonstrated that elevated maternal cell-free DNA molecules in the blood of women affected by PE were indeed of NETotic origin, thereby providing the necessary proof for our original hypothesis (71, 74).

Furthermore, in a translational study conducted together with the groups of Wagner and Karumanchi (Harvard, Boston, USA) it was determined that overt NETs formation could indeed contribute to PE-like symptoms or fetal loss in a murine model system (75). NETS clearly contributed to the development of these conditions, as they were absent in genetically modified mice incapable of undergoing NETosis. These data provide crucial additional credence to the hypothesis that overt neutrophil activity, particularly in the form of NETosis, can contribute to the development of severe complications of pregnancy, or even result in fetal loss (76).

COMPLEX MULTI-MODAL REGULATION OF NETOSIS DURING THE COURSE OF NORMAL PREGNANCY

Since a mild inflammatory state occurs in human pregnancy characterized by neutrophil activation (60), we recently examined the NETotic response during the course of normal gestation (77). Our data indicated that during pregnancy circulatory neutrophils exhibited an enhanced propensity to undergo NETosis, which increased progressively during the course of gestation. This feature was mediated in part by the action of G-CSF (granulocyte colony stimulating factor), the levels of which increased concomitantly during pregnancy (77).

A fascinating observation made in this study concerned the multi-modulation of neutrophil activity and NETosis by pregnancy associated sex hormones. In this context, NETosis is enhanced by chorionic gonadotropin during the first trimester of pregnancy, whereas toward term a complex interaction arises, in

that it is stimulated by estrogen, but restrained by progesterone. In these neutrophils, extensive histone citrullination is evident, yet despite the presence of this key requirement in the NETotic cascade, they are unable to progress to full NETs formation. This hindrance appears to be mediated via the inability of NE to migrate to the nucleus by the action of progesterone. Previous studies have indicated that nuclear localisation of NE is essential for chromatin decondensation via the proteolytic action of this enzyme on histones, particularly the linker activity of histone H1 (64).

In this manner, progesterone appears to have a unique ability to regulate a vital step required for NETosis, maintaining circulatory neutrophils in a highly primed pro-NETotic, yet restrained state, ready to respond immediately and vigorously to an infection. Furthermore, as both phagocytosis and degranulation by neutrophils are enhanced in pregnancy, our data indicate that the innate arm of the immune system is highly active during the course of gestation to safeguard maternal and fetal wellbeing.

A possible downside of maintaining such a primed pro-active state is that it may be highly susceptible to inappropriate NETotic triggering by aberrant conditions. This could lead to the initiation of PE. Such an event could occur in pregnant women with systemic lupus erythematosus, where flares are strongly associated with the development of PE (78, 79). This supposition is supported by a recent report describing the presence of NETs in placentae of such cases with lupus induced PE (80).

NETS IN DIABETES: BYSTANDERS OR CONTRIBUTORS TO ASSOCIATED PATHOLOGIES?

Recent reports indicated that neutrophil activity, specifically NETosis, may be altered in diabetes [reviewed in (81)]. Unfortunately there was some confusion in initial reports as to whether NETosis was enhanced or reduced under diabetic or hyperglycemic conditions (81). Despite this preliminary confusion, these reports, however, may provide insight into the associated pathologies, such as reduced wound healing ability or increased susceptibility to bacterial infections in diabetes.

In an initial study examining NETosis in diabetes, it was suggested that the increased prevalence of infections with *Burkholderia pseudomallei* and resulting in diabetic patients was due to a defect in NETosis (82). It was noted that neutrophils isolated from diabetic patients exhibited a reduced ability to generate NETs following stimulation with phorbol ester, usually a powerful trigger of the NETotic cascade. This translated into a diminished ability by neutrophils from diabetic cases to trap and kill *B. pseudomallei* micro-organisms (82).

A similar observation was made by Joshi et al., who determined that although spontaneous NETosis was enhanced under hyperglycaemic conditions, it was reduced when such neutrophils were stimulated with lipopolysaccharides (LPS) (83). In addition, in a recent report by Raposo-Garcia et al., it was observed that neutrophils and macrophages from individuals with T2DM displayed a reduced capacity to engulf and destroy *Mycobacterium tuberculosis* bacilli (84). These findings provide compelling evidence that neutrophil activity is altered in diabetes, and could serve to explain the increased sensitivity of these patients to infections.

In contrast, however, Menegazzo et al., using neutrophils isolated from healthy donors, determined that NET formation was enhanced by hyperglycaemia (25 mM glucose), and further increased following stimulation with phorbol ester (85). These authors furthermore detected evidence for increased NETosis in T2DM patients by the examination of surrogate markers for NETosis in plasma.

Altered neutrophil activity may also contribute to other pathologies associated with diabetes. In this context, Wong et al. argued that aberrant NETosis in diabetes (T2DM) could contribute to impaired wound healing (86). In their seminal study, they observed that diabetes promoted enhanced NETs formation, which was further increased by calcium influx promoted ionomycin (86). This facet was linked to increased expression of PAD4 in neutrophils from diabetic individuals. As discussed above, this enzyme is required for histone citrullination, a key step initiating chromatin decondensation, preparing the cell for subsequent NETosis (65). In a murine model system, they observed that tissue wounding lead to a pronounced influx of NETting neutrophils, a feature more pronounced under diabetic conditions (86). In their experimental setting wound healing was significantly increased in both normal and diabetic mice in which the PAD4 had been "knocked-out" by genetic ablation, indicating NETs involvement.

These findings were largely corroborated by an extensive investigation of diabetic foot ulcers by Fadini et al., who detected NETs-derived products in affected human tissue specimens (87). In a murine model ulcer formation and healing could be improved by pharmacological inhibition of PAD4 enzyme activity.

In summary, these data provide compelling evidence that aberrant NETosis in diabetes could contribute to associated pathologies such as increased susceptibility to infection and reduced wound healing capability. They also provide tempting therapeutic approaches, such as the use of PAD4 inhibitors to modulate NETosis in order to address these current issues.

NOT ONLY NETS: EXOGENOUS NEUTROPHIL ELASTASE USURPS IRS1 SIGNALING

In a hallmark study, Houghton et al. determined a possible mechanism whereby infiltrating neutrophils contribute to tumor growth (88). The basis for this study was the finding that an inflammatory milieu has a profound influence on tumor growth (89, 90). This appears to be largely due to infiltrating immune effector cells, including macrophages and neutrophils, facilitated by tumor chemokine release. These reports also indicate that auspicious neutrophil infiltrates are associated with poor prognosis (89, 90).

In a murine model system for lung cancer, NE significantly influenced tumor growth and murine survival (88). The most

notable aspect of this analysis was that none of the mice died in which the NE gene (*Elane*) had been ablated, whilst all mice with an intact *Elane* gene had demised during the 30 weeks study period. Furthermore tumor size and burden was also significantly higher in mice with an intact *Elane* gene than ablated counterparts.

To gain insight into the underlying mechanism the authors examined the interaction between PMN and tumor cell lines. Here they made the key finding that NE released by infiltrating neutrophils was sequestered into neighboring tumor cells, enhancing proliferation. The authors determined that once internalized this potent proteolytic enzyme lead to the degradation of IRS1 (insulin receptor substrate 1), a key regulator of the mitogenic pathway triggered by PDGF. In a series of experiments they demonstrated that removal of IRS1 in the tumor cells line investigated lead to unrestricted growth factor independent mitosis, whereas the overexpression of IRS1 or inhibition of NE proteolytic activity reduced proliferation. Hence, infiltrating neutrophils appear to promote tumor growth by the uptake of exogenous NE, via the uncoupling of a key regulatory pathway.

These findings paved the way for similar analyses in other pathologies, including diabetes. In T2DM, evidence for such a mechanism was provided by Talukdar et al. (91), by observing that hepatocytes treated with exogenous NE became insulin resistant. They furthermore observed that mice fed a high fat diet (HFD) had improved insulin resistance and glucose tolerance, when the NE gene was ablated. Akin to the observation made in cancer cells, it was determined that exogenous uptake of NE by liver cells interfered with IRS1 signaling (91).

Subsequently, in an in-depth translational study, Mansuy-Aubert et al., observed increased activity of NE in obese human individuals. This feature was also evident in HFD obese mice, underscoring the value of this model system. Of note was that increased NE activity was coupled with a reciprocal reduction in the level of the NE inhibitor, alpha-1 antitrypsin (A1AT). This imbalance would increase enzymatic activity of liberated NE. In knockout and transgenic animals they obtained additional evidence that NE plays a key role in obesity induced insulin resistance in that NE knockout mice were resistant to HFD. This feature was also evident when NE activity was blocked pharmacologically, or by overexpression its natural inhibitor, A1AT, in transgenic mice.

NEUTROPHILS AND GDM: INTERPLAY BETWEEN HYPERGLYCAEMIA, TNFα, AND EXOGENOUS ELASTASE

In our analysis of neutrophil activity during normal pregnancy discussed above (77), we noted an unprecedented degree of NETosis in a sample, that we assumed to be have been drawn from a normal healthy pregnant woman. A reappraisal of the case history indicated that it was from a pregnancy affected by GDM, a facet diagnosed after to the time of sample collection (92).

Intrigued, we set out to verify this phenomenon in a larger cohort, the analysis of which indicated that GDM was indeed associated with an excessive NETotic response when compared to matching healthy controls (92).

This was observed using both freshly isolated neutrophils, where we observed that GDM derived cells displayed a significantly increased propensity to undergo spontaneous NETosis; and by the use of surrogate serum markers (cell free nucleosomes and/or complexes with neutrophil granular proteins) for NET formation (74, 93), where increased levels of these analytes were detected in GDM cases (92).

We were moreover able to compare the NETotic response in cases with T1DM and T2DM to that in GDM, which indicated that this was greatest in the latter, intermediate in T1DM and lowest in T2DM (92). NETosis in all classes of diabetes was, however, significantly greater than in healthy matching controls; while only that in GDM exceeded basal levels in normal pregnancy. These data underscore potential differences between the various forms of diabetes based on neutrophil activity.

Our examinations indicated that high glucose conditions (25 mM) did indeed promote an enhanced level of NETosis in freshly isolated neutrophils, but that it on its own it could not account for the high levels evident in GDM.

In order to understand the mechanism leading to this very high level of NETosis in GDM, we made use of an *in-vitro* BeWo trophoblast cell culture system. Since previous studies suggested that the placenta produces TNFα in GDM (50), we examined the effect of hyperglycaemic (25 mM glucose) conditions on BeWo cells. Such treatment elevated TNFα production by BeWo cells. Additionally, appropriate culture supernatants exerted a pronounced pro-NETotic effect on freshly isolated normal neutrophils. Since we also determined that circulating levels of TNFα were also increased in the plasma of GDM cases, it appears that this potent pro-inflammatory cytokine renowned for its potential to prime neutrophils (94, 95), played a vital role in promoting the pro-NETotic phenotype occurring in GDM.

Since numerous other cytokines could be produced by the placenta under high glucose conditions, and by analogy BeWo cells, we sought to confirm that TNFα was responsible for the observed pro-NETotic effect. This was achieved using infliximab, a clinically employed biologic agent used to counter TNFα activity in auto-inflammatory diseases such as rheumatoid arthritis. The addition of infliximab was determined to reduce the pro-NETotic effect of both GDM sera and high-glucose BeWo culture supernatants. It therefore appears that TNFα plays a pivotal role in priming neutrophils for NETosis in GDM.

Interestingly, we detected a significantly increased neutrophil infiltration in GDM placentae, both by immunohistochemistry as well as quantitative PCR for NE mRNA. Granted recent findings concerning the interaction of exogenous NE with surrounding tissues we examined whether exogenous NE could influence trophoblast behavior akin to what had previously been observed in cancer cells or diabetic hepatocytes (88, 96). Our analysis of BeWo trophoblast cells indicated that exogenous NE lead to decreased expression of IRS1 (92). A decrease in the amount of this key regulatory protein was also observed in affected placentae, concomitant with an increase in elastase expression. Hence, our data appear to mirror the effect of exogenous on IRS1 expression observed in previous studies (92).

We furthermore determined that NE altered the glucose response of BeWo cells, by reducing the expression of GLUT4 glucose transporter protein. This led to a diminished ability of NE treated cells to respond to insulin, evident by a decrease in glucose uptake.

An important aspect of our study was that circulating levels of A1AT, the natural inhibitor of NE proteolytic activity, were decreased in GDM cases. Accordingly, the action of NE liberated by degranulation or NETosis could potentially be more vigorous than under normal conditions, where this activity is held in check by A1AT (92). As we shall see below, A1AT holds another trick or two up its sleeve.

A1AT: ELASTASE INHIBITOR OR MODULATOR OF NEUTROPHIL ACTIVATION?

A1AT, also termed serpin A1, is a protease inhibitor with a high specificity for neutrophil enzymes including NE, cathepsin G, and proteinase-3 (97, 98). It is probably best known for its deficiency (alpha-1 anti-trypsin deficiency/AATD) in affected individuals, where symptoms include chronic obstructive pulmonary disease (COPD), liver disease and in rare instances, panniculititis (97, 98).

AATD patients also exhibit other inflammatory characteristics, which cannot be derived merely from loss of protease inhibitor function. These include the ability to regulate the inflammatory action of IL-1β, IL-6, TNFα, and IL-8. On the other hand a number of studies using exogenous or transgenic A1AT have observed tolerogenic activities that cannot be attributed solely to protease inhibition (97, 98).

This was most strikingly observed when examining the action of a recombinant form of A1AT without elastase inhibiting properties in a murine pulmonary inflammation model, where it was as effective as normal functional A1AT (99).

Further evidence of a pleiotropic activity by A1AT is that AATD is frequently associated with overt neutrophil activation in the absence of infection, being especially sensitive to the action of TNFα (97, 98). In this context, a recent report indicated that augmentation with A1AT in AATD individuals diminished neutrophil activity, by specifically reducing degranulation via affecting the binding of TNFα to its receptor [**Figure 1**; (100)].

Other reports indicating that A1AT possesses pleotropic activities, other than protease inhibition include the observation that exogenous A1AT reduces ROS production in stimulated neutrophils (101), or that A1AT disrupts neutrophil migration by binding to IL-8, a key chemokine (102). In addition, A1AT may blunt the immune response by binding to danger signals or damage associated molecular patterns (DAMPS) such as 70kDa heat shock protein (HSP70) (97).

As we have seen above, an imbalance between NE and A1AT has recently been implicated in obesity induced insulin signaling and energy metabolism, suggesting this pair maybe involved in the metabolic cascade initiating T2DM (96). This and other studies have prompted the exploration of recombinant A1AT molecules for the treatment of inflammatory cascades associated with the development of T2DM (103).

In the context of pregnancy, it is worth noting that circulatory A1AT levels increase during the course of gestation (97), while low circulating levels are associated with both recurrent and spontaneous abortion (104), as well as cases with severe PE (105). As the latter pathologies are associated with overt neutrophil activity (61, 76), it is currently unclear what the contribution of reduced A1AT levels is. An important facet concerning the regulatory action of A1AT in GDM, where we have noted a reduction in circulating A1AT levels, is that the activity of A1AT is diminished by high glucose conditions (97).

LEPTIN—MORE THAN AN ADIPOKINE?

Leptin, the satiety hormone, was originally described as a hormone produced by adipose tissue, regulating hunger and whose action is deregulated in obesity (106). In the interim leptin was established as an important molecule in immune regulation, most evident by virtue of its absence leading to an enhanced susceptibility to infection (106). Leptin has been described to trigger monocyte proliferation, activation, and release of pro-inflammatory cytokines, including TNFα (106).

Although neutrophils do express the short form of the leptin receptor (Ob-Ra), this is not sufficient to trigger their activation by leptin. Rather their activation by this cytokine is dependent on an interaction with monocytes and the presence of TNFα (106). Leptin has been shown to promote neutrophil migration, a feature attributed to the production of TNFα and CXCL1 by affected tissues (107). In addition leptin has been shown to enhance neutrophil longevity by suppressing apoptosis of mature neutrophils, which could be an important contributing factor for inflammatory processes (108). It is currently not clear if leptin modulates neutrophil functions, such as NETosis or degranulation.

Leptin plays a crucial role in pregnancy, where it is produced by placenta, in prodigious amounts (106, 109). In reproduction, leptin is implicated in the process of implantation, and during gestation it is implicated in regulating trophoblast differentiation, maturation and apoptosis, as well as the expression of key tolerogenic molecules such as HLA-G (106).

Leptin expression is enhanced in GDM placenta, apparently by the action of insulin or high glucose, correlating with enhanced expression of other pro-inflammatory cytokines such as TNFα (106).

Although there is some evidence that circulating levels of leptin are elevated in advance of detectable glucose intolerance, suggesting that it may be a useful screening marker to detect pregnancies at risk of GDM, these results are currently not conclusive and require further examination [**Figure 2**; (106, 110)].

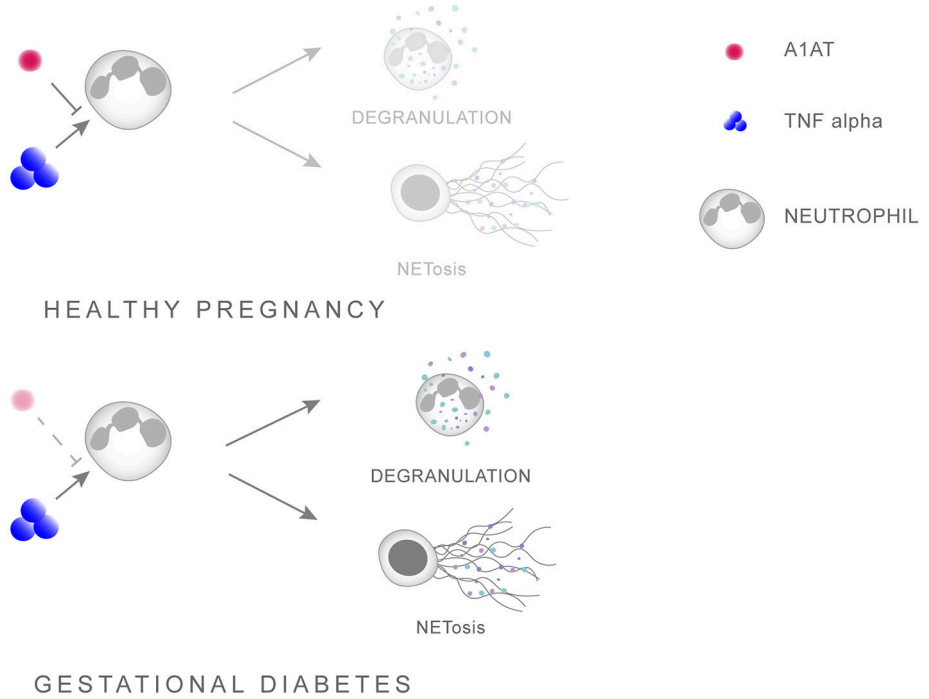

FIGURE 1 | Scheme illustrating the interaction between A1AT and TNFα in regulating neutrophil priming. A1AT hinders the ability of TNFα to interact with its receptor, thereby reducing the extent of neutrophil priming and subsequent degranulation. In GDM this system is skewed in favor of overt neutrophil priming due to enhanced TNFα levels concomitant with reduced A1AT concentrations.

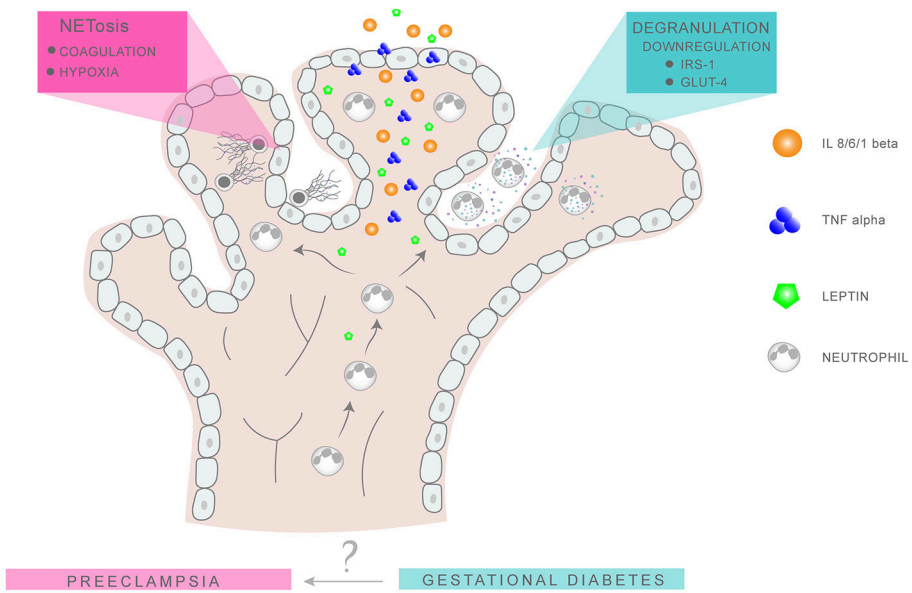

FIGURE 2 | Putative neutrophil activity in GDM and PE placentae. Neutrophil migration into the intervillous space is favored by the chemokine action of leptin, IL-8, and TNFα. In GDM, the release of NE via degranulation results in the degradation of IRS1 and GLUT4 in surrounding tissues, leading to an imbalance in glucose metabolism and insulin response. In PE, excessive NETs formation promotes placental occlusion and hypoxia, leading to extensive tissue damage.

In the context of this review it is interesting to note that circulatory leptin levels are also enhanced in PE, being more pronounced in eoPE than loPE cases (111). Akin to GDM, leptin levels increase prior to development of PE symptoms, as suggested by a recent analysis of 387 first trimester samples, of which 120 developed PE (112).

Although no formal proof is present at the moment, these data do suggest that the concomitant expression of leptin and TNFα in GDM placenta, similar to that in PE, could have pronounced effects on neutrophil activity, by promoting migration to this tissue and possibly activation.

CALPROTECTIN—A NEW PLAYER IN DIABETES ASSOCIATED COAGULOPATHIES

As we have seen above, neutrophils can contribute to diabetes-associated pathologies in a number of ways (81). A recent study has, however, indicated that calprotectin can play a role in the development of diabetes-associated coagulopathies. In their report, Kraakman et al. noted that neutrophils released calprotectin under hyperglycaemic conditions (113). Calprotectin is a dimer of the two calcium binding proteins S100A8 and S100A9 (114). It is abundantly expressed in circulatory neutrophils, where it constitutes up to 60% of the soluble cytosolic protein content. Its main function appears to be an antimicrobial action via the chelation of calcium, zinc, and manganese (114). Calprotectin has been identified as an alarmin, acting as an agonist of TLR4. In this capacity it plays a pivotal role in enhancing inflammatory responses by augmenting other DAMP (damage associated molecular patterns) or PAMP (pathogen associated molecular pattern) signals (114).

In this recent study on experimentally induced murine diabetes, it was determined that calprotectin can bind to the RAGE (receptor for advanced glycation end-products) receptor on liver Kupffer cells, thereby triggering their activation and leading to the production of thrombopoietin (TPO) (113). In the bone marrow, TPO leads to increased platelet production via the activation of megakaryocytes. These freshly released reticulocytes are larger than more mature platelets, contain mRNA and are highly responsive to agonist stimulation. Via the increased expression of p-selectin, activated platelet reticulocytes can readily interact with integrin receptors on leucoytes such as neutrophils, thereby promoting the formation of aggregates (113, 115). These platelet-leucocyte aggregates are a crucial event in the cascade leading to the formation of artherothrombosi (113). In an elegant manner, this study provides a mechanism and possible therapeutic target for diabetes associated cardiovascular disease.

These findings are of considerable interest in the context of PE, a disorder characterized by widespread endothelial dysfunction and cardiovascular damage (18). Consequently, it is worth noting that elevated calprotectin levels were previously noted in pregnancies with GDM and in pregnancies affected by PE Sugulle et al. (116). In addition, elevated expression of calprotectin was noted in placentae from cases with recurrent fetal loss, as well as in hypertensive disorders of pregnancy, most notably in cases with severe PE (117–119).

By a similar mechanism to that observed in T2DM described above (113), it is possible that elevated levels of calprotectin in PE or GDM will stimulate Kupffer cells to produce TPO, thereby leading to an influx of reticulocytes into the maternal circulation. Since neutrophils are primed in normal pregnancy and highly activated in cases with PE (76, 120), these leucocytes will readily interact with activated reticulocytes, thereby providing the basis for pronounced or severe thrombotic lesions so prevalent in this enigmatic disorder.

SUMMARY AND CONCLUSION

Pregnancies complicated by GDM, or other diabetic conditions, are associated with poor outcome and are frequently affected by further complications such as PE, a severe life-threatening condition (6, 31). GDM and especially PE are significant risk factors for adverse post-partum maternal and fetal health. In this review we have attempted to discern whether overt neutrophil activity and NETosis in pregnancies affected by GDM, could contribute to the subsequent development of PE.

Granted the complexity of PE, a condition best described as a syndrome with at least two distinct forms, and the transient nature of GDM, this is an worthy if not impossible task, and it is clear that many factors or facets are missing from a global picture.

In this overview we have highlighted the role of excessive neutrophil activation and NETosis in the etiology of PE, and related these to analogous features evident in GDM. Factors contributing to deregulated neutrophil activity in GDM appear to include hyperglycaemia and the interplay between elevated TNFα levels with a concomitant reduction in its potential regulator A1AT. Of considerable interest are the manifold contributions of NE, especially concerning IRS1, to the pathology of PE and GDM.

Further factors contributing to aberrant neutrophil activation include leptin, whose placental expression is enhanced by high glucose conditions, as well as IL-1β produced via the action of TNFα on adipocytes.

That not all cases with GDM develop PE should serve as a reminder that the described pro-inflammatory features are *per sé* not sufficient to trigger PE, but rather can be viewed as a pivotal components of a cascade, the full dimensions of which are beyond the scope of our current understanding. It is to be hoped that this deficit in our understanding will be changed by the successful implementation of systems biology approaches, thereby finally ushering in the promise of efficacious therapies dreamt of as we entered the post-genomic era. In this manner a significant contribution will be made to ensure optimal long-term fetal and maternal wellbeing.

AUTHOR CONTRIBUTIONS

SH wrote the manuscript. LV made the figures. LV, SB, XY, EH, NT, PH, OL, IH, SR commented and expanded the manuscript.

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Liver Enzymes in Early to Mid-pregnancy, Insulin Resistance, and Gestational Diabetes Risk: A Longitudinal Analysis

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Background: Liver enzymes may be implicated in glucose homeostasis; liver enzymes progressively change during pregnancy but longitudinal data during pregnancy in relation to insulin resistance and gestational diabetes (GDM) risk are lacking. We investigated longitudinal associations of γ-glutamyl transferase (GGT) and alanine aminotransferase (ALT) with insulin secretion and resistance markers across early to mid-pregnancy and subsequent GDM risk.

Methods: Within the prospective Pregnancy Environment and Lifestyle Study cohort, 117 GDM cases were ascertained and matched to 232 non-GDM controls in a nested case-control study. Fasting blood samples were collected at two clinic visits (CV1, gestational weeks 10–13; CV2, gestational weeks 16–19). Linear mixed model and conditional logistic regression were used, adjusting for major risk factors for GDM.

Results: In repeated measure analysis, after adjusting for confounders including body mass index and waist-to-hip ratio, GGT per standard deviation increment was associated with elevated fasting glucose and HOMA-IR (% change = 1.51%, 95% CI 0.56–2.46% and 7.43%, 95% CI 1.76–13.11%, respectively) and decreased adiponectin (% change = −2.86%, 95% CI−5.53 to −0.20%) from CV1 to CV2. At CV1 and CV2, GGT levels comparing the highest versus lowest quartile were associated with 3.01-fold (95% CI 1.32–6.85) and 3.51-fold (95% CI 1.37–8.97) increased risk of GDM, respectively. Progressively increased (<median at CV1, ≥median at CV2) and stably high (≥median at both CV1 and CV2) GGT levels were associated with 3.89- and 2.39-fold increased risk of GDM, compared to stably low levels (<median at both CV1 and CV2), respectively (both $P < 0.05$). Similar but non-significant trends were observed for ALT.

Conclusion: Elevated levels of GGT in early and mid-pregnancy, even within the conventional normal range, and its progressive increase from early to mid-pregnancy may be implicated in the pathogenesis of GDM, highlighting its potential to inform early screening or preventive strategies to mitigate subsequent risk of GDM.

Keywords: gestational diabetes, liver enzymes, longitudinal associations, pregnancy, repeated measures

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INTRODUCTION

Gestational diabetes (GDM), the most common metabolic dysfunction during pregnancy, affects ∼15% of pregnant women worldwide (1). The alarming rise in its prevalence over recent decades (2, 3) may be fueling the growing global epidemic of type 2 diabetes (4), becoming a major public health concern. While the underlying etiology remains to be fully understood, β cell dysfunction and thus failure to compensate for insulin resistance induced by pregnancy have been implicated in GDM development (5).

The liver, a major site of insulin action and clearance, plays an important role in maintaining glucose and insulin homeostasis, and thus is recognized as a major target of injury induced by insulin resistance and other metabolic impairments (6). Liver enzymes, specifically γ-glutamyl transferase (GGT, a marker for alcohol-related liver disease and non-alcoholic related liver fat) and alanine aminotransferase (ALT, a marker for hepatocellular damage), even within the normal range, have been linked to a multitude of cardiometabolic diseases including type 2 diabetes (7–9). Nonetheless, data among pregnant women are limited.

Importantly, emerging, yet sparse data indicate that liver enzymes may undergo progressive physiologic changes during pregnancy due to alterations in hormone homeostasis and hemodilution, which concomitantly impact hepatic function (10–12). Thus, a comprehensive understanding of the role of liver enzymes in GDM pathogenesis requires longitudinal investigations throughout pregnancy. Nonetheless, longitudinal and prospective data on liver enzymes throughout early to mid-pregnancy in relation to subsequent risk of GDM are lacking. Further, despite the emerging data that indicate elevated liver enzymes even within the normal range may stimulate insulin resistance among non-pregnant individuals free of diabetes (13), little is known about the patterns of liver enzymes across gestation in relation to insulin secretion and resistance prior to the diagnosis of GDM and subsequent risk of GDM.

Therefore, to address the critical evidence gaps, we aimed to investigate the longitudinal associations of liver enzymes GGT and ALT with markers and indices of insulin secretion and resistance with repeated measures from early to mid-pregnancy and subsequent risk of GDM, in a case-control study nested within a prospective cohort of pregnant women.

MATERIALS AND METHODS

Study Population and Design

The study participants were from the Pregnancy Environment and Lifestyle Study (PETALS), a longitudinal prospective multi-racial/ethnic cohort of pregnant women. The study design and scope have been described in detail elsewhere (14). Briefly, after weekly search of the electronic health records, pregnant women aged 18–45 years, of all races/ethnicities, carrying a singleton, and without recognized pre-existing diseases (i.e., diabetes, cancer, hepatitis C, or liver cirrhosis) were recruited before gestational week 11 at five participating medical centers of Kaiser Permanente Northern California. Questionnaire data and fasting blood specimens were longitudinally collected during early and mid-pregnancy at clinical visit 1 (CV1; gestational weeks 10–13) and CV2 (gestational weeks 16–19), respectively. The study was approved by the human subjects committee of the Kaiser Foundation Research Institute. Informed consent was obtained from all participants.

From October 2013 to June 2016, 1,708 pregnant women were enrolled and delivered a singleton, of which 1,616 (95%) were screened for GDM, serving as the source cohort. To investigate the pathophysiology of GDM, we conducted a nested case-control study within the PETALS cohort, including117 GDM cases and 232 controls individually matched at a ratio of 1:2 (with missing blood samples from 2 out of 234 controls). Matching factors included race/ethnicity (Caucasian, non-Caucasian minorities), age (±5 years), calendar time of enrollment (±3 months), and gestational weeks at blood collection (±3 weeks). Measurements of serum liver enzymes and markers of insulin secretion and resistance were obtained using fasting blood specimens collected at CV1 and CV2, respectively.

Ascertainment of Outcome

In this clinical setting, pregnant women are universally screened for GDM by a 50-g, 1-h glucose challenge test (GCT) around gestational weeks 24–28. Among pregnancies with GCT values above 7.8 mmol/L, a diagnostic 100-g, 3-h oral glucose tolerance test was performed after a 12-h overnight fast and GDM was ascertained according to the Carpenter and Coustan criteria with two or more values meeting or exceeding the following thresholds: fasting glucose 5.3 mmol/L, 1-h 10.0 mmol/L, 2-h 8.6 mmol/L, and 3-h 7.8 mmol/L (15). Serum glucose measurements for diagnosis of GDM were performed using the hexokinase method at the KPNC regional laboratory, which participates in the College of American Pathologists' accreditation and monitoring program (2).

Measurement of Liver Enzymes

Fasting blood samples were collected after an 8-12 h overnight fast at CV1 (gestational weeks 10–13) and CV2 (gestational weeks 16–19) and were stored at −80°C until being thawed immediately before assay. All assays were performed at the Lipid and Apolipoprotein Laboratory at the University of Washington (Seattle, WA) without knowledge of GDM status. All measurements were performed in duplicate and results were reported as the mean. Serum concentrations of GGT and ALT were measured on a Roche Modular P Analytics Chemistry auto-analyzer using Roche Diagnostics reagents (Roche Diagenticas Inc., Indianapolis, IN).

Markers of Insulin Secretion and Resistance

Serum glucose insulin concentrations were measured using a glucose analyzer (YSI 2300 STAT Plus, Yellow Springs, OH) and Millipore radioimmunoassay (St Charles, MO), respectively. The updated homeostatic model assessment for β-cell function (HOMA2-β) and insulin resistance (HOMA2-IR) were calculated by the computer-based models accounting for variations in hepatic and peripheral glucose resistance (16, 17). Adiponectin, as an indicator of insulin resistance (18), was measured by a

commercially available radioimmunoassay (Millipore). All the inter- and intra-assay coefficients of variation were <6.2%.

Covariates

Data on demographic, lifestyle, and clinical factors were obtained from structured questionnaires administered at CV1 and extracted from medical records. Covariates were *a priori* selected: family history of diabetes (yes, no), pre-gestational hypertension (yes, no), alcohol consumption before and/or during pregnancy (≥1 drink/day or not), and pre-pregnancy body mass index (BMI, <18.5, 18.5–24.9, 25.0–29.9, ≥30.0 kg/m^2), and waist-to-hip ratio (WHR, ≥0.85 or <0.85) as an indicator of abdominal obesity (19) which has been linked to both elevated liver enzymes and insulin resistance (20). Matching factors age (years), race/ethnicity (non-Hispanic White, African American, Hispanic, Asian/Pacific Islander, other), and gestational week of blood collection (weeks) were also included as covariates to account for residual confounding due to matching ranges and to derive conservative risk estimates. Additional covariates including education, parity, smoking before/after pregnancy, physical activity during pregnancy, diet during pregnancy, prenatal supplement use, and fetal sex were considered but were not retained in the final models failing the inclusion criteria of ≥10% change in the main effect estimates.

Statistical Analysis

Differences in participant characteristics and log-transformed serum GGT and ALT concentrations at the two clinic visits prior to GDM diagnosis between GDM cases and matched controls were assessed by linear mixed models for continuous variables with a random effect for the matched case-control pairs, and by binomial or multinomial logistic regression with generalized estimating equations for binary or multilevel categorical variables. Comparisons of log-transformed GGT or ALT concentrations between clinic visits were obtained by paired *t*-test within cases and controls, respectively.

We first assessed the continuous associations between liver enzymes and markers of insulin secretion and resistance among the entire sample. Specifically, in repeated measures analysis, we assessed the longitudinal associations between time-varying liver enzymes and time-varying markers and indices implicated in glucose and insulin homeostasis (i.e., glucose, insulin, HOMA2-β, HOMA2-IR, and adiponectin) using linear mixed models with subject-specific random intercepts, an auto-regressive covariance structure, and also a random effect for the matched cases-control pairs, adjusting for aforementioned covariates and GDM status. Natural log transformations were performed on aforementioned markers and indices to approximate normal distributions; β coefficients indicated the percent difference in these markers per standard deviation (SD) increase in serum GGT or ALT across CV1 and CV2. Further, stratified analysis was conducted to explore heterogeneity in effects by GDM status among cases and controls, respectively.

Conditional logistic regression models adjusting for covariates were fitted to assess the associations of GGT or ALT at CV1 and CV2 with subsequent risk of GDM, respectively. We analyzed each liver enzyme by categorizing the measurements into quartiles based on the distribution among controls and also by treating each enzyme as a continuous variable standardized by the SD of the measurements among controls. Tests of linear trend were conducted by using the median value for each quartile and fitting it as a continuous variable in the conditional logistic regression models. Further, to investigate the effect of progression and regression of GGT or ALT across early (CV1) to mid-pregnancy (CV2) on subsequent risk of GDM, we assessed the risk estimates associated with joint categories of GGT or ALT levels above or below the respective median at CV1 or CV2 based on distributions among controls. Specifically, stably low was defined as concentrations below median (low) at both CV1 and CV2 (reference group), progression as low at CV1 and high (≥median) at CV2, regression as high at CV1 and low at CV2, and stably high as above median at both visits.

To examine whether insulin resistance could partially explain the associations of GGT or ALT levels in early and mid-pregnancy and their respective changes across early to mid-pregnancy with subsequent risk of GDM, we conducted sensitivity analysis by additionally adjusting for HOMA2-IR as a marker of insulin resistance. Further, to test the robustness of our results against the potential impact of pathophysiologically elevated liver enzymes, we conducted sensitivity analyses by excluding women with GGT or ALT levels above the normal range established among non-pregnant women [GGT >33 U/L (21)] and ALT >19 U/L (22), due to lack of normal references tailored to pregnant women. We also assessed the potential effect modification by status of overall overweight/obesity (BMI ≥ 25 kg/m^2), abdominal obesity (WHR ≥ 0.85), and race/ethnicity (non-Hispanic White, African American, Hispanic, Asian/Pacific Islander, other). Interaction was examined by likelihood ratio test. Moreover, we excluded women with hepatitis C at the enrollment given that GGT and ALT are biomarkers more specific to chronic hepatitis C compared to hepatitis A or B (23, 24). Nonetheless, we also conducted sensitivity analysis by further excluding women with self-report or physician diagnosis of hepatitis A or B ($n = 3$). All analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Compared to non-GDM controls, women with GDM were more likely to have pre-gestational hypertension and a higher prepregnancy BMI but less likely to have alcohol use during 3 months before pregnancy (**Table 1**). Compared to non-GDM controls, GDM cases had significantly higher levels of GGT at both CV1 (gestational weeks 10–13) and CV2 (weeks 16–19) and higher levels of ALT at CV1. Across CV1 and CV2, GGT concentrations tended to decrease regardless of GDM status, whereas ALT concentrations slightly increased although to a non-statistically significant extent (**Figure 1**).

Among the entire sample, in repeated analysis, increased GGT but not ALT concentrations over early to mid-pregnancy were

TABLE 1 | Participant characteristics among gestational diabetes cases and non-gestational diabetes controls: a nested case-control study within the PETALS prospective pregnancy cohort, 2013-2016.

	GDM case ($n = 117$)	Non-GDM control ($n = 232$)	*P*-value[a]
Age, *n (%)*, years			0.59
18-24	5 (4.3)	16 (6.9)	
25-29	24 (20.5)	51 (22.0)	
30-34	56 (47.9)	114 (49.1)	
≥35	32 (27.4)	51 (22.0)	
Race/Ethnicity, *n (%)*			0.76
Non-Hispanic White	23 (19.7)	49 (21.1)	
African American	7 (6.0)	22 (9.5)	
Asian	45 (38.5)	74 (31.9)	
Hispanic	42 (35.9)	87 (37.5)	
Education, *n (%)*			0.77
High school or less	12 (10.3)	23 (9.9)	
Some college	47 (40.2)	91 (39.2)	
College graduate or above	57 (48.7)	118 (50.9)	
Missing	1 (0.9)	0 (0.0)	
Parity, *n (%)*			0.88
0	48 (41.0)	93 (40.1)	
1	40 (34.2)	84 (36.2)	
≥2	29 (24.8)	52 (22.4)	
Missing	0 (0.0)	3 (1.3)	
Pre-pregnancy body mass index, *n (%)*, kg/m^2			<0.0001
<18.5	1 (0.9)	6 (2.6)	
18.5-24.9	20 (17.1)	100 (43.1)	
25.0-29.9	36 (30.8)	56 (24.1)	
≥30.0	60 (51.3)	70 (30.2)	
Family history of diabetes, *n (%)*	36 (30.8)	53 (22.8)	0.13
Pre-gestational hypertension, *n (%)*	11 (9.4)	10 (4.3)	0.05
Smoking during 1 mo preceding pregnancy, *n (%)*	11 (9.4)	16 (6.9)	0.63
Smoking in early pregnancy, *n (%)*	1 (0.9)	1 (0.4)	0.39
Alcohol use during 3 mos preceding pregnancy, *n (%)*	48 (41.0)	131 (56.5)	0.004
Alcohol use in early pregnancy, *n (%)*	13 (11.1)	44 (19.0)	0.07

Data are presented as n (%) for categorical variables.

[a]*Obtained by binomial/multinomial logistic regression with generalized estimating equations for binary/multilevel categorical variables, accounting for matched case-control pairs.*

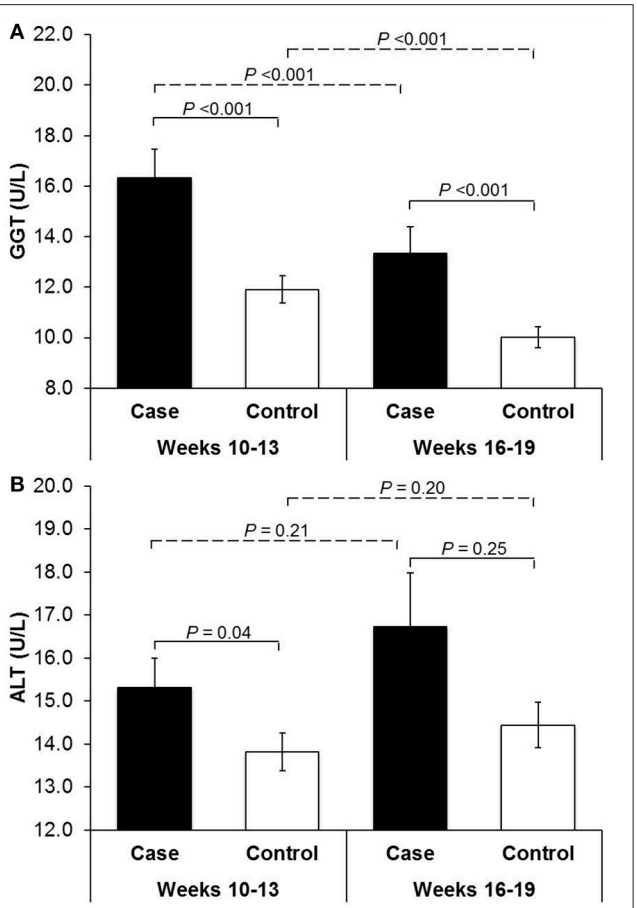

FIGURE 1 | Serum mean concentrations of **(A)** γ-glutamyl transferase and **(B)** alanine aminotransferase among women with gestational diabetes and their matched controls at gestational weeks 10–13 and 16–19. ALT, alanine aminotransferase; GGT, γ-glutamyl transferase. *P* values for case-control comparisons of the log-transformed GGT or ALT levels were obtained by linear mixed-effect linear regression models with a random effect for the matched case-control pairs at gestational weeks 10–13 and 16–19, respectively. *P* values for between-visit comparisons were obtained by paired *t*-test within cases and controls, respectively.

associated with markers of insulin resistance (**Table 2**). Overall, per SD (9.2 U/L) increase of GGT from early to mid-pregnancy was associated with 1.51% increase in fasting glucose ($P = 0.002$), 8.36% increase in insulin ($P = 0.009$), 7.43% increase in HOMA2-IR ($P = 0.010$), and 2.86% decrease in adiponectin ($P = 0.035$), after adjusting for aforementioned covariates and GDM status. No associations were observed for GGT in relation to HOMA2-β. When stratified by GDM status, significant associations were only evident among GDM cases but not controls. Similar trends were observed in longitudinal associations between ALT and these markers; however, risk estimates were not statistically significant.

We further examined associations of liver enzymes in early to mid-pregnancy with subsequent risk of GDM, respectively (**Table 3**). At CV1 (gestational weeks 10–13) and CV2 (weeks 16–19), GGT in the highest versus the lowest quartile was associated with a 3.01- and 3.51-fold increased risk of GDM after adjusting for covariates, respectively (both *P*-for-trend < 0.05, model 2). A linear relationship was observed when liver enzymes were parameterized continuously, with a 1.43- and 1.45-fold increased risk of GDM per SD increase in GGT levels at gestational weeks 10–13 and 16–19, respectively (model 2). On the other hand, at weeks 10-13, ALT comparing the highest vs. lowest quartile was significantly associated with a 2.05-fold [95% confidence interval (CI) 1.06, 3.99; *P*-for-trend = 0.035; model 2]; however, the significant association did not persist after adjusting for

TABLE 2 | Adjusted percent difference in glucose and insulin homeostasis markers per one standard deviation increase in serum γ-glutamyl transferase or alanine aminotransferase concentrations from early to mid-pregnancy[a].

Liver enzymes	Glucose and insulin homeostasis markers	All ($n = 349$)		GDM cases ($n = 117$)		Non-GDM controls ($n = 232$)	
		β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
GGT (U/L)[b]	Glucose (mg/dL)	1.51 (0.56, 2.46)	0.002	2.23 (0.48, 3.99)	0.013	0.56 (−0.62, 1.74)	0.349
	Insulin (μU/mL)	8.36 (2.11, 14.61)	0.009	11.35 (2.21, 20.49)	0.015	5.56 (−3.51, 14.64)	0.228
	HOMA2-β	2.17 (−1.34, 5.68)	0.224	2.80 (−2.00, 7.59)	0.250	2.11 (−3.11, 7.321)	0.427
	HOMA2-IR	7.43 (1.76, 13.11)	0.010	9.84 (2.05, 17.62)	0.014	5.03 (−3.43, 13.48)	0.243
	Adiponectin (ng/mL)	−2.86 (−5.53, −0.20)	0.035	−3.90 (−7.81, −0.01)	0.048	−0.48 (−2.74, 1.77)	0.673
ALT (U/L)[c]	Glucose (mg/dL)	−0.02 (−0.86, 0.82)	0.958	−0.20 (−1.81, 1.41)	0.806	0.32 (−0.63, 1.27)	0.508
	Insulin (μU/mL)	1.82 (−3.58, 7.23)	0.508	1.72 (−7.10, 10.53)	0.701	1.58 (−5.50, 8.67)	0.660
	HOMA2-β	1.21 (−1.68, 4.10)	0.411	2.31 (−1.80, 6.42)	0.268	0.16 (−3.85, 4.16)	0.938
	HOMA2-IR	2.23 (−2.62, 7.08)	0.367	3.01 (−4.39, 10.42)	0.422	1.26 (−5.30, 7.83)	0.705
	Adiponectin (ng/mL)	−0.56 (−2.16, 1.04)	0.491	−0.52 (−2.84, 1.79)	0.655	−0.48 (−2.74, 1.77)	0.673

ALT, alanine aminotransferase; GGT, γ-glutamyl transferase; HOMA2-β, updated homeostatic model assessment of β-cell function; HOMA2-IR, updated homeostasis model assessment of insulin resistance.

[a]Repeated measures analysis was adjusted for age, race/ethnicity, gestational week at blood collection, family history of diabetes, pre-gestational hypertension, alcohol use before and/or during pregnancy, pre-pregnancy body mass index, waist-to-hip ratio, and gestational diabetes (not in the stratified analysis).

[b]Standard deviation of GGT: 9.2 U/L.

[c]Standard deviation of ALT: 8.4U/L.

covariates. In the sensitivity analysis with additional adjustment for HOMA2-IR as a marker of insulin resistance (model 3), the results were slightly attenuated but remained significant.

Across CV1 and CV2 from early to mid-pregnancy, compared to stably low levels (<median 10 U/L at CV1 and <median 8 U/L at CV2) of GGT, progressively increased levels (<median at CV1 and ≥median CV2) and stably high levels of GGT (≥median at both visits) were associated a 3.89-fold (95% CI 1.13, 13.30) and 2.39-fold (95% CI 1.18, 4.86) increased risk of GDM, whereas progressively regressed (≥median at CV1 and <median CV2) GTT levels was not associated with subsequent risk of GDM (**Figure 2**). No significant associations were observed for changes in ALT levels across CV1 and CV2. In sensitivity analyses excluding women with elevated liver enzymes based on the normal range established among non-pregnant women ($n = 13$ and 6 for GGT >33 U/L; $n = 51$ and 57 for ALT >19 U/L at CV1 and CV2, respectively), results did not appreciably change (data not shown). Likewise, the results were robust against additional adjustment for HOMA2-IR.

There was significant interaction between GGT and abdominal obesity; the GGT-GDM association was only significant among women with high GGT concentrations (≥median 10 U/L at CV1 or 8 U/L at CV2) and WHR ≥0.85 (*P*-for-interaction = 0.001 and 0.025 at CV1 and CV2, respectively) (**Table S1**). No significant effect modification was observed for the association between GGT and GDM risk by race/ethnicity (*P*-for-interaction = 0.21). Despite a non-significant association between ALT and GDM risk, combination of high ALT concentrations and overall overweight/obesity or abdominal obesity illustrated a synergistic effect. Compared to women with low ALT (<median 12 U/L at either CV1 or CV2) and BMI <25 kg/m^2, women with high ALT levels (≥median 12 U/L) and BMI ≥25 kg/m^2 had a 4.20- and 3.29-fold increased risk of GDM at CV1 and CV2, respectively. Further, in the sensitivity analysis further excluding women with recognized hepatitis A or B ($n = 3$), the results remained similar.

DISCUSSION

In this case-control study nested within the prospective PETALS cohort, elevated GGT levels as early as the first trimester, even within the conventional normal range established of non-pregnant individuals, were significantly associated with markers of insulin resistance and increased risk of subsequent GDM but not markers of insulin secretion. Further, despite an overall decreasing trend of GGT over early to mid-pregnancy, progressively increased GGT levels from early to mid-pregnancy were associated with almost 4-fold increased risk of GDM.

We are the first study to our knowledge to longitudinally and prospectively examine liver enzymes from early to mid-pregnancy in relation to markers of insulin secretion and resistance and subsequent risk of GDM. Previous studies were largely cross-sectional and based on retrospective data with single measurements coinciding with the time of GDM diagnosis (25–28), precluding conclusions regarding the temporal sequence. Our findings are consistent with data from one study which linked pregravid GGT but not ALT measured on average 7 years preceding the index pregnancy to increased risk of subsequent GDM (29). In contrast, two previous studies reported significant and positive associations between ALT in the first trimester and subsequent GDM risk, although data were not adjusted for important confounders such as WHR as an indicator of abdominal obesity (30, 31), which has been linked to both elevated liver enzymes and insulin resistance (20). Notably, we also observed significant crude associations between ALT at

TABLE 3 | Crude and adjusted odds ratio (95% CI) of gestational diabetes associated with γ-glutamyl transferase or alanine aminotransferase during early-to-mid pregnancy.

	Crude model	Model 1[a]	Model 2[b]	Model 3[c]
Gestational weeks 10–13				
GGT, U/L				
Q1: 3–7[d]	1	1	1	1
Q2: 8–10	1.51 (0.75, 3.05)	1.76 (0.83, 3.70)	2.05 (0.88, 4.78)	1.97 (0.80, 4.85)
Q3: 11–14	2.22 (1.07, 4.61)	2.31 (1.06, 5.04)	2.82 (1.17, 6.76)	2.75 (1.10, 6.88)
Q4: 15–81	3.78 (1.93, 7.39)	3.81 (1.84, 7.91)	3.01 (1.32, 6.85)	2.93 (1.26, 6.80)
P-for-trend	<0.001	<0.001	0.011	0.016
Per SD increment	1.58 (1.25, 1.99)	1.58 (1.23, 2.03)	1.43 (1.08, 1.90)	1.39 (1.03, 1.87)
ALT, U/L				
Q1: 5–9[d]	1	1	1	1
Q2: 10–12	1.35 (0.71, 2.59)	1.47 (0.73, 2.94)	1.38 (0.63, 3.02)	1.28 (0.57, 2.91)
Q3: 13–16	1.34 (0.67, 2.65)	1.65 (0.79, 3.44)	1.08 (0.47, 2.49)	1.03 (0.43, 2.47)
Q4: 17–54	2.05 (1.06, 3.99)	2.44 (1.18, 5.02)	1.73 (0.75, 3.95)	1.56 (0.66, 3.70)
P-for-trend	0.035	0.015	0.247	0.367
Per SD increment	1.31 (1.04, 1.64)	1.34 (1.04, 1.71)	1.17 (0.87, 1.55)	1.11 (0.82, 1.49)
Gestational weeks 16–19				
GGT, U/L				
Q1: 3–6[d]	1	1	1	1
Q2: 7–8	2.86 (1.36, 6.02)	3.09 (1.30, 7.36)	3.03 (1.23, 7.46)	3.01 (1.21, 7.29)
Q3: 9–12	3.10 (1.35, 7.11)	3.27 (1.42, 7.55)	3.36 (1.30, 8.65)	3.30 (1.24, 8.14)
Q4: 13–47	4.02 (1.87, 8.65)	3.92 (1.73, 8.86)	3.51 (1.37, 8.97)	3.41 (1.31, 9.02)
P-for-trend	0.001	0.002	0.024	0.030
Per SD increment	1.55 (1.20, 1.99)	1.56 (1.19, 2.06)	1.45 (1.06, 1.98)	1.41 (1.04, 1.93)
ALT, U/L				
Q1: 4–10[d]	1	1	1	1
Q2: 11–12	0.74 (0.31, 1.79)	0.73 (0.29, 1.85)	0.68 (0.24, 1.93)	0.67 (0.23, 1.93)
Q3: 13–16	0.78 (0.40, 1.52)	0.89 (0.44, 1.80)	0.55 (0.24, 1.27)	0.53 (0.21, 1.26)
Q4: 17–71	1.35 (0.72, 2.53)	1.35 (0.68, 2.70)	1.12 (0.51, 2.42)	1.11 (0.48, 2.46)
P-for-trend	0.31	0.305	0.628	0.652
Per SD increment	1.17 (0.93, 1.48)	1.18 (0.91, 1.53)	1.15 (0.87, 1.52)	1.10 (0.83, 1.51)

ALT, alanine aminotransferase; GGT, γ-glutamyl transferase; Q, quartile; SD, standard deviation.

[a]Adjusted for age (years), race/ethnicity (White, African American, Asian/other, Hispanic), gestational week at blood collection (weeks), family history of diabetes (yes, no), pre-gestational hypertension (yes, no), and alcohol use before and/or during pregnancy (≥1 drink/day or not).

[b]Adjusted for covariates in Model 1, pre-pregnancy body mass index (<18.5, 18.5–24.9, 25.0–29.9, ≥30.0 kg/m^2), and waist-to-hip ratio (≥0.85 or not).

[c]Sensitivity analysis: Adjusted for covariates in Model 2 and updated homeostasis model assessment of insulin resistance.

[d]Quartiles are classified based on distributions of biomarkers among non-gestational diabetes controls.

gestational weeks 10–13 and subsequent GDM risk, whereas significant associations did not persist after additionally adjusting for covariates including WHR. Indeed, in our study the association between GGT and GDM appeared to be moderated by abdominal obesity, and in the stratified analysis, only present among women with WHR ≥0.85.

Importantly, our longitudinal data illustrated notable physiologic changes in liver enzymes during pregnancy, particularly a decreasing trend of GGT in contrast to a slightly upward but non-significant trend of ALT from early to mid-pregnancy. This observation was consistent with previous data among 103 healthy pregnant women, likely in response to increases in sex steroid levels and alterations in free fatty-acid metabolism and consequent alterations in hepatic function induced by pregnancy (10–12). Our sensitivity analysis restricted to women with GGT or ALT levels within the normal range established of non-pregnant women found similar results. Taken together, these data call for evaluation of the normal range for liver enzymes during pregnancy, given that high GGT levels even within the conventional non-pregnant normal range were associated with significantly increased risk of GDM. Further, despite the overall decreasing trend of GGT, an increase in GGT from early to mid-pregnancy may prompt further evaluation with respect to subsequent GDM risk. Notably, GDM is conventionally screened for and diagnosed at gestational weeks 24–28, leaving little time for effective interventions or treatment. In this regard, identification of pre-diagnostic markers and its progressive trends for subsequent GDM is warranted (32),

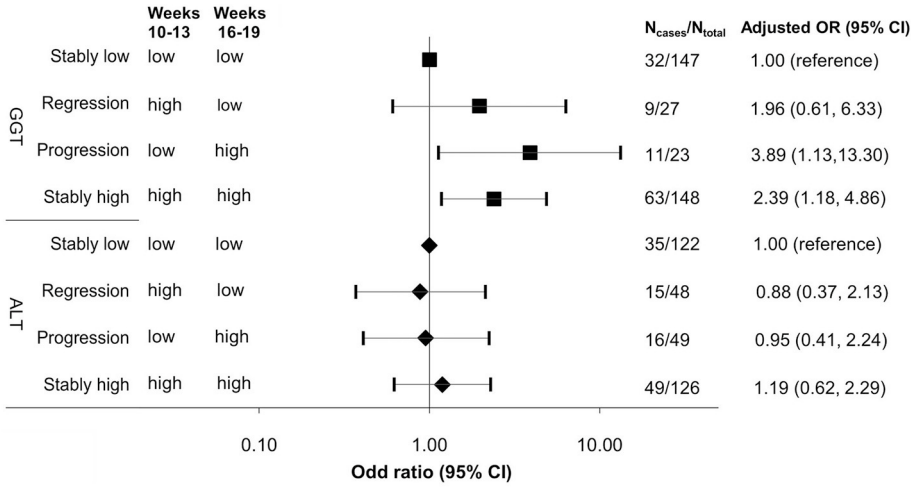

FIGURE 2 | Adjusted odds ratio (95% CI) of GDM risk associated with progression and regression of γ-glutamyl transferase and alanine aminotransferase from early to mid-pregnancy, ALT, alanine aminotransferase; GGT, γ-glutamyl transferase; stably low, levels <median at both visits; regression: levels ≥median at visit 1 (12 U/L for ALT, 10 U/L for GGT) and <median at visit 2 (12 U/L for ALT, 8 U/L for GGT); progression: levels <median at visit 1 and ≥median at visit 2; stably high: levels ≥median at both visits; early pregnancy: clinic visit 1 (gestational weeks 10–13); mid-pregnancy: clinic visit 2 (gestational weeks 16–19). Risk estimates were adjusted for age (years), race/ethnicity (White, African American, Asian/other, Hispanic), difference in gestational week at blood collection between clinic visits 1 and 2 (weeks), family history of diabetes (yes, no), pre-gestational hypertension (yes, no), alcohol use before and/or during pregnancy (≥1 drink/day or not), pre-pregnancy body mass index (<18.5, 18.5–24.9, 25.0–29.9, ≥30.0 kg/m^2), and waist-to-hip ratio (≥0.85 or not).

which may be utilized to inform early screening or preventive strategies.

Although the exact pathophysiological pathways underlying GDM development remain to be elucidated, our longitudinal data on repeated measures of GGT and ALT from early to mid-pregnancy in relation to markers and indices of insulin secretion and resistance may provide mechanistic insight. In line with our finding that GGT is associated with elevated insulin resistance, animal data demonstrated that hepatic GGT overexpression may induce insulin resistance (33). Concomitantly, epidemiological data among non-pregnant individuals have linked elevated serum GGT to increased insulin resistance and intrahepatic lipids (13, 34) but not β-cell function (34). Moreover, in contrast to ALT, which is predominantly localized in hepatocytes and thus a specific marker for liver injury, GGT is a ubiquitous epithelial enzyme involved in extracellular catabolism of antioxidant glutathione and thus a marker of oxidative stress (35, 36), which in turn may induce insulin resistance (37). Thus, different downstream pathways and cellular processes may partially explain the stronger association of GGT versus ALT with GDM risk. Likewise, outside of pregnancy, synthesized results in a meta-analysis of 10 prospective cohorts with measurements of both GGT and ALT indicate that GGT is a more sensitive marker for incident diabetes (7). Notably, in our sensitivity analysis with additional adjustment for HOMA2-IR as a marker of insulin resistance, the results were slightly attenuated but remained robust, suggesting that insulin resistance may not fully explain the positive association between GGT levels and risk of GDM. Future investigations on other mechanistic pathways may be warranted.

Our study has several notable strengths. The prospective study design is vital to ascertaining the temporal sequence of liver enzymes during early to mid-pregnancy in relation to subsequent GDM risk. Furthermore, longitudinal measurements of markers and indices involved in glucose and insulin homeostasis were also available in the present study, providing a unique opportunity to gain mechanistic insight into the role of liver enzymes in GDM development. We also obtained detailed data on conventional risk factors for GDM including demographic, medical, and lifestyle factors (including physical activity and diet) to minimize potential residual confounding. Finally, we had simultaneous measurements of GGT and ALT. Direct comparison of risk estimates associated with GGT and ALT in the same study setting may shed light onto the existing debate regarding the superior predictive ability of these liver enzymes in hyperglycemic status (7).

Some potential limitations of our study merit discussion. First, we did not have direct assessment of visceral fat to account for its possible residual confounding. Nonetheless, we used WHR in early pregnancy as a proxy, which has been demonstrated as a simple and reliable surrogate measure for intra-abdominal or visceral fat (38). Second, we did not have direct measurement of glucose and insulin homeostasis via the euglycemic clamp technique or insulin suppression test; however, it is experimentally intensive and impractical at large-scale epidemiological investigations. Thus, we utilized the updated computerized models of HOMA2-β and HOMA2-IR, which has been demonstrated reliable and valid to assess longitudinal changes in β-cell function and insulin resistance (17). Further, we conducted sensitivity analyses restricted to women within the normal range of GGT or ALT established among non-pregnant

women (21, 22), due to the lack of tailored normal ranges for pregnant women. Indeed, our unique data demonstrated the progressive gestational changes in liver enzymes (particularly GGT), highlighting the importance of further evaluation of physiological normal ranges of these liver enzymes during pregnancy.

In summary, elevated GGT levels as early as gestational weeks 10–13 were significantly associated with markers of insulin resistance and increased risk of subsequent GDM, suggesting that incipient perturbations in glucose and insulin homeostasis are already underway prior to conventional time for GDM screening and diagnosis. Further, from early to mid-pregnancy, despite an overall decreasing trend of GGT, progressively increased levels of GGT elevated subsequent risk of GDM. Our findings suggest the pathophysiological role of GGT as early as the first trimester in GDM development, highlighting its potential to inform early screening or preventive strategies to mitigate subsequent risk of GDM.

AUTHOR CONTRIBUTIONS

YZ and AF conceived the study concept. YZ performed the statistical analysis and drafted the original manuscript. MH contributed to data interpretation and revised the manuscript. CQ contributed to statistical analysis and data interpretation. JF contributed to data management and analysis. All authors contributed to the interpretation of data discussed in the manuscript, revised the manuscript and approved its final version to be published. YZ and AF are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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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.2018.00581/full#supplementary-material

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SYSTEMATIC REVIEW
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Lifestyle Intervention for the Prevention of Diabetes in Women With Previous Gestational Diabetes Mellitus: A Systematic Review and Meta-Analysis

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Background: Type 2 diabetes is increasing among the young, and gestational diabetes (GDM) offers a unique opportunity for diabetes prevention. We aimed to systematically review postpartum randomized trials to summarize the benefits of lifestyle interventions for women with previous GDM.

Methods: We searched for RCTs involving women with previous GDM that compared lifestyle interventions—diet, physical activity or breastfeeding—at postpartum with usual care up to May 2018.

Results: Of 1,895 abstracts identified, we selected 15 studies investigating incidence of diabetes or changes in glycemia. Most interventions focused on changes in diet and physical activity, only one also on incentive to breastfeeding. Meta-analysis of 8 studies investigating incidence of diabetes revealed a homogeneous (I^2 = 10%), reduction of 25% (RR = 0.75; 95%CI: 0.55–1.03) borderline statistically significant. Only trials offering intervention soon after delivery (<6 months post-partum) were effective (RR = 0.61; 95%CI: 0.40–0.94; p for subgroup comparison = 0.11). Overall, no benefit was found regarding measures of glycemia. Although moderate reductions in weight (MD = −1.07 kg; −1.43–0.72 kg); BMI (MD = −0.94 kg/m^2; −1.79 −0.09 kg/m^2); and waist circumference (MD = −0.98 cm; −1.75 −0.21 cm) were observed, effects were larger with longer follow-up.

Conclusions: Summary results of the available evidence support benefits of lifestyle interventions at postpartum for women with previous GDM. Benefits, although smaller than those of major trials based in older subjects receiving intensive interventions, appear clinically relevant for this young subset of woman. Further studies are needed to improve the quality of the evidence and to further tailor interventions to this specific setting.

Keywords: diabetes, gestational, diabetes mellitus, life style, primary prevention, women

INTRODUCTION

The International Diabetes Federation (IDF) estimates that at least 425 million persons in the world have diabetes (1).From 1980 to 2014 the global age–standardized prevalence of diabetes in adults more than doubled in men and increased almost 60% in women (2). If these trends continue, the World Health Organization (WHO) goal of halting the rise of diabetes by 2025 will not be achieved (2). The increasing burden of diabetes challenges individuals, families and health systems globally.

Diabetes can be prevented or delayed with intensive lifestyle changes offered to high-risk people, as indicated as indicated by the following now classical studies. The Da Qing Diabetes Prevention Study, after 6 years of lifestyle intervention, reduced the incidence of diabetes by 31, 46, and 42% in the groups of diet, exercise and diet plus exercise, respectively (3),and benefits extended over 20 years after the intervention was discontinued (4). The Finnish Diabetes Prevention Study (DPS) and the Diabetes Prevention Program (DPP) both showed a reduction of 58% in the incidence of diabetes mellitus in individuals with impaired glucose tolerance after an average of 3 years of lifestyle interventions focusing on diet and physical activity (5, 6). A recent systematic review of 43 studies evaluating the long-term sustainability of diabetes prevention approaches showed that the superiority of lifestyle interventions over medications observed at the end of the trial persisted for many years (7). The review included 49,029 participants with mean age of 57.3 (±8.7) years, indicating that the younger age group has been little evaluated.

Of great concern, prevalence of type 2 diabetes is increasing among the young, a phenomenon potentially increasing the burden of disease owing to the longer duration of diabetes and the apparently high incidence of chronic complications of those so affected (8, 9). Thus, diabetes prevention starting earlier than the settings of most published trials is of paramount importance. Gestational diabetes mellitus (GDM) offers a unique opportunity for diabetes prevention in younger adults. First, the diagnosis of GDM confers an increased risk of diabetes and its complications which appears to be mediated at least in part by subsequent weight gain and lack of a healthy lifestyle (10). Initial studies testing the efficacy of lifestyle interventions suggest benefit (11–25), but few systematic reviews have been carried out so far (26–28), with only one attempting to assess diabetes as an outcome (26).

We aim to systematically review and summarize the benefits of lifestyle interventions in the prevention of diabetes as well as in reduction of plasma glucose levels and anthropometry measures in women with recent GDM, as evaluated in postpartum randomized controlled trials.

METHODS

Protocol and Registration

This is a systematic review and meta-analysis of randomized controlled trials (RCTs), registered with the International Prospective Register of Ongoing Systematic Reviews (PROSPERO) under the number CRD42018092440, and following the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA Statement) (29) and the Cochrane Handbook for Systematic Reviews of Interventions.

Eligibility Criteria

The review included all RCTs involving women with previous GDM (as defined by any recognized diagnostic criteria) that compared lifestyle interventions—diet and/or physical activity and/or breastfeeding—with usual care without pharmacological treatment. We included only trials assessing the incident of diabetes mellitus (primary outcome) or glycemic levels ((mean change from baseline of fasting or 2 h glucose, or HbA_{1C}), our surrogate outcomes. We excluded studies including women with current or previous diagnosis of type 1 or type 2 diabetes, using pharmacological interventions or having recruitment strategies that were not based on a recent diagnosis of GDM.

Literature Search

We searched PubMed, Cochrane Central Register of Controlled Trials, Web of Science and EMBASE databases in May, 2018. The search string for PubMed was: ("Diabetes, Gestational" [Mesh] OR "Diabetes, Pregnancy-Induced" OR "Diabetes, Pregnancy Induced" OR "Pregnancy-Induced Diabetes" OR "Gestational Diabetes" OR "Diabetes Mellitus, Gestational" OR "Gestational Diabetes Mellitus") AND ("Exercise"[Mesh] OR Exercises OR "Physical Activity" OR "Activities, Physical" OR "Activity, Physical" OR "Physical Activities" OR "Exercise, Physical" OR "Exercises, Physical" OR "Physical Exercise" OR "Physical Exercises" OR "Diet"[Mesh] OR Diets OR "Body Weight"[Mesh] OR "Weight, Body" OR "Weight Loss"[Mesh] OR "Loss, Weight" OR "Losses, Weight" OR "Weight Losses" OR "Weight Reduction" OR "Reduction, Weight" OR "Reductions, Weight" OR "Weight Reductions" OR "Life Style"[Mesh] OR "Life Styles" OR Lifestyle OR Lifestyles) AND ("controlled study" OR trial*).These terms were adjusted to fit the requirements of each electronic database. We screened the list of references of the included studies and of systematic reviews to check for other possible studies to be included.

We did not include terms for the primary outcome to enhance the search sensitivity. We made no restrictions regarding language or publication date.

Data Extraction

Initially, two reviewers (DS, GL) independently analyzed titles and abstracts of each paper retrieved to identify potential eligible studies. Inconsistencies were discussed and studies not clearly meeting the inclusion criteria were excluded. Disagreements were resolved by discussion with a third reviewer (PG) whenever necessary.

A standard data form was used to extract the following information: study population, demographic data and baseline characteristics of participants, details of the intervention and the control counterpart, results, moments of measurement; and information for assessment of risk of bias.

Relevant missing information was requested from the original authors. Procedures for estimation of missing data were performed whenever possible (29). If data were still insufficient

after these processes, the outcome was included in descriptive analysis only.

Outcomes

The primary outcome was incidence of diabetes mellitus. We also reported change in glycemic levels (mean fasting or 2 h glucose, or HbA_{1C}). Secondary outcomes were changes in the anthropometric measures of weight and waist circumference.

Risk of Bias (Quality) Assessment

Three reviewers in pairs (DS, GL, PG) independently assessed the quality of the studies. The disagreements were resolved by consensus or with the consultation of an additional author (WC).

We evaluated the risk of bias as described in the Cochrane Handbook for Systematic Reviews of Interventions using the Cochrane Collaboration tool (29), with the following criteria: random sequence generation (selection bias); allocation concealment (selection bias); blinding (performance bias and detection bias) considering blinding of participants, personnel and those performing outcome assessment; incomplete outcome data (attrition bias); selective reporting (reporting bias); and other biases.

Data Analysis

We estimated relative risks for the incidence of diabetes mellitus. For continuous outcomes, we estimated mean differences from baseline. When standard deviations for changes were missing, we made imputations considering a conservator correlation equal to zero. We used random effects models with DerSimonian and Laird estimators for analyses of all outcomes. All statistical tests were two-sided and significance was defined as $P < 0.05$. We assessed statistical heterogeneity of treatment effects across studies using the I^2 metric statistics. The statistical analyses were performed used R version 3.5.0 (R Foundation for Statistical Computing). In addition, publication bias was examined using funnel plot and the Egger test (Stata 11.0, StataCorp, College Station, TX).

RESULTS

Study Selection and Patient Characteristics

The flowchart for the selection and exclusion of studies is presented in **Figure 1**. After removing duplicates, we found a total of 1,895 abstracts from where 38 articles were considered as potentially eligible and assessed through full-text reading. We then excluded 23 additional studies, remaining with a total of 15 studies. The reasons for exclusion were: not a randomized controlled trial ($n = 4$) (30–33), not reporting our primary outcomes ($n = 13$) (34–46), study population not meeting our inclusion criteria specification ($n = 4$) (47–50) and different reports from the same study ($n = 2$) (51, 52).

The 15 studies included in the systematic review are described in **Table 1**. All articles were published within the last 10 years, except one (11). Studies took place in the United States (11–13), Australia (14–16, 25), China (17–20), Spain (21), Malaysia (22), Israel (23), and Ireland (24). The number of women who were randomized in individual studies ranged from 28 to 573, with 8 studies including at least 100 participants (11, 16–21, 23). Ten studies specified eligibility criteria regarding the risk of diabetes: postpartum glucose intolerance (11, 17, 18, 20–22, 24), overweight or obesity (13, 22, 24), low level of physical activity (12, 25), altered lipid profile (24), high waist circumference (22, 24), family history of diabetes (22), use of insulin during pregnancy (17) or hypertension (24).

Duration of follow-up was 6 months or less in 5 studies (12, 14, 15, 18, 22), 1 year in 5 studies (13, 16, 19, 24, 25), and 2 years or more in 5 studies (11, 17, 20, 21, 23).

Most of the interventions focused on changes in diet and physical activity. Only one study mentioned incentive to breastfeed (13). Three studies focused solely on the effectiveness of physical activity intervention (12, 15, 25) and one only on diet (22). Standard/brief advice on diet and/or exercise was considered to be comparable with usual care and accepted as the control comparison. Different ways of delivering the intervention were applied: Nine established remote contact (11–16, 18, 20, 25) (by phone, internet or postcards); four performed group sessions (14, 16, 23, 24), and eleven had individual face-to-face contacts (15–25). From those which held individual meetings, two conducted home visits (16, 18) and the others held the sessions in the clinic/hospital.

Eight trials had data to estimate incident diabetes (11, 13, 17–21, 25). Eleven trials measured glycemic control (11, 12, 14–16, 18, 19, 21–24), and all trials investigated the effect on body weight. Overall, considerable heterogeneity was evident between studies in relation to several key characteristics, namely, the method of the intervention, the time lag since the pregnancy complicated by GDM, the degree of risk beyond having GDM, and the duration of follow-up.

Quality Assessment of Included Studies

Table 2 presents items necessary to assess risk of bias in each study according to the Cochrane Collaboration risk of bias tool for RCTs. Considering all studies included, 60% described adequate random sequence generation (12–18, 22, 24) and 40% allocation concealment (13–16, 22, 24). We did not evaluate blinding of staff performing the interventions due to the nature of lifestyle interventions. Only 26% of the studies mentioned blinding of the outcome assessors (12–14, 22), and it was frequently unclear whether blinding extended to all staff involved (laboratory technicians, staff making anthropometric assessments, data analysts). About half of the studies described exclusions and losses during follow-up (12–14, 16, 17, 21, 22) and a similar proportion reported intention-to-treat analysis (13, 14, 16, 17, 21, 22, 24). Some studies (11, 19, 20) though not mentioning intention to treat analysis or reasons for losses or exclusions, presented few such events, thus minimizing the possibility of bias due to incomplete outcome data.

Main Results

Meta-analysis of the 8 studies reporting incident diabetes (**Figure 2**) revealed a borderline statistically significant relative

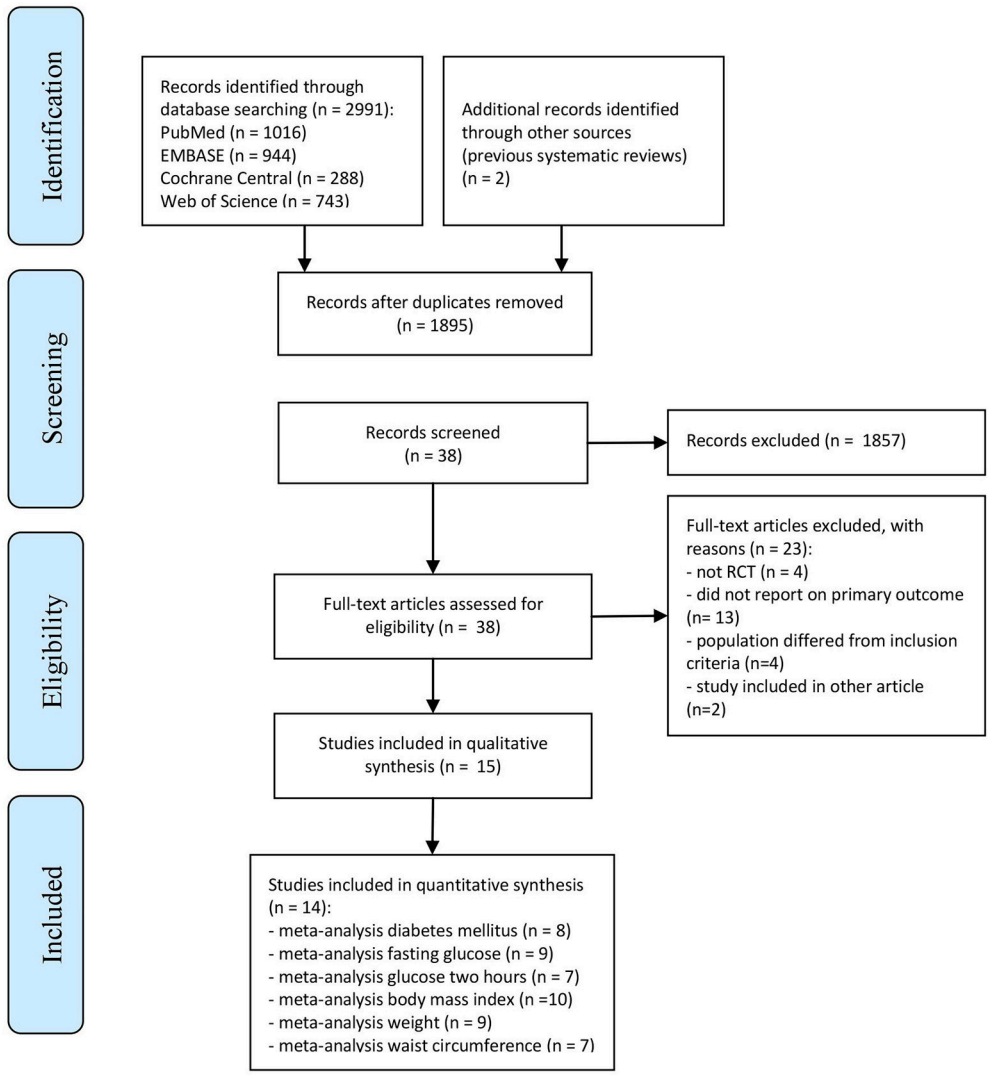

FIGURE 1 | Flow chart summarizing the process for the identification of the eligible studies.

reduction of 25% (RR = 0.75; 95%CI: 0.55–1.03) in incidence with intervention. The results were homogeneous across studies (I^2 = 10%). When stratified by time of randomization, only studies initiating earlier in the post-partum period showed a significant reduction (RR = 0.61; 95%CI: 0.40–0.94; p for subgroup comparison = 0.33). The overall absolute difference in incidence between groups was −0.04 (95%CI: −0.09; 0.01).

Figure 3 shows a funnel plot for the 8 studies reporting incidence of diabetes. We can observe a general funnel shape indicating that studies of lower precision were spread evenly on both sides of the average, suggesting absence of publication bias. The Egger test also indicated absence of publication bias ($p = 0.47$).

Figures 4, **5** showed a lack of effect of lifestyle interventions in mean fasting and 2h plasma glucose, with a non-significant difference from baseline on fasting glucose (MD = −0.13; 95%CI: −0.36; 0.09) mmol/L and on 2 h glucose (MD = −0.12; 95%CI: 0.47; 0.23) mmol/L for 2 h glucose. Only 3 studies reported HbA1c, without positive results.

Figures 6, **7** showed that the life style intervention had a moderate statistically significant greater reductions in mean weight (MD = −1.07; 95%CI: −1.43; −0.72) kg and BMI (MD = −0.94; 95%CI: −1.79; −0.09) kg/m^2, respectively, effects being larger with longer follow-up. **Figure 8** also show a statistic significant greater reduction in waist circumference (MD = −0.98; 95%CI: −1.75; −0.21) cm, also larger with longer follow-up.

DISCUSSION

Evidence here summarized reveal that lifestyle changes started after a pregnancy complicated by GDM produce a 25%

TABLE 1 | Characteristics of the included studies.

First author and year	Country	Sample size (randomized)	Period of intervention following partum: start point; endpoint.	Inclusion criterion in addition to recent GDM	Intervention duration/ Follow-up	Focus of interventions	Mode of interventions	Outcome measures
Cheung et al. (25)	Australia	43	Not specified; 4 years	Sedentary habits	1 year	Exercise	1 individual meeting (clinic visit) pedometer 5 telephone contacts 7 postcards	DM BMI
Hu et al. (19)	China	444	1 year; 6 years	–	1 year (in this article; total follow-up 2 years)	Diet and exercise	6 individual meetings (clinic visits)	DM FBG OGTT HbA_{1C} BMI WC
Ji et al. (18)	China	144	Shortly after partum; not specified.	Postpartum IGT or IFG	4 months	Diet and exercise	• 4 individual meetings (home visits) • Diary (diet, exercise, weight, postprandial blood glucose) • 3 telephone contacts	DM FBG OGTT HbA_{1C} BMI Weight
Kim et al. (12)	United States	49	Not specified; 3 years	Sedentary habits	3 months	Exercise	Web content Internet forum Pedometer Text messages	FBG OGTT BMI Weight WC
McIntrye et al. (15)	Australia	28	Shortly after partum; not specified.	–	3 months	Exercise	1 individual meeting (clinic visit) 7 telephone contacts	FBG Weight WC
Nicklas et al. (13)	United States	75	Shortly after partum; not specified.	BMI > 22/24 kg/m^2 and< 50 kg/m^2	1 year	Diet and exercise; breastfeeding	12 web modules Lifestyle coach available by phone or email Support material (laptop, scale, measuring cups and spoons, membership to the gym)	DM BMI Weight
O'Dea et al. (24)	Ireland	50	1year post partum; 3 years	IGT, IFG or insulin resistance (HOMA) + 2 of the following: hypertension, high total cholesterol, triglycerides or LDL-C, low HDL-C, BMI >30 kg/m^2, WC>88 cm.	12–16 weeks of intervention/1year of follow-up	Diet and exercise	1 individual meeting (clinic visit) 12 group sessions with individual meeting at the end of each group session	FBG OGTT BMI Weight WC
O'Reilly et al. (16)	Australia	573	Notspecified; 1 year	–	1 year	Diet and exercise	1 individual meeting (home visit) 5 group sessions 2 telephone contacts	FBG BMI Weight WC
Peacock et al. (14)	Australia	31	6 months after partum; 2years	–	3 months	Diet and exercise	4 nutrition coaching workshops Pedometer Text messages if the participant uploaded accelerometry data	FBG BMI Weight WC

(Continued)

TABLE 1 | Continued

First author and year	Country	Sample size (randomized)	Period of intervention following partum: start point; endpoint.	Inclusion criterion in addition to recent GDM	Intervention duration/ Follow-up	Focus of interventions	Mode of interventions	Outcome measures
Pérez-Ferre et al. (21)	Spain	260	Shortly after partum; not specified.	Exclusion of postpartum IFG	3 years	Diet and exercise	Training exercise (at the hospital, 20 training sessions in 10 weeks) 4 individual meetings (clinic visits)	DM FBG HbA_{1C} BMI WC
Shek et al. (17)	China	450	Shortly after partum; not specified.	Postpartum IGT; exclusion if insulin use during pregnancy	3 years	Diet and exercise	7 individual meetings (clinic visits) Diary (diet and exercise) of 5 days prior to each visit	DM BMI
Shyam et al. (22)	Malaysia	77	Shortly after partum; not specified.	Family history of diabetes and: BMI >23 kg/m^2 or WC 80 cm or IGT or IFG	6 months	Diet	1 individual meeting (clinical visit)	FBG OGTT BMI Weight WC
Wein et al. (11)	United States	200	Shortly after partum; not specified.	Postpartum IGT	7.1 to 81 months (median of 51months)	Diet and exercise	3 monthly telephone contacts	DM FBG OGTT BMI
Yu et al. (20)	China	126	Shortly after partum; not specified.	Postpartum IGT/IFG	2 years	Diet and exercise	4 individual meetings (clinic visits) 4 telephone contacts	DM BMI
Zilberman-Kravits et al. (23)	Israel	180	3–4 months after partum; not specified.	–	2 years	Diet and exercise	3 individual meetings (clinic visits) 3 to 4 group sessions	FBG BMI Weight WC

IFG, impaired fasting glucose; IGT, impaired glucose tolerance; DM, diabetes mellitus; FBG, fasting blood glucose; OGTT, standard 2 h oral glucose tolerance test; HbA_{1C}, glycated hemoglobin; BMI, body mass index; WC, waist circumference; GDM, gestational diabetes mellitus; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

TABLE 2 | Risk of bias among included studies.

	Adequate random sequence generation	Allocation concealment	Blinding of outcome assessment	Description of losses and exclusions	Intention-to-treat analysis	Free from selective reporting
Cheung et al. (25)	Not informed	Not informed	Not informed	No	No	Unclear[d]
Hu et al. (19)	Not informed	Not informed	Not informed	No	No	Yes
Ji et al. (18)	Yes	Not informed	Not informed	No	No	Unclear[d]
Kim et al. (12)	Yes	Not informed	Yes[a]	Yes	No	No
McIntrye et al. (15)	Yes	Yes	Not informed	No	No	Yes
Nicklas et al. (13)	Yes	Yes	Yes[b]	Yes	Yes	Yes
O'Dea et al. (24)	Yes	Yes	Not informed	No	Yes	Unclear[d]
O'Reilly et al. (16)	Yes	Yes	Not informed	Yes	Yes	Yes
Peacock et al. (14)	Yes	Yes	Yes[b]	Yes	Yes	Yes
Pérez-Ferre et al. (21)	Not informed	Not informed	Not informed	Yes	Yes	Yes
Shek et al. (17)	Yes	Not informed	Not informed	Yes	Yes	Unclear[d]
Shyam et al. (22)	Yes	Yes	Yes[ac]	Yes	Yes	Yes
Wein et al. (11)	Not informed	Not informed	Not informed	No	No	Unclear[d]
Yu et al. (20)	Not informed	Not informed	Not informed	No	No	Unclear[d]
Zilberman-Kravits et al. (23)	No	Not informed	Not informed	No	No	Yes

[a] *Blinding of staff obtaining anthropometry.*
[b] *Blinding of data analysts.*
[c] *Blinding of laboratory technicians.*
[d] *Study registration or published protocol not found.*

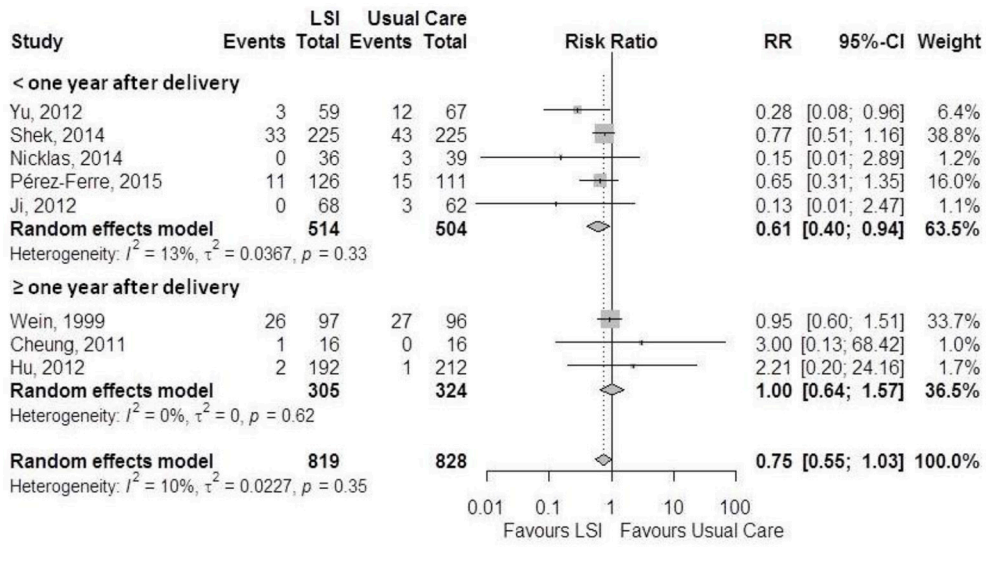

FIGURE 2 | Meta-analysis of the effects of lifestyle interventions (LSI) in diabetes incidence according to post-partum time at randomization.

(RR = 0.75; 95%CI: 0.55–1.03) reduction in diabetes risk which reaches borderline statistical significance. Effects appeared to be larger when the interventions were initiated within 6 months after birth (RR = 0.61; 95%CI: 0.40–0.94; p for subgroup comparison = 0.33). We found small but statistically significant reductions in weight, BMI and waist circumference, particularly with longer periods of intervention. In contrast, we found no change with intervention for final fasting or 2 h glucose values.

The only previous meta-analysis reporting effects on diabetes incidence among women with recent gestational diabetes (26) included four of the eight trials here summarized. It did not report relative risks but found an absolute risk difference of (RD = −5.02%; 95%CI: −9.24; −0.80), consistent with the size of the risk reduction we found. With regard to weight changes, the previous meta-analysis (27) found a similar difference mean weight reduction (MD = −1.06; 95%CI: −1.68; −0.44) kg. We found no meta-analysis

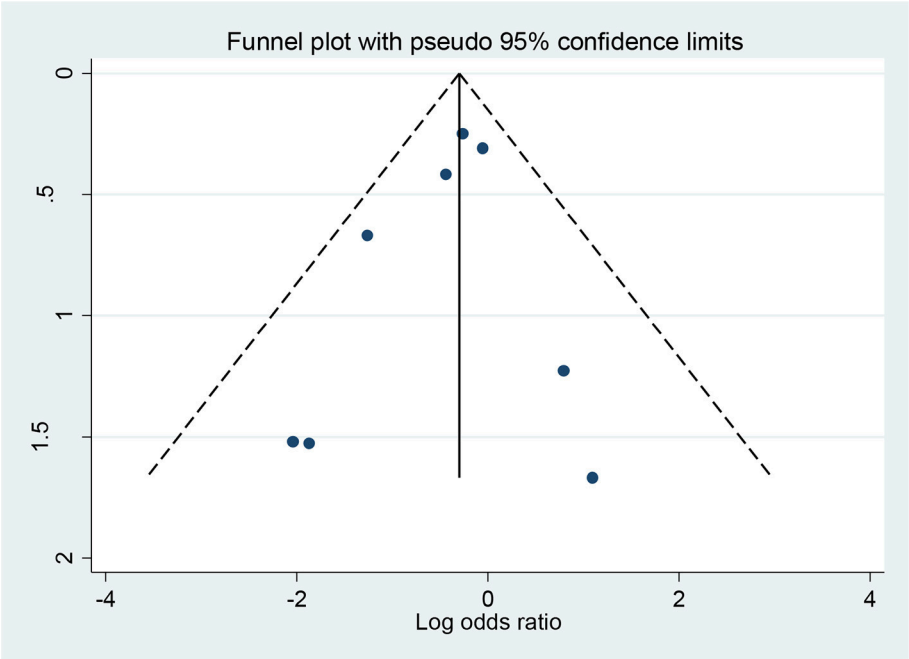

FIGURE 3 | Funnel plot, using data from 8 trials with information for diabetes incidence. Log-odds ratios all displayed on the horizontal axis.

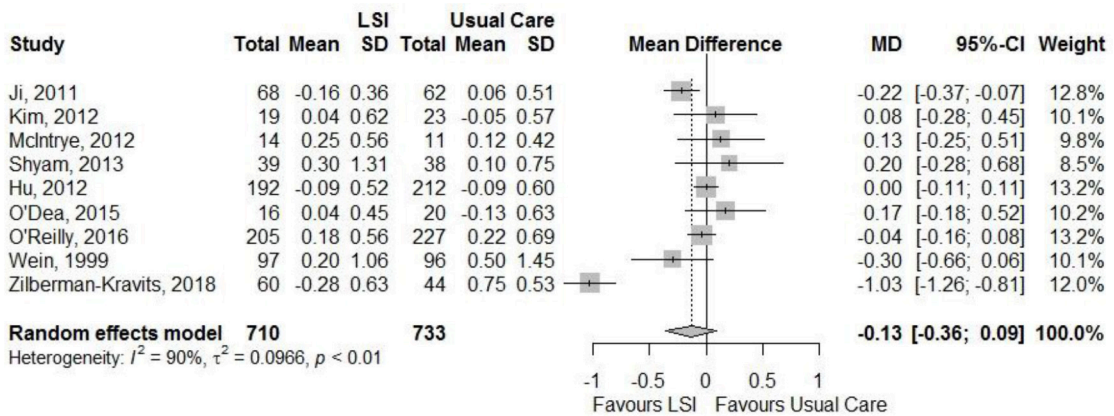

FIGURE 4 | Meta-analysis of the effects of lifestyle interventions (LSI) in fasting glucose change (mmol/L) from baseline to the end of follow up.

reporting effects on BMI, waist circumference, 2h glucose during an oral glucose tolerance test or HbA_{1C}. The only one reporting a summarized effect on fasting plasma glucose, like ours did not find a statistically significant difference in reduction (MD = −0.05; 95%CI: −0.21; 0.11 mmol/L).

The fact that reductions in incidence here reported were somewhat greater when the intervention initiated sooner after birth (RR = 0.61 vs. 1.00; p = 0.11) may reflect stronger motivation to initiate lifestyle changes when women are closer to their GDM treatment during pregnancy. However, the number of studies initiating later is small to reach a conclusion. We have no explanation for the small size of changes in mean glucose values, but as numbers are not large, it is possible that outliers in glucose values, once diabetes developed, could influence these glucose means. Additionally, heterogeneity across studies for these outcomes was large.

We found a consistently greater effect in studies with longer follow-up across the three anthropometric measures. In these studies, the period of intervention was also of greater duration, which suggests the importance of maintaining support for lifestyle changes for a longer period, particularly given the women's frequently overwhelming tasks of

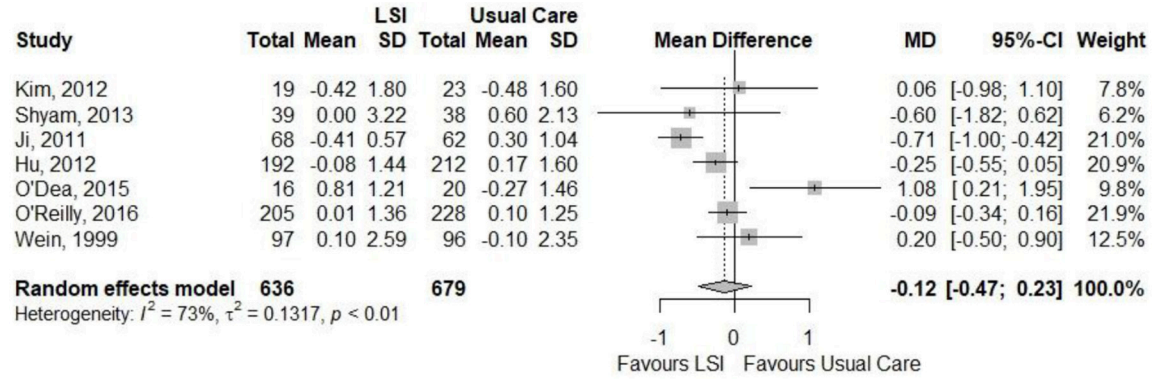

FIGURE 5 | Meta-analysis of the effects of lifestyle interventions (LSI) in 2-h glucose change (mmol/L) from baseline to the end of follow up.

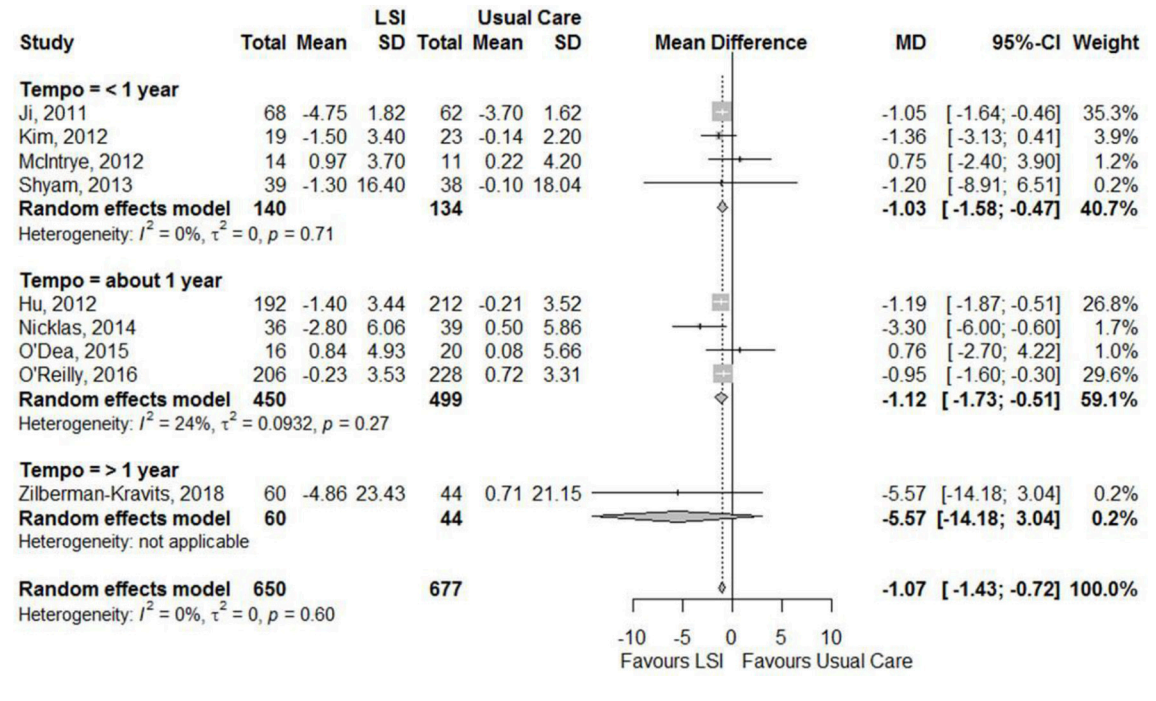

FIGURE 6 | Meta-analysis of the effects of lifestyle interventions (LSI) in weight change (kg) from baseline to the end of follow up, according to the duration of follow-up.

motherhood. Of note also, since breastfeeding is often being performed during the post-partum period, weight loss recommended is usually small, thus requiring a longer period than the usual weight loss programs to reach weight loss goals.

There are several ongoing trials which may complete data collection and publish their results in the next three to 4 years (53–56). Up to now, this is the most comprehensive summary reporting on the feasibility and effectiveness of lifestyle modifications soon after birth of mothers with gestational diabetes. Compared to the only previous meta-analysis reporting diabetes as an outcome (26), we have increased the number of studies involved, as well as the scope of the outcomes assessed.

Although effects are small, benefits are clinically relevant, since seemingly minimal changes in anthropometric measures over a short period translate into a 25% risk reduction of diabetes in women who are, on average, only 30 years old. We hope that these ongoing trials of longer duration and with greater support for lifestyle changes will produce larger effects, perhaps with results approaching the relative risk reduction of 53% found in *post-hoc* analyses focusing on women with previous gestational diabetes (47), treated about 9 to 10 years after the target pregnancy in the similarly more robust and longer DPP study.

Our study has strengths and limitations. First, the number of women randomized (1647) and the number of events (180) are still small, resulting in only borderline statistical significance. Of

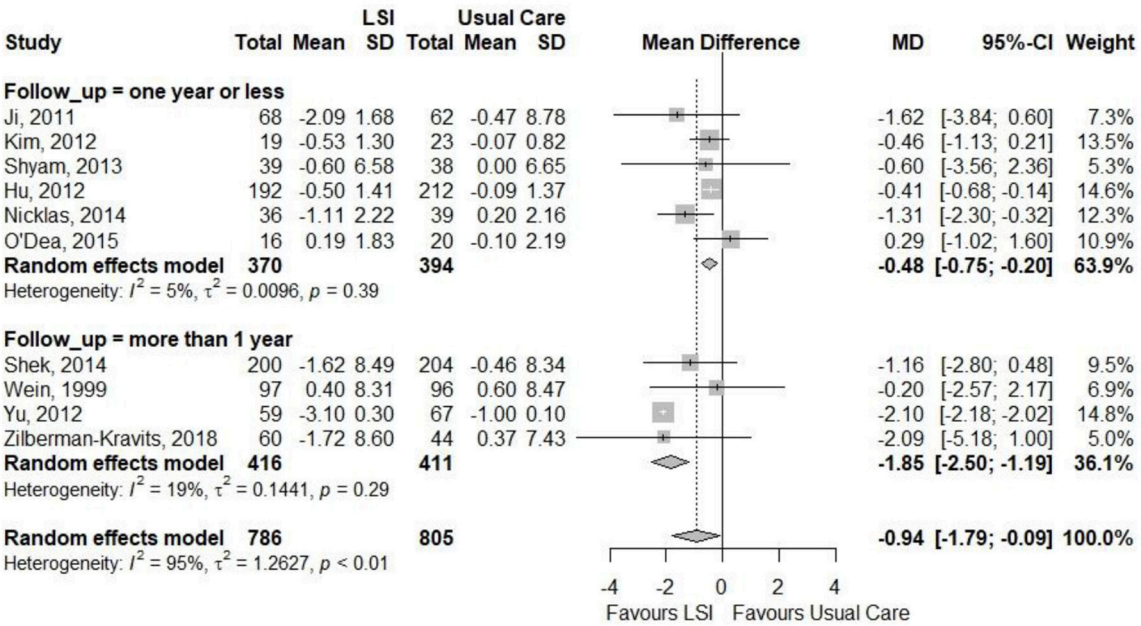

FIGURE 7 | Meta-analysis of the effects of lifestyle interventions (LSI) in BMI change (kg/m²) from baseline to the end of follow up, according to the duration of follow-up.

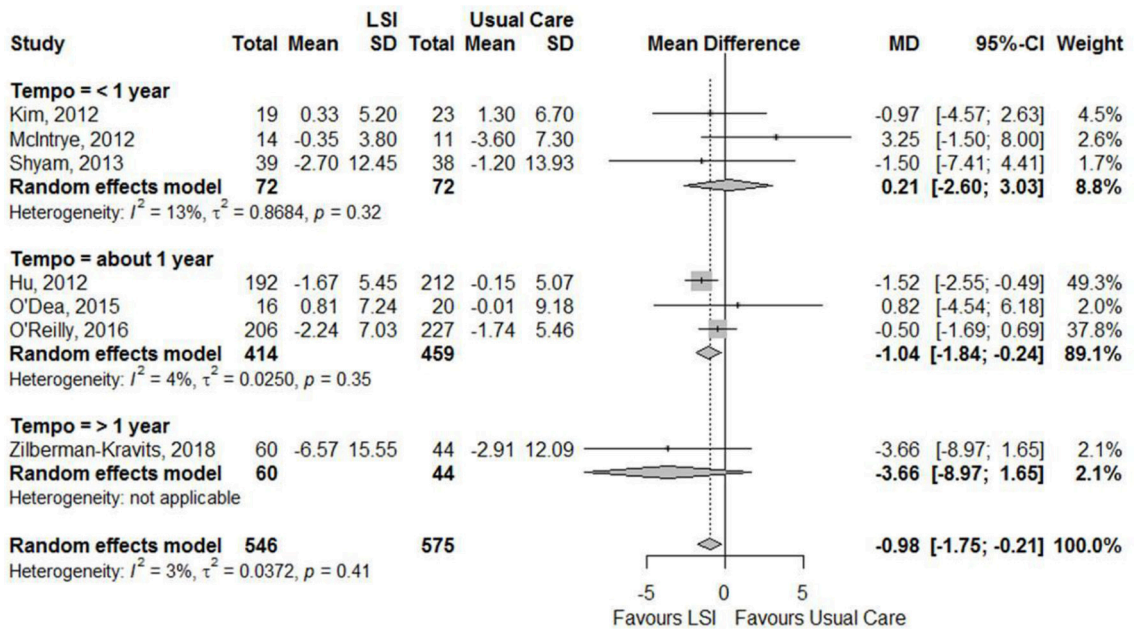

FIGURE 8 | Meta-analysis of the effects of lifestyle interventions (LSI) in waist circumference change (cm) from baseline to the end of follow up, according to the duration of follow-up.

note however, funnel plot and Egger test indicated small chance of publication bias. The effect of 25% reduction in the incidence of diabetes is small but potentially clinical relevant. As suggested by the absolute risk difference we found, 4%, the number needed to treat is 25 women, in other words, we need to treat 25 women with GDM at postpartum with similar interventions to prevent one case of diabetes. Finally, the quality of most studies included in this review is not high and sample size often limited to less than 70 women. These limitations highlight the need for further studies to provide more accurate summary results.

In conclusion, our comprehensive meta-analysis suggests an effect of lifestyle intervention after a pregnancy complicated by gestational diabetes. The effect is smaller than those of the classic studies of lifestyle intervention to prevent diabetes in older subjects when offered more intensive interventions. Nonetheless, the benefits here reported for younger women with previous GDM suggest that interventions to prevent diabetes are feasible and may have potential clinical. Additional studies are needed to further tailor the delivery of lifestyle interventions to this particular period of life and to improve the quality of the evidence for their effectiveness when offered to women with GDM after pregnancy.

AUTHOR CONTRIBUTIONS

MS, PG, and WC designed the study. DS, GL, PG, and RM contributed to the literature search and data extraction. PG and PZ performed data analyses. BD, MS, PG, PZ, and WC participated in the interpretation, writing, and proofreading of the manuscript.

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Dietary Protein Intake, Meat Consumption, and Dairy Consumption in the Year Preceding Pregnancy and During Pregnancy and Their Associations With the Risk of Gestational Diabetes Mellitus: A Prospective Cohort Study in Southwest China

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Background: Gestational diabetes mellitus (GDM) has become a public health problem in China.

Objective: To examine the association of dietary protein intake before and during pregnancy with the risk of GDM.

Design: Dietary intake before pregnancy and during the first and second trimesters of pregnancy was assessed using food frequency questionnaires in a prospective cohort of pregnant women. To screen GDM, participants underwent an OGTT test during 24–28 weeks of gestation. Cox proportional hazards were used to estimate RRs and 95% CIs for the associations between tertiles of dietary protein and the source of protein intake in different time windows with GDM status.

Results: Higher intake of total protein [RR (95% CI): 1.92 (1.10–3.14), p for trend = 0.04] or animal protein [1.67 (1.19–2.93), p for trend = 0.03] in mid-pregnancy was associated with higher risk of GDM. Vegetable protein intake before or during pregnancy was not related to GDM risk (p for trend > 0.05). Moreover, in the mid-pregnancy, participants with higher meat consumption or dairy consumption had a higher risk of GDM.

Conclusion: Our study indicated that higher dietary intakes of total protein and animal protein in mid-pregnancy were associated with an increased risk of GDM among pregnant Chinese women.

Keywords: gestational diabetes mellitus, insulin resistance, protein intake, longitudinal cohort, meat consumption, dairy consumption

INTRODUCTION

Gestational diabetes mellitus (GDM) is a common complication of pregnancy characterized by glucose intolerance with onset or first recognition during pregnancy (1). For mothers, GDM is not only associated with adverse perinatal outcomes (2), it is also related to a higher risk of GDM during subsequent pregnancies (3, 4) and type 2 diabetes and premature cardiovascular disease in the medium and long term (5). For offspring, GDM pregnancy confers a greater risk of developing obesity, diabetes, hypertension, cardiovascular disease in youth, and adult life (6, 7). According to the diagnostic criteria of 2011 issued by the Ministry of Health (MOH) China (8), which endorsed the new criteria by the IADPSG (1), the prevalence of GDM in China is 17.5% in 2013 (9), which is higher than the prevalence in Europe and the USA (10, 11). Given the substantial relevance of GDM for medium and long-term health of both mother and offspring, it is crucial to identify modifiable risk factors which contribute to GDM prevention among Chinese population.

GDM is a disease of disturbed glucose homeostasis (1). In pregnancy, the sensitivity of insulin is reduced by 50–70% (12), while compensatory increase in insulin secretion is 2–2.5 times to maintain normal blood glucose level (13). Consequently, pregnancy represents a physiological state of insulin resistance. Evidence supports that chronic insulin resistance is a central component of the pathophysiology of GDM (14). Dietary proteins might play a vital role in the pathogenesis of insulin resistance, acting as gluconeogenic precursors thereby stimulating hexosamine biosynthesis, or activating the mTOR-signaling pathway (15, 16). Hence, an understanding of the role of the dietary protein intake on the GDM development may have important public health implications. Several studies have found that a diet during mid-pregnancy with a higher protein intake among Asian women (17, 18) or higher protein intake among US women before pregnancy (19) is related to higher GDM risk, while a previous study among Canada women did not find the relationship between protein intake and GDM (20). The reasons for these inconsistent results may be due to the various sample sizes [205 (20) to 15,294 (19)], the focused observation time window, the diagnostic criteria for GDM [clinical examination: WHO standard (1999) (17), IADPSG standard (2010) (18), NDDG standard (1979) (20), and self-reported (19)] or the ethnicity of the research participants [Asians (17, 18), Americans (19), Canadians (20)].

To date, all cohort studies analyzing the link between maternal individual nutrients and GDM risk addressed dietary intake only in one time window, i.e., either before pregnancy (19, 21–28) or during pregnancy (29–31). However, individual dietary protein intake level may change during pregnancy (32). An increase in the intake of protein rich foods during pregnancy was reported in UK (33), Portugal (34), Switzerland (35), Hungary (36), and Asia (37), especially a remarkable increase in protein intake during mid-pregnancy was observed among Portuguese (34) and Swedes (35). In terms of public health, it would thus be intriguing to clarify whether there is a critical time window for dietary protein intake, taking into consideration pre-pregnancy intake and intake across the two pregnancy trimesters preceding the diagnosis of GDM.

Therefore, using data from the Nutrition in Pregnancy and Growth in Southwest China (NPGSC) prospective cohort study, we examined whether dietary protein intake during potentially critical periods, i.e., the year preceding pregnancy, 1st or early 2nd trimester were associated with GDM risk.

MATERIALS AND METHODS

Study Sample

We used data from the NPGSC study, which is a prospective cohort study initiated in January 2014 to investigate the relevance of maternal nutrition before and during pregnancy on health outcomes of mother (e.g., gestational diabetes mellitus) and child (e.g., birth weight, birth length, and body composition development in childhood). Using a sampling design stratified by urban and rural locations, a representative sample of pregnant women was drawn from public hospitals with obstetric services in Southwest China (Sichuan Province, Yunnan Province, and Guizhou Province). Within each urban area 2 public hospitals were randomly selected, while 2–3 public hospitals were randomly selected within each rural area. In total, 27 study centers (12 urban hospitals and 15 rural hospitals) were included until December 2017. The study was approved by the Ethics Committee of Sichuan University.

At each center, pregnant women were invited during their first visit for routine ultrasound examination at gestational weeks 9–11. To facilitate follow-up, only women who had lived in their current residence for at least 2 years were eligible to participate. The overall response rate was 91.2%.

Data collection of NPGSC study was performed 3 times before birth (the first routine ultrasound examination, Q1; gestational weeks 20–22, Q2; gestational weeks 33–35, Q3) and 8 times in infancy and childhood (**Figure 1**). At Q1, approached by trained interviewers and local nurses, each woman was asked to complete a self-administered questionnaire to collect information on her birth characteristics, demographic characteristics, medical history, lifestyle (e.g., smoking behavior, alcohol consumption, tea/coffee consumption), employment, annual family income, and family history of chronic diseases. In

addition, participants were interviewed by trained investigators in a face-to-face interview with respect to their diet [one food frequency questionnaire (FFQ) covering the consumption over the past 12 months before pregnancy and one 24-h dietary recall addressing intake over the past 24 h] and physical activity (one questionnaire covering physical activity and sedentary behavior over the past 12 months before pregnancy and from the start of the pregnancy, separately). At Q2 and Q3, one 24-h dietary recall each inquiring intake over the past 24 h and one FFQ addressing consumption during the previous 12 weeks, as well as one physical activity questionnaire were administeredby trained investigators in face-to-face interviews.

Maternal anthropometrics measures (body weight before pregnancy and during pregnancy), clinical measures, as well as information on current and past pregnancy outcomes, complications, and infant abnormalities recorded in the Medical Birth Registry were linked to the study database. Furthermore, anthropometric measures of offspring and information of cognitive development test which have been followed in infancy and childhood and recorded in health registries were linked to the study database. At Q1, all participants provided written informed consent for all examinations as well as for linkage of their data from the Medical Birth Registry and the data of their offspring from the health registries.

From 2014 to 2017, 1,0126 pregnant women were recruited in the NPGSC Study. For the current analysis, we used information on diet, anthropometry and clinical measures collected between 2014 and 2017 from mothers living in the Sichuan Provence and Guizhou Province (18 study centers: 8 urban hospitals and 10 rural hospitals). The Ministry of Health China recommends screening by fasting plasma glucose (FPG) test at the first prenatal visit to rule out previously undiagnosed preexisting diabetes. Of the 6,886, 73 women with preexisting diabetes mellitus before pregnancy were excluded. To be included in this analysis, participants had to have delivered a live, singleton baby and to have answered the first general questionnaire resulting in 6,686 women. Of them, 6,502 had provided three FFQs for the dietary intakes before pregnancy, at 1st trimester, or at 2nd trimester. We further excluded women with an implausible energy intake ($<$500 or $\geq$3,500 kcal/day) (38) ($n = 106$), as well as those with missing information on parity ($n = 36$), maternal occupation ($n = 23$), or annual family income ($n = 38$). In total, 6,299 women with complete information were included in this analysis (**Figure 2**).

Nutrition Assessment

Since only FFQ covering consumption over the past 12 months before pregnancy can be used for the estimation of dietary intake before pregnancy, the present analyses is based dietary data collected by FFQs. At each Q1, Q2, and Q3, a modified validated 128-item FFQ (39) was used to inquire how often, on average (never to $\geq$5 times/d) the participants had consumed the respective food groups [e.g., white rice, brown rice, red rice, wheat noodle, steamed bread, bread, whole grain foods, potatoes, cakes, vegetables, fruits, subtropical fruits, dairy and dairy products, soybeans, and its products, nuts (walnuts, almonds, cashews, peanuts, and other nuts), meat (pork, beef, chicken, or lamb), eggs, fish and shrimp, and beverages (including drinking water, mineral water, tea and herbal tea, lemonades, fruit drinks (diluted and sugar-sweetened fruit juices), ice teas, soft drinks, and sports drinks)], using standard serving sizes. The participants were offered a range of different serving sizes for each food and beverage item. To each foods item, vegetable protein, and animal protein were categorized. In our study, the most important determinant of animal protein were meats, fish and shrimp, eggs, dairy and dairy products, and for vegetable protein were beans (sum of soybeans and its products), and nuts. Visual aids such as standard serving bowls, plates and glasses were displayed to the participants to improve the accuracy of the estimated portion sizes. The frequency and amount of consumption of each food or beverage per unit of time were converted into food consumption per day. Total energy and nutrient intakes were calculated using the continuously updated in-house nutrient database (40) reflecting the composition of Chinese Foods (41). This nutrient database includes any food item ever recorded in previous studies conducted and is based on information from standard nutrient tables, product labels (e.g., most convenience foods) or recipe simulation based on the labeled ingredients and nutrients (e.g., commercial mixed dishes).

Diagnostic Criteria for GDM

At 24–28 weeks of gestation, participants underwent a 2-h 75-g oral-glucose-tolerance test (OGTT) after an overnight fast, and venous plasma glucose levels at 0, 1, and 2 h were measured. A diagnosis of GDM was made if any one of the following values was met or exceeded: 0 h (fasting), $\geq$5.1 mmol/L; 1 h, $\geq$10.0 mmol/L; and 2 h, $\geq$8.5 mmol/L, according to the diagnostic criteria for GDM recommended by the Chinese Ministry of Health (8). The diagnostic criteria were in line with the criteria proposed by the International Association of Diabetes and Pregnancy Study Groups.

Quality Control

All study laboratories successfully completed a standardization and certification program. The coefficient of variation within and between study laboratories was $<$5% for each marker. All laboratory equipment was calibrated and blinded duplicate samples were used. All data were double entered into the database. Participants were informed about all clinical data within 36 h after collection.

Other Clinical Examinations

Self-reported pregravid weight was recorded on the day of registration. Body weight was measured with an ultrasonic meter (Dingheng, Zhengzhou, China) to the nearest 100 g by trained nurses according to standard procedures at enrollment and at regular intervals (in 4-week intervals from enrolment to week 25, every 2-week until week 33 weekly thereafter) to birth (**Figure 1**). These information and maternal height (measured to the nearest 0.1 cm by trained nurses at enrollment) were recorded in the Medical Birth Registry.

For all participants, gestational age (GA) was assessed during the first ultrasound scan (Eub 5500, Hitachi; Eub 7500, Hitachi; Logiq E9, GE) on the day of registration, which was conducted

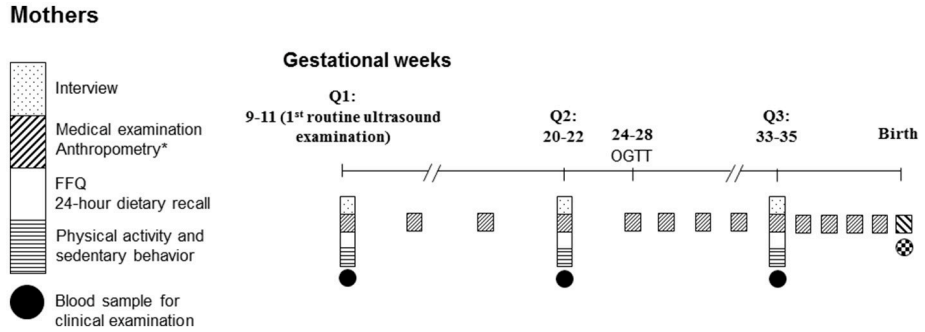

FIGURE 1 | The study examination schedule.

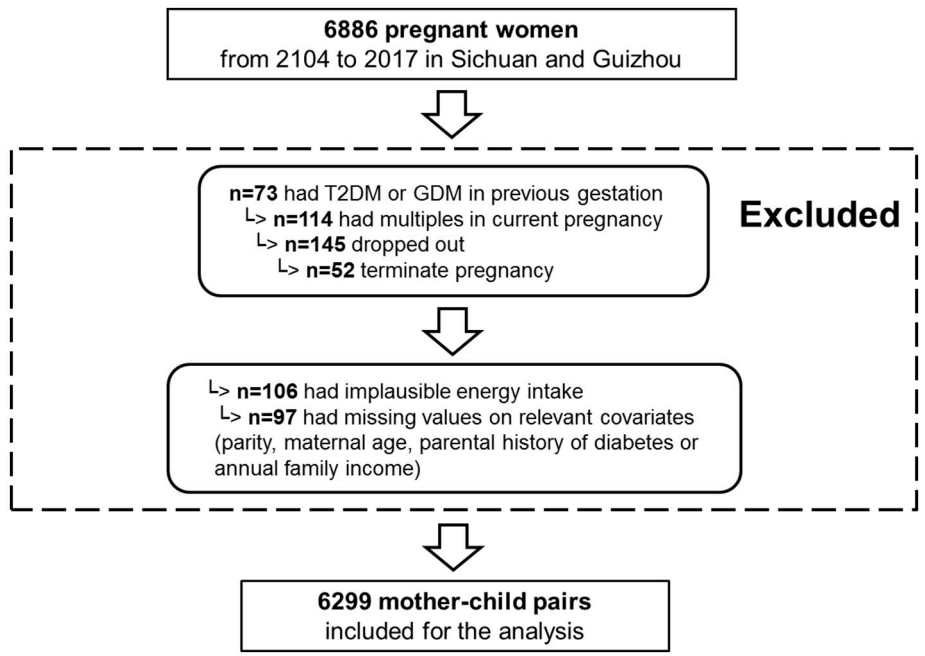

FIGURE 2 | Flowchart for the study sample.

in a standard manner by trained ultrasonographers. GA was estimated by combining ultrasonography data with self-report on the last menstrual period: if both measures were available and there was agreement (±14 days) self-report data was used, otherwise, ultrasound data were used.

Statistical Analysis

SAS® procedures (version 9.2, SAS Inc., Cary, NC) were used for all data analyses. All analyses were performed with a significance level at $p < 0.05$.

Because we were interested in the critical time window regarding the relevance of dietary protein intakes for GDM onset, we conducted separate analyses using dietary data collected before pregnancy and during the 1st trimester and 2nd trimester of pregnancy. Dietary protein intakes in these 3 different periods were expressed each as residuals from their regression on energy intake.

Energy-adjusted residuals of dietary protein intakes were grouped into tertiles to illustrate their associations with the risks of GDM using Cox proportional hazards analysis. In the basic models, dietary protein intakes were the independent predictors. The following variables potentially affecting these associations were considered: gestational age, age, parity, location (urban/rural), family history of diabetes, maternal education level (12 or more years of schooling; yes/no), maternal occupation (no, yes: part-time worker, or full-time worker), monthly personal income (<3,000 CNY, 3,000–6,000 CNY, >6,000 CNY), and pregravid BMI, physical activity, smoking/passive smoking before or during pregnancy (never, past, current: 1–15, 16–24, or >24 cigarettes/d), alcohol consumption before or during pregnancy (0, 0.1–9.9, 10.0–19.9, 20.0–29.9, ≥30 g/d), gestational

TABLE 1 | General characteristics[a] of the study participants.

Characteristics	Values (n = 6,299)
Urban [n (%)]	3,030 (48.1)
Maternal age (years)	26.5 (3.8)
Pregravid BMI (kg/m^2)	20.7 (2.4)
Gestational diabetes mellitus [n (%)]	1,203 (19.1)
Single mother [n (%)]	157 (2.5)
High education level[b] [n (%)]	3,666 (58.2)
Moderate personal monthly income[c] [n (%)]	3,281 (52.1)
PREVIOUS MEDICAL HISTORY AND FAMILY HISTORY	
Polycystic ovarian syndrome [n (%)]	119 (1.9)
Primiparous (n, %)	4,585 (72.8)
Family history of diabetes [n (%)]	1,492 (23.7)

[a] *Values are means (SD) or frequencies.*
[b] *School education at least 12 years.*
[c] *Personal income per month at least ≥3,000 CNY (Chinese Yuan), which is an average level among the general population in Southwest China.*

weight gain during pregnancy, and intakes of carbohydrate and fat at the same time point of dietary protein. Each potential confounder was initially considered separately and included if it substantially modified the association of dietary protein with GDM or significantly predicted the outcome variable. Thus, maternal age and parity were retained in model 2. In a further step, we adjusted for parental history of diabetes (yes or no), current smoking (combination of passive smoking and active smoking, yes or no), polycystic ovarian syndrome (PCOS, yes or no), family income, total energy intake, fat intake, carbohydrate intake and physical activity. Animal protein and vegetable protein were mutually adjusted for one another. Relative risk (RR) were calculated for the respective tertiles and a test for trend was performed using the respective continuous variables.

RESULTS

General Characteristics

Maternal characteristics are presented in **Table 1**. The mean age of participants was 26.5 years and the mean pre-gravid BMI was 20.7 kg/m^2. GDM was diagnosed in 1,203 of 6,299 pregnancies. 23.7% of our participants reported family history of diabetes, 41.8% of them had less than a college education.

Women in the 2nd trimester had gained more weight compared with women in the 1st trimester (**Table 2**). Participants were more likely to work outside before pregnancy (64.3%) than participants in the 1st trimester (48.2%) or in the 2nd trimester (45.0%). Compared to those in pre-pregnancy, women in the early pregnancy reduced physical activity sharply and in the second trimester basically returned to physical activity level as it before pregnancy. Dietary fat intake, protein intake, animal protein intake and vegetable protein intake in the mid pregnancy were higher than those in the pre-pregnancy or in the early pregnancy, while their energy intake from carbohydrate was lower than those in the pre-pregnancy and in the early pregnancy. Not surprisingly, consumption of meats, eggs, dairy and dairy products, fish and shrimp were greatest in the mid pregnancy as well as beans and nuts consumption.

Relative Risk for GDM by the Dietary Protein Intake Before Pregnancy and During the 1st Trimester and the 2nd Trimester

Higher intake of total protein, animal protein and vegetable protein before pregnancy and in the early pregnancy were not associated with risks for GDM (**Table 3**). In mid pregnancy, higher total protein intake or animal protein intake was associated with higher risks for GDM: women with highest total protein intake or animal protein intake had an approximately 92% (67%) higher risk for GDM than those in the lowest total protein intake tertile or animal protein intake tertile. Vegetable protein intake in the 2nd trimester was not related to GDM risk.

Relative Risk for GDM by the Major Dietary Protein Sources in the Mid Pregnancy

In our population, animal protein accounted for the majority and the major source of animal protein is from meats, fish and shrimp, eggs, dairy, and dairy products. The main contributors to vegetable protein were beans and nuts.

In the mid pregnancy, higher meat consumption, dairy, and dairy products were associated with higher risk for GDM: participants with highest meat consumption or dairy consumption had an approximately 95% (115%) higher odds for GDM than those in the lowest tertile of meat or dairy consumption (**Figure 3**).

DISCUSSION

Our study indicated that higher dietary protein intake and animal protein intake in mid-pregnancy, but not in early pregnancy or before pregnancy, were associated with risks for GDM in a Chinese population. In addition, the main dietary protein sources in 2nd trimester such as meats or dairy products were significantly associated with a higher risk of GDM.

To our knowledge, this is the first report on dietary changes before and during pregnancy among Chinese women. Compared to the dietary protein intake before pregnancy, a clear increase was seen in pregnancy, especially it in the 2nd trimester, among our sample. This is in line with the data from Sweden women (n = 50) (35) (14.4% of energy vs. 16.8% of energy) and Portugal mother (n = 249) (34) (17.6% of energy vs. 18.4% of energy). In addition, our finding of the main dietary source of protein are broadly consistent with that observed among British in The Southampton Women's Survey (n = 1,490) and Hungarian women (n = 349) (33, 36). The reason for this result may be due to physiological changes, traditional beliefs and dietary recommendations during pregnancy (32).

Physical activity is a well-established risk factor for the development of GDM (42). In present study, we were not surprised to find that with the increase of gestational age, the work of pregnant women was significantly reduced, and physical activity during pregnancy (especially in the first trimester)

TABLE 2 | Anthropometric, behaviors, and nutritional data[a] at the pre-pregnancy and during pregnancy in participants (n = 6,299).

	Pre-pregnancy	1st trimester	2nd trimester
ANTHROPOMETRIC DATA			
Body weight (kg)	53.7 (5.3)	55.1 (4.6)	61.3 (5.7)
Gestational weight gain (kg) during each trimester	–	1.2 (2.1)	5.5 (2.7)
BEHAVIORS			
Multivitamin use [*n* (%)]	3,716 (59.0)	6,179 (98.1)	5,026 (79.8)
Work outside [*n* (%)]	4,050 (64.3)	3,036 (48.2)	2,834 (45.0)
Physical activity (MET-h/wk)[b]	18.6 (10.4)	13.2 (8.7)	17.2 (10.2)
Self-reported passive smoking [*n* (%)]	3,149 (50.0)	2,103 (33.4)	1,700 (27.0)
Self-reported active smoking [*n* (%)]	170 (2.7)	31 (0.5)	0 (0.0)
Self-reported alcohol drinking [*n* (%)]	1,650 (26.2)	120 (1.9)	19 (0.3)
DIETARY INTAKE[c]			
Total Energy (kcal/d)	1658.2 (516.9)	1639.8 (472.0)	1986.9 (555.3)
Carbohydrate (% of energy)	52.6 (8.7)	52.7 (8.2)	48.6 (7.6)
Fat (% of energy)	31.9 (8.0)	31.7 (7.3)	33.6 (6.5)
Protein (% of energy)	15.5 (2.3)	15.6 (2.3)	17.8 (2.6)
Animal protein (% of energy)	9.2 (2.8)	9.3 (2.8)	11.2 (2.7)
Vegetable protein (% of energy)	6.3 (1.6)	6.3 (1.6)	6.6 (1.6)
Meats (g/d)	94.0 (27.0)	83.9 (21.9)	128.5 (24.2)
Eggs (g/d)	49.8 (3.9)	41.6 (5.7)	64.7 (4.1)
Dairy and dairy products (g/d)	217.9 (27.7)	259.1 (21.5)	377.1 (23.9)
Fish and shrimp (g/d)	23.9 (6.6)	21.0 (8.1)	75.5 (5.9)
Beans (g/d)	15.9 (20.8)	17.5 (33.3)	18.4 (29.4)
Nuts (g/d)	9.7 (20.6)	19.2 (17.0)	23.3 (16.7)

[a] *Values are means (SD).*
[b] *Metabolic equivalent hours of activity per week.*
[c] *All nutritional data represent crude values.*

was also reduced. The effect of mid-pregnancy protein on the incidence of GDM may be enhanced by decline physical activity. However, after analysis, there was still a significant risk of GDM associated with high levels of protein even when the decline in physical activity was accounted for.

Our data do in addition suggest that the relation of dietary protein intake to risk of GDM is particularly pronounced in the time window closer to GDM diagnosis, at least for Chinese women, i.e., only protein intake during mid-pregnancy rather than in pre-pregnancy or in early pregnancy is relevant for risk of GDM. Pregnancy is characterized by a series of metabolic changes, including the development of physiological insulin resistance. During early pregnancy, glucose tolerance is normal or even slightly improved, and peripheral sensitivity to insulin and hepatic basal glucose production is normal (43), however, in later gestation, maternal adipose tissue depots decline, while post-prandial free fatty acid levels increase, and insulin mediated glucose disposal worsens by 50–70% compared with pre-pregnancy (12). Dietary proteins which may modulate insulin sensitivity (15) are thus of specific relevance on the glucose homeostasis for the pregnant women, with respect to the physiological insulin resistance. However, data from the NHS II Cohort Study (19) suggested that dietary protein intake or animal protein intake before pregnancy was related to higher GDM risk. The reason of the inconsistence of our findings with NHS II data might be the intake level among study population (15.5% in our participants vs. 19.14% in US women). The other study in Guangzhou China inconsistently demonstrated that protein-rich pattern was not associated with the risk of GDM, which may be attributed to the different dietary patterns in South China and Southwest China. In addition, unlike our study of single nutrients, there are interactions between nutrients in dietary patterns (44).

In the current study, we discovered that an increase in the intake of animal protein during the 2nd trimester was associated with higher risk of GDM which is in line with previous findings on a relevance of protein intake in mid pregnancy for GDM confirming this for both 1,247 women from Singapore (17) and 1,261 pregnant women from China (18). Although the mechanisms between high dietary animal protein intake and GDM risks are unclear, the observed association with GDM is biologically plausible. It could be caused by several nutrients, such as iron and amino acids. An overload of iron can cause insulin resistance leading to increase in the production of hepatic glucose (45, 46), subsequently reducing the secretion of insulin (47). Considering that an increase in the consumption of animal protein may contribute to increased body iron load, the association between a higher animal protein intake and gestational diabetes in the mid-pregnancy may be partly explained markers of body iron load. Second, the

TABLE 3 | Dietary protein intakes before and during pregnancy and risk of gestational diabetes[a] (n = 6,299).

Variable	Protein intake in tertile			
	T1	T2	T3	*p*
PRE-PREGNANCY				
Total protein				
Median (% energy/d)	13.40	15.36	17.42	–
Model 1	1.00	1.91 (1.04–3.56)	1.17 (0.61–2.27)	0.08
Model 2	1.00	1.86 (1.01–3.49)	1.14 (0.60–2.21)	0.10
Model 3	1.00	2.07 (1.09–4.01)	1.59 (0.76–3.39)	0.09
Animal protein				
Median (% energy/d)	6.69	9.15	11.75	–
Model 1	1.00	1.36 (0.75–2.49)	0.90 (0.48–1.70)	0.37
Model 2	1.00	1.39 (0.76–2.56)	0.90 (0.76–2.56)	0.33
Model 3	1.00	1.60 (0.83–3.13)	1.28 (0.56–2.92)	0.36
Vegetable protein				
Median (% energy/d)	4.89	6.21	7.79	–
Model 1	1.00	1.09 (0.59–2.05)	1.33 (0.73–2.46)	0.64
Model 2	1.00	1.11 (0.60–2.09)	1.33 (0.73–2.47)	0.64
Model 3	1.00	1.21 (0.62–2.37)	1.45 (0.69–3.07)	0.63
EARLY PREGNANCY				
Total protein				
Median (% energy/d)	13.69	15.44	17.47	–
Model 1	1.00	1.04 (0.56–1.95)	1.38 (0.75–2.53)	0.52
Model 2	1.00	0.97 (0.51–1.83)	1.31 (0.72–2.43)	0.55
Model 3	1.00	0.79 (0.41–1.54)	0.89 (0.43–1.86)	0.79
Animal protein				
Median (% energy/d)	6.95	9.22	11.52	–
Model 1	1.00	0.89 (0.50–1.67)	1.19 (0.66–2.17)	0.65
Model 2	1.00	0.86 (0.46–1.61)	1.13 (0.62–2.07)	0.68
Model 3	1.00	0.62 (0.30–1.27)	1.07 (0.75–1.69)	0.54
Vegetable protein				
Median (% energy/d)	4.75	6.10	7.71	–
Model 1	1.00	0.74 (0.41–1.36)	0.71 (0.39–1.30)	0.48
Model 2	1.00	0.80 (0.44–1.47)	0.73 (0.40–1.34)	0.58
Model 3	1.00	0.94 (0.48–1.81)	0.90 (0.43–1.86)	0.96
MID PREGNANCY				
Total protein				
Median (% energy/d)	15.51	17.45	20.24	–
Model 1	1.00	1.24 (1.03–2.40)	2.11 (1.15–2.96)	0.04
Model 2	1.00	1.19 (1.01–2.31)	2.07 (1.12–2.90)	0.04
Model 3	1.00	1.18 (1.02–2.37)	1.92 (1.10–3.14)	0.04
Animal protein				
Median (% energy/d)	8.36	11.10	13.76	–
Model 1	1.00	1.45 (1.13–2.35)	1.83 (1.17–2.49)	0.05
Model 2	1.00	1.48 (1.14–2.44)	1.81 (1.16–2.47)	0.04
Model 3	1.00	1.38 (1.12–2.93)	1.67 (1.19–2.93)	0.03
Vegetable protein				
Median (% energy/d)	5.16	6.45	8.00	
Model 1	1.00	0.77 (0.41–1.43)	1.04 (0.57–1.88)	0.60
Model 2	1.00	0.76 (0.41–1.42)	1.01 (0.56–1.42)	0.61
Model 3	1.00	0.87 (0.46–1.67)	1.33 (0.69–2.58)	0.42

[a] *Values are medians and intakes were calculated as the percentage of energy by tertile. Model 1: crude model. Model 2: additionally adjusted for age, pregravid BMI, and parity. Model 3: additionally adjusted for family history of diabetes (yes or no), current smoking (yes or no), PCOS (yes or no), family income, total energy intake, fat intake, carbohydrate intake, and physical activity.*

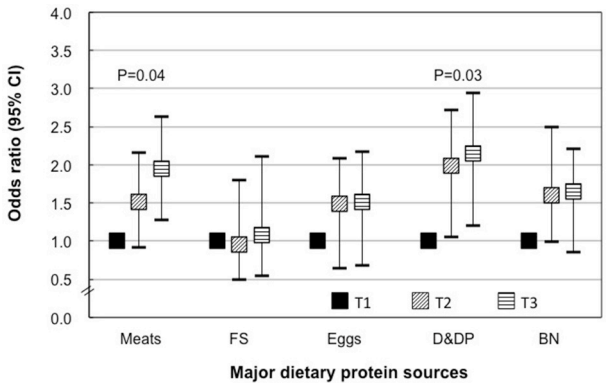

FIGURE 3 | Specific dietary protein intakes during mid pregnancy and risk of gestational diabetes. T, tertile; FS, fish and shrimp; D&DP, dairy and dairy products; BN, beans and nuts. Data shown are OR and 95% confidence interval. Models were adjusted for additionally adjusted for age, pregravid BMI, parity, family history of diabetes (yes or no), current smoking (yes or no), PCOS (yes or no), family income, total energy intake, fat intake, carbohydrate intake, and physical activity.

ingestion of meals rich in animal protein can cause a significant increase in the plasma concentration of BCAAs (branched-chain amino acids) with a notably altered metabolism as observed in conditions characterized by insulin resistance, insulin deficiency, or both (48).

Moreover, we found that higher consumption of meat and dairy products, two major dietary sources of animal protein, were associated with higher risk of GDM. Meat is a principal food source of dietary protein and its potential mechanisms may involve advanced glycation end-products (49) or gamma-glutamyl-transferase (50) with high meat intake. Dairy products have been identified as potent insulin secretagogues, as their consumption stimulates acute hyperinsulinemia (51). For patients with hyperglycemia and type 2 diabetes, hyperinsulinemia caused by consumption of dairy products may be beneficial or even protective for regulating blood glucose levels (52). However, long-term hyperinsulinemia from the consumption of dairy products may have adverse long-term effects on healthy individuals, including insulin resistance (53).

Given that the substantial relevance of GDM for medium and long-term health of both mother and offspring, the influences of dietary protein intake and animal protein intake as well as meat and dairy consumption on GDM as those observed in our study will identify the modifiable dietary risk factors and critical time window which contribute to GDM development and thus have important public health implications in view of the high GDM prevalence in the Chinese population.

Some limitations of our study should be mentioned. Southwestern China is home for many ethnic minorities. We could not examine the association in each ethnic minority group. However, involvement of such an ethnically diverse population may increase generalizability of our findings. Secondly, there is still controversy in the diagnostic criteria of GDM. In the current study, GDM was assessed according to the IADPSG criteria which has been endorsed by the American Diabetes Association

(1) and Chinese Ministry of Health (8). However, how the diagnostic criteria will affect the observed association between dietary protein intake and GDM warrants further investigation. Thirdly, assessing diet based on the memory-based questionnaire has its limitations, but we think the best which could be done in the circumstances. Instead keeping a food diary and assessing the protein and another nutrients intake would be a better way, but very cumbersome and difficult. Finally, we cannot rule out the possibility of residual confounding from unmeasured or unknown factors.

The strengths of this analysis include its prospective nature data, large sample size, high response rate, and detailed measurement of both dietary data and clinical measures in conjunction with the ability to adjust for a number of major potential confounders. Notably, unlike previous studies on the association between protein intake and GDM in only one specific period (pre-pregnancy or mid pregnancy), we carefully chose our study period to cover the time period ranging from the year preceding pregnancy to birth, which make it possible to investigate the critical time window of the impact of dietary protein intake on GDM risk. Specifically, we used the same sample for analysis of the different potentially critical periods, therefore minimizing the heterogeneity among different women. Finally, compared to the 24-h recalls, the validated FFQs might better reflect the habitual dietary intake over a period of time.

In conclusion, our study indicated that higher dietary intakes of total protein and animal protein in mid-pregnancy were associated with an increased risk of GDM among pregnant Chinese women. The findings in this study can be defined and examined by random prospective studies in the future.

AUTHOR CONTRIBUTIONS

YL performed the analyses and wrote the manuscript. YG, XZ, DY, DZ, LQ, and RZ coordinated the study centers. WB and GC supervised the analyses. GC conceived the project. All authors critically reviewed the manuscript for important intellectual content.

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GENERAL COMMENTARY
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Commentary: Research Gaps in Gestational Diabetes Mellitus: Executive Summary of a National Institute of Diabetes and Digestive and Kidney Diseases Workshop

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Keywords: gestational diabetes, metabolic programming, hyperglycemia in pregnancy, maternal outcome, fetal outcome

A Commentary on

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Diabetes mellitus diagnosed in pregnancy, or gestational diabetes mellitus (GDM), is a problem of increasing public health importance in the United States and worldwide. GDM is associated with significantly increased risks of adverse perinatal outcomes as well as an increased risk of subsequent type 2 diabetes (T2D) in the mother, and obesity in offspring (1–3). Its prevalence is also increasing with the current obesity epidemic and advancing age of motherhood (1). Furthermore, based on the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study, there appears to be a strong linear relationship between increasing maternal glucose concentrations (below those currently used to diagnose overt diabetes) and adverse neonatal outcomes without any clear inflection point (4). Data from the HAPO follow-up study at 8–12 years after the pregnancy will also soon provide further insight into the relationship between glucose levels below the current GDM diagnostic threshold and subsequent T2D in mothers and obesity in the offspring.

Importantly, many unanswered questions exist about the role of maternal glucose intolerance in fetal metabolic imprinting, and whether identifying and effectively treating maternal dysglycemia earlier in pregnancy than the current "standard of care" assessment of maternal dysglycemia at 24–28 weeks gestation would mitigate potential short- and long-term effects on the mother and/or offspring. Specifically, little is known about variations in maternal glycemia prior to 20 weeks gestation. As such, questions such as the following persist: (i) do glycemic measures (e.g., various degrees of maternal glucose intolerance as measured by oral glucose tolerance testing [OGTT], hemoglobin A1c [HbA1c], or continuous glucose monitoring [CGM]), or other biomarkers in early gestation predict GDM in later pregnancy and/or other adverse outcomes in the mother and/or the offspring; and (ii) would early diagnosis and treatment of maternal dysglycemia improve short- and long-term outcomes for the mother and her child?

To address these issues, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) at the National Institutes of Health convened an international workshop in August 2017 involving obstetricians, maternal-fetal medicine specialists, internists, and endocrinologists with expertise in GDM to address the current gaps in GDM research. Areas of particular focus included the lack of knowledge on when dysglycemia manifests during pregnancy and the

potential importance of early diagnosis of GDM, and evidence for and against different treatment strategies and therapeutic goals in the management of GDM. As summarized in a recently published Executive Summary of the workshop (5), appropriate diagnostic criteria for the diagnosis of GDM unfortunately remain poorly defined, and an effect of early diagnosis and treatment of GDM on the risk of adverse perinatal and long-term outcomes has not been well demonstrated. Furthermore, despite many small randomized controlled trials of glucose-lowering medication treatment in GDM, gaps remain in understanding how best to utilize pharmacological therapy in GDM, as evidenced by discrepancies among professional society treatment guidelines. The comparative effectiveness of insulin, metformin, and glyburide in the management of GDM also remains uncertain, particularly with respect to long-term maternal and fetal outcomes. The NIDDK workshop participants also identified additional topics in need of further research, including phenotypic heterogeneity in GDM and novel and individualized treatment approaches. Interest in the topic of pregnancies complicated by GDM (as well as pre-existing diabetes) is also highlighted by the American Diabetes Association including in its July 2018 issue of *Diabetes Care* a Special Article Collection entitled "Reconsidering Pregnancy With Diabetes."

In summary, the many unanswered questions posed above highlight some, but certainly not all, of the knowledge gaps in the field of GDM as it pertains to the mother and her offspring. As the field moves forward, filling these gaps will better elucidate our understanding of the physiology of glucose homeostasis during pregnancy, as well as our understanding of pathophysiological changes and the timing and approach of medical management of maternal glucose intolerance to mitigate short- and long-term adverse effects on the mother and the offspring. Although advances in diabetes technologies (such as CGM) have the potential to facilitate these efforts (6), their large-scale use in women with or at-risk for GDM is limited at best. However, these technological advances provide opportunities for interrogating gestational glycemia—both untreated and in response to therapy—in ways that were previously undoable or impractical. As the American Diabetes Association so aptly alluded to on the cover of its July 2018 issue of *Diabetes Care*, it is time to reconsider pregnancy with diabetes—in all facets.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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REVIEW
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Maternal Diabetes and Fetal Programming Toward Neurological Diseases: Beyond Neural Tube Defects

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The purpose of this review was to search for experimental or clinical evidence on the effect of hyperglycemia in fetal programming to neurological diseases, excluding evident neural tube defects. The lack of timely diagnosis and the inadequate control of diabetes during pregnancy have been related with postnatal obesity, low intellectual and verbal coefficients, language and motor deficits, attention deficit with hyperactivity, problems in psychosocial development, and an increased predisposition to autism and schizophrenia. It has been proposed that several childhood or adulthood diseases have their origin during fetal development through a phenomenon called fetal programming. However, not all the relationships between the outcomes mentioned above and diabetes during gestation are clear, well-studied, or have been related to fetal programming. To understand this relationship, it is imperative to understand how developmental processes take place in health, in order to understand how the functional cytoarchitecture of the central nervous system takes place; to identify changes prompted by hyperglycemia, and to correlate them with the above postnatal impaired functions. Although changes in the establishment of patterns during central nervous system fetal development are related to a wide variety of neurological pathologies, the mechanism by which several maternal conditions promote fetal alterations that contribute to impaired neural development with postnatal consequences are not clear. Animal models have been extremely useful in studying the effect of maternal pathologies on embryo and fetal development, since obtaining central nervous system tissue in humans with normal appearance during fetal development is an important limitation. This review explores the state of the art on this topic, to help establish the way forward in the study of fetal programming under hyperglycemia and its impact on neurological and psychiatric disorders.

Keywords: diabetes, pregnancy, fetal programming, neurological disorders, psychiatric disease

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INTRODUCTION

Diabetes is a group of metabolic diseases characterized by deficient insulin secretion and/or action which leads to hyperglycemia, and, in turn, to abnormal metabolism of carbohydrates, fats, and proteins in insulin target tissues (1). Depending on pathogenesis, diabetes can be classified as type 1, type 2, gestational diabetes mellitus (GDM), or other types of diabetes. Worldwide, ∼5–10%

of diabetic patients have type 1 diabetes, in which complete insulin deficiency stems from β-cell autoimmune destruction as a result of genetic susceptibility or viral antigens. Type 2 affects 90–95% of diabetic patients and is characterized by either insulin resistance or defective secretion. Other, less common, types of diabetes are associated with monogenetic defects in β-cell function, pancreatic injuries, and drug abuse, among other causes (1).

GDM is commonly diagnosed in the second or third trimester of pregnancy, and its prevalence varies between 1 and 17% depending on the studied population and the diagnostic test used (1, 2). Due to the ongoing epidemic of obesity and diabetes in women of childbearing age, the American Diabetes Association (ADA) has established that women with GDM risk factors must be tested for diabetes during the second trimester of pregnancy, using standard diagnostic criteria. Furthermore, that diabetes in women in the first trimester should be classified as type 2 diabetes (1). However, pregnant women with no risk factors can develop diabetes as early as the first trimester (3–5), making it evident that earlier testing is required in some cases.

As a result of GDM, women and their offspring confront a series of problems including fetal death, spontaneous abortion, congenital malformations, fetal-placental abnormalities, and altered fetal programming (6–8). Fetal programming is defined as the development of pathologies during childhood and adulthood that originate during fetal development (9). In this sense, maternal diabetes has been linked to offspring that develop obesity, diabetes, neurodegenerative and psychiatric diseases, as well as low intellectual and verbal coefficients, language and motor impairments, attention deficit with hyperactivity disorder, and problems in psychosocial development (10–15). However, many of these relationships remain unclear, have not been well-studied and have not been related to fetal programming (**Figure 1**).

During this review, we realized that existing information regarding the mechanisms of action between hyperglycemia during fetal development and the outcomes is limited. Most reports are focused on *in utero* hyperglycemia and neural tube defects (NTD). Also, studies on viable "normal" offspring and strategies to prevent the effects of maternal diabetes are scarce; an understandable problem given that several extrinsic and intrinsic factors (including embryo susceptibility, among others), may contribute to CNS fetal programming in specific cell types, locations, and times.

Bibliographic Search

We searched for experimental and clinical evidence regarding the effect of hyperglycemia on the development of the CNS and fetal programming related to neurological diseases, but excluding evident NTD. We searched PubMed (https://www.ncbi.nlm.nih.gov/pubmed) for studies on humans or other animals, published in English in a variety of article types (classical article, clinical study, comparative study, evaluation studies, journal article, meta-analysis, and technical report) published between 1990/01/01 and 2018/01/31. We used a combination of the following keywords: maternal diabetes or pregnancy+hyperglycemia, in combination with neurological fetal programming, neurological outcomes, fetal neural development, or neural tube.

From a total of 525 articles, 76 remained after we eliminated those that contained the phrase "neural tube defect," those that were not related to CNS development and/or function, duplicated articles, and those published in a language other than English.

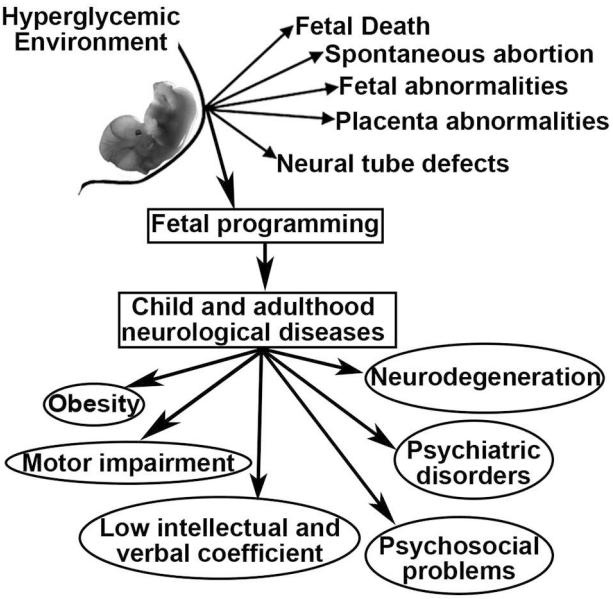

FIGURE 1 | Possible outcomes related to intrauterine hyperglycemia.

OBESITY AND DIABETES

Childhood obesity and type 2 diabetes are closely related to GDM. A systematic review that included 20 observational studies involving a total of 26,509 children showed that maternal hyperglycemia (GDM and type 1 diabetes) was associated with obesity and abnormal glucose tolerance in offspring. Interestingly, while higher body mass index was reported for the children of GDM mothers during childhood, the same was reported from prepuberty to adolescence in children from mothers with type 1 diabetes. Furthermore, offspring from GDM mothers had high 2-h plasma glucose from prepuberty to early adulthood, and those from mothers with type 1 diabetes had a high rate of type 2 diabetes from years 2 to 5 and early adulthood (16). On the other hand, the effect of diabetes during gestation in offspring can be generationally transmissive through the maternal line. Hanafi et al. (17) showed that rats with grand-maternal diabetes showed impaired glucose sensing, increased oxidative stress, insulin resistance, and impaired

Abbreviations: GDM, gestational diabetes mellitus; NTD, neural tube defects; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; AgRP, agouti-related peptide; ARC, arcuate nuclei; PVN, paraventricular nuclei; CART, cocaine and amphetamine-related transcripts; Shh, Sonic hedgehog; Nkx2.1, thyroid transcription factor 1; NSCs, neural stem cells; ASD, Autism spectrum disorder.

glucose tolerance in F1 and F2, with more prominent effects in F2.

In an effort to study the mechanisms involved in the development of obesity in offspring from diabetic mothers, the plasma content of hormones involved in food intake and energy expenditure were measured in an Austrian cohort of children with a mean age of 6 years (male:female = 36:40) born from mothers with GDM, pre-gestational diabetes, and nondiabetic women. No differences were found in the plasma content of hormones involved in food intake and energy expenditure such as ghrelin, leptin, adiponectin, neuropeptide Y (NPY), peptide YY, and growth differentiation factor 15. However, using multiple regression analysis, the authors found that body mass index, leptin, and GDF-15 had independent effects on insulin resistance (18). It is worth mentioning that both GDF-15 and leptin are synthesized by white adipose tissue; the first decreases food intake, while the latter suppresses appetite and increases energy expenditure (19, 20). Furthermore, leptin is a hormone that acts via receptors in the hypothalamus, and the activation of leptin receptors in pro-opiomelanocortin (POMC) and agouti-related peptide (AgRP)/NPY neurons within the arcuate nuclei (ARC) lead to increased AgRP and POMC expression reducing food intake (19, 21). The absence of changes in plasma leptin in obese children from GDM, but its relation with body mass index may be explained through an impaired function in the leptin receptor expressed in the CNS, a phenomenon called, leptin resistance.

POMC is the precursor of α-melanocortin stimulating hormone, which in turn (through the activation of type 3 and 4 melanocortin receptors) is the most important component of the network responsible for controlling appetite, energy expenditure, glucose homeostasis and lipid metabolism in the hypothalamus (19, 22, 23). This peptide functions as an anorexigenic factor in ARC and paraventricular nuclei (PVN), predominantly reducing appetite (24). AgRP and NPY are orexigenic factors inducing hyperphagia and obesity (25–27).

The interaction between peripheric leptin and the melanocortin systems in the ARC and PVN is essential for the circuits that regulate food intake, energy expenditure, glucose, and lipid metabolism. Furthermore, it can be suggested that changes in hypothalamic fetal ontogenesis could be taking place in the diencephalon exposed to hyperglycemia, affecting the postnatal function of hypothalamic circuits because of the effects reported at early and late development in offspring from GDM and Type 2 diabetes mothers on leptin level, glucose homeostasis and BMI, mentioned above (16, 18). Indeed, several studies have shown that high glucose levels promote an inadequate organization of hypothalamic ARC and PVN, as well as malformation of the ventromedial hypothalamic nucleus in rats (28–31). Moreover, chicken embryos exposed to high glucose concentrations (30 mM) have lower glucose tolerance in neurons located in the hypothalamic infundibulum the equivalent anatomic area of the ARC (32).

On the other hand, the expression of hypothalamic neuropeptides in sheep ARC exposed to intrauterine hyperglycemia increases the expression of POMC and the cocaine and amphetamine-related transcripts (CART) at 81 and 140 days of gestation (33, 34). Such changes may affect energy balance regulation in later life, affecting food intake and energy balance. To our knowledge, there is no postnatal evidence of an altered pathway for central energy balance in sheep, but there are some clues provided in the murine model. Offspring from diabetic rats and mice have increased susceptibility to body weight dysregulation and obesity due to: increased expression of the orexigenic (NPY and AgRP) and decreased expression of the anorexigenic (α-MSH) peptides in the ARC (30, 35), leptin resistance in 10-day old pups, decreased fiber density of AgRP and α-MSH peptides, as well as in the PVN, and increased food intake and body weight (31). These findings suggest that offspring born from diabetic dams showed leptin resistance in first-order neurons within the ARC, less synaptic transmission into the PVN and, consequently, obesity (**Figure 2**).

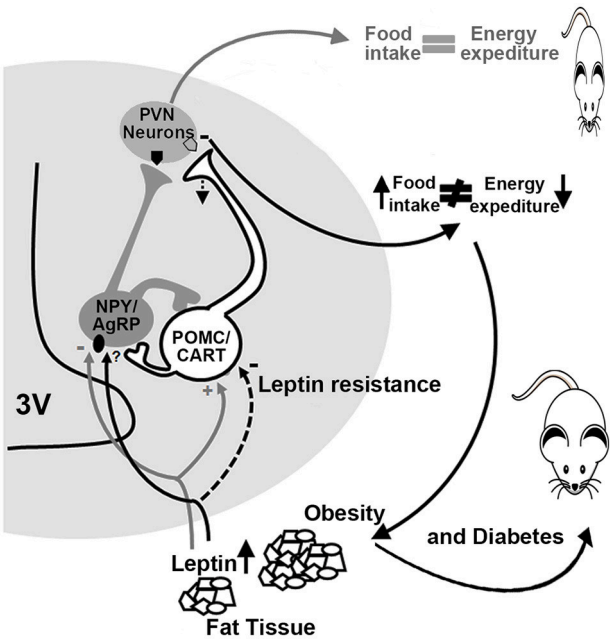

FIGURE 2 | Altered energy and food intake balance in diabetic dams offspring. In normal conditions (gray lines and arrows), leptin activates POMC/CART neurons (pro-opiomelanocortin/cocaine- and amphetamine-regulated transcript) and inhibits NPY/AgRP neurons (neuropeptide Y/agouti-related protein) promoting satiety and a balance between food intake and energy expenditure. In diabetic offspring (black lines and arrows), a decrease in the activation of POMC/CART neurons takes place due to leptin resistance, which reduces melanocortin release at the PVN (paraventricular nuclei), promoting an increase in food intake leading to obesity and diabetes. 3V, third ventricle.

Altered levels of morphogens and transcription factors important in hypothalamic organization may affect hypothalamic fetal development under hyperglycemic conditions. During early fetal development, the hypothalamus emerges from the diencephalon, and its adequate formation depends on the precise regulation of molecular and cellular mechanisms orchestrated by regional morphogenetic organizers in the neural tube. The diencephalon is separated from surrounding regions by the influence of organizing signals (morphogens) such as

Wingless/integrins, Sonic hedgehog (Shh), bone morphogenetic proteins and fibroblast growth factors, and later by the expression of the thyroid transcription factor 1 (Nk×2.1, a downstream gene of Shh) and a key factor for hypothalamic neuron development (36, 37).

Interestingly, Shh has been evaluated by *in situ* hybridization and qRT-PCR during brain development in embryos from streptozotocin-induced diabetic mice, at embryo day (E) 8.5 and E11.5. Normally, at E8.5 Shh expression is restricted to the ventral medial plate in the forebrain, but its domain is expanded and its expression increases in embryos from diabetic mice. At E11.5 in control animals, Shh expression is localized in the ventral diencephalon and telencephalon, but in the diencephalon from diabetic animals its expression is stronger and expanded toward the dorsal telencephalon. Thus, there is an increased and expanded expression of Nk×2.1 to the telencephalon (38). In addition, an increase in cell proliferation and neurogenesis has been reported in ventral telencephalon/diencephalon in E11.5 to diabetic mice (39).

These data suggest that early changes in the ventral-dorsal patterning and increased neurogenesis are contributing to defects in the fetal and postnatal hypothalamus in murine, chicken and sheep models; an aspect worthy of further study.

COGNITION

Cognition is involved in the regulation of emotional and social cues, including the formal measures of intelligence such as memory and attention. Cognitive functions studied in infants from diabetic mothers and offspring from different animal models include language (animal communication), learning, memory, motor coordination, perception, and problem-solving. All of these are functions that are coordinated in a complex manner by different anatomical structures such as the cerebral cortex, amygdala, hippocampus, and basal ganglia. However, the relationship between diabetes during pregnancy and impaired cognitive function after birth remains controversial. Some studies in humans have shown that maternal diabetes contributed to cognitive dysfunction in school-age children, which has been associated with changes in cell migration and differentiation during brain development (12, 40, 41). For example, Ornoy et al. (12) reported that children under 9 years old born to GDM women had lower scores in verbal tasks and fine and gross motor skills. Bolaños et al. (42) found that *in utero* hyperglycemia was associated with a lower average IQ and poor performance in working memory skills, such as graphic and visuospatial tasks, in children from 7 to 9-years-old born to control and GDM women: They concluded that GDM leads to minor neurological deficits in children (42).

Working memory is the result of proper coordination between the prefrontal cortex and hippocampus; structures that arise from the dorsal telencephalon during embryo development.

Hyperglycemia during hippocampal development decreases synaptic plasticity and reduces memory durability in male rats (43, 44), presumably due to a delay in normal hippocampal development regulated by insulin and insulin growth factor-1 receptors that lead to structural, behavioral, and cognitive abnormalities (45).

It is worth mentioning that the hippocampus is very vulnerable to several neurotoxic insults, including fetal hypoxia and iron deficiency, both of which are phenomena reported in fetuses from diabetic mothers (46–49). One way to evaluate early postnatal hippocampal function is through the measurement of event-related potentials (ERPs) generated by visual or auditory stimuli. ERPs are divided into two categories: sensory, which are early waves, peaking within the first 100 ms after the stimulus, and cognitive, which are waves generated later due to information processing (50). Using this tool, recognition memory (visual and auditory stimuli) was evaluated in 6-month-old infants from diabetic mothers, finding robust evidence of a memory deficit (51, 52).

During embryo development, the cerebral cortex and hippocampus arise from the dorsal telencephalic neuroepithelium. As in other areas of the CNS, intrinsic and extrinsic factors coordinate correct patterning during development by promoting the self-renewal of neural stem cells (NSC). These then will specify into neurons, astrocytes, and oligodendrocytes, to develop the functional areas of the cerebral cortex and the characteristic hippocampus anatomy. Diabetes during pregnancy in mice promotes changes in the expression of proliferative and differentiative related genes during brain development. However, NTD embryos or complete litters have been used, which makes it difficult to relate changes in gene expression during fetal development and postnatal impaired cognitive functions in offspring without NTD. Moreover, contrasting data have been reported regarding the effect of hyperglycemia on embryo cell proliferation, differentiation, and survival. Thus, an increase in cell proliferation without affection on cell death, which promotes thickening and deformation of the dorsal telencephalon in embryos to diabetic mice, was reported at E11.5. However, increased BrdU incorporation was not observed (38, 39). On the other hand, there are several reports which suggest increased cell death using the same model and embryo data (53–57). Despite some discrepancies, changes in cell proliferation, migration, and differentiation may be taking place in a different and complex manner, and even depending on the embryos susceptibility. Thus, researchers need to separate non-NTD from NTD embryos in order to define both shared and different mechanisms, and, in turn, propose and establish specific prevention, treatment, and diagnosis strategies beyond NTD.

Even though the telencephalon is the most studied structure during development in health and disease, few studies have analyzed the effect of hyperglycemia in embryos with "normal" (non-NTD) development. However, the use of cortical NSC obtained from the dorsal telencephalon can be a useful tool to study the effect of hyperglycemia. Fu et al. (39), studied the effect of high glucose (30 mM) on the proliferation and differentiation of E13 cortical NSC from normal pregnant mice, and showed that high glucose in proliferative NSC promoted increased cell death and reduced cell proliferation,

together with increased neuron, astrocyte, and oligodendrocyte differentiation in differentiated cells. They concluded that NSC cultured in high glucose led to cell-cycle arrest and apoptosis and influenced lineage specification, through a mechanism in which Shh, Bmps and the Notch/Delta pathway are involved (39).

The effect of high glucose on cell phenotype must be more complex, and other factors may be involved, such as micro RNAs (miRNAs, molecules with a potential use in therapeutic as well as noninvasive biomarkers), neurotransmitters and epigenetic mechanisms (chromatin modification, histone methylation, or acetylation and DNA methylation). The level of telencephalic development-related miRNAs was evaluated in serum from control and GDM women whose fetuses were not diagnosed with NTD. Interestingly, GDM led to higher levels of proliferative and neurogenic miRNAs (miR-183-5p, miR-200b-3p, miR-125-5p, and miR-1290). Moreover, gene ontology *in-silico* analysis revealed alterations in cell proliferation and neuron differentiation (5). Furthermore, an array study containing ∼2,383 probes was used to analyze the *in vitro* and *in vivo* effects of high glucose in mice forebrain NSC. Results showed that high glucose deregulated 104 and 25 miRNAs *in vivo* and *in vitro,* respectively. Neurogenic miRNAs: miR-30 family, miR-125-5p, miR-124, and miR-128 were upregulated under both conditions (58). Furthermore, miR-183, considered a proliferative miRNA, was downregulated in NSC obtained in embryos from diabetic mice (59, 60). Although the authors focused on the miRNA-30 family, because it is known to be involved in schizophrenia, ASD, axon extension and guidance, and other neurodevelopmental disorders (61–63), it is clear that changes in miRNAs expression may have an extensive regulatory effect. One miRNA may regulate hundreds or thousands of RNAm, which may, in turn, affect cell proliferation, differentiation, migration, and death, through a complex network of epitrascriptomic regulation occurring in parallel.

The expression of miRNAs under hyperglycemic conditions may be regulated by epigenetic factors. To explore this circumstance under high glucose *in utero* and *in vitro,* Shyamasundar et al. (64) isolated E13.5 dorsal telencephalic tissue from diabetic and control pregnant mice litters or NSC from control mice that were maintained under normal or high-glucose conditions during 48 h (40 mM). They reported that high glucose *in vivo* and *in vitro* alters chromatin reorganization due to an increase in histone H3K9 trimethylation and global DNA methylation, and provokes a decrease in histone H3K9 acetylation. Decreased gene expression due to high glucose was expected because H3K9 can turn genes on by becoming acetylated, and can silence when methylated. However, the authors reported that high glucose in NSCs in embryos from diabetic mice promoted a significant increase in the expression of doublecortin and Pafah1b1 (Platelet-activating factor acetylhydrolase isoform 1b, subunit 1), molecules essential for neuron migration and differentiation; this effect could be explained because no change in the CpG methylation status of the gene promoter was observed (55, 64, 65). They further determined that the decreased miR-200a, miR-200b, miR-466a-3p, and miR-466d-3p miRNAs were responsible for the changes observed in Dcx and Pafah1b1 (64), suggesting the epigenetic regulation of these miRNAs. Interestingly, a lower level of mir-200b was reported in serum samples from diabetic pregnant women at the third trimester, with a negative diagnosis for NTD (5).

Neurotransmitters may also contribute to increased neurogenesis observed in the cortical neuroepithelium of embryos under hyperglycemia with non-NTD. Our group reported that histamine increases cortical and mesencephalic NSC neurogenesis through H_1-receptor (H_1R) activation. Both this neurotransmitter and H_1R showed a significant increase in the cortical neuroepithelial of embryos from diabetic pregnant rats (66–68). In mammals, histamine is a neurotransmitter/neuromodulator in the adult brain, acting through G-protein coupled receptors [H_1R, H_2RH_3R, and H_4R] (69, 70). During cortical development, histamine displays high concentration as well as a high expression of H_1R and H_2R (71–73). On the other hand, in cortical NSC, HA promotes cell proliferation and neuron differentiation through H_1R and H_2R activation, respectively (66, 74–76).

In the diabetic model, the cortical neuroepithelium of embryos without NTD showed increased neurogenesis (E14) as well as histamine concentration (E14) and H_1R expression (E12). Interestingly, the systemic administration at E12 of chlorpheniramine (H_1R antagonist/inverse agonist) partially prevented increased dorsal telencephalic neurogenesis in embryos from diabetic rats, suggesting the participation of this receptor in the impaired neurogenesis observed in embryos from the diabetic model (68). The relevance of the above findings is also supported by evidence on the effect of antihistamine drugs on controlling glycemia under diabetic conditions (77).

MOTOR BEHAVIOR

Early school-age children born from mothers with GDM present motor impairment (12). As with cognition, several regions of the brain control motor activity, including the cerebral cortex and the cerebellum. As mentioned above, the dorsal telencephalon showed increased neurogenesis during fetal development. However, to our knowledge, postnatal studies on the anatomy and functional neurochemistry of the motor cortex have not been reported. In contrast, we found a few studies regarding the effect of hyperglycemia during embryo development and its effect on the cerebellum. A reduction in cerebellum size has been observed, which correlates with a decreased number of Purkinje and granular cells, and the reduced size of the molecular and granule cell layers in offspring of the streptozotocin-induced diabetic model in rats (**Figure 3**) (78, 79). Under these conditions, an increase in the synaptic length and dendritic spine was detected at postnatal days 30 and 70 (78), which suggests that the reduction in cerebellum size is compensated by an increase in the size of the dendritic spine. Nevertheless, it may also indicate inadequate cerebellar synaptic maturation (80).

Although postnatal cytoarchitectonic changes are reported in diabetic offspring, to our knowledge there is no evidence that

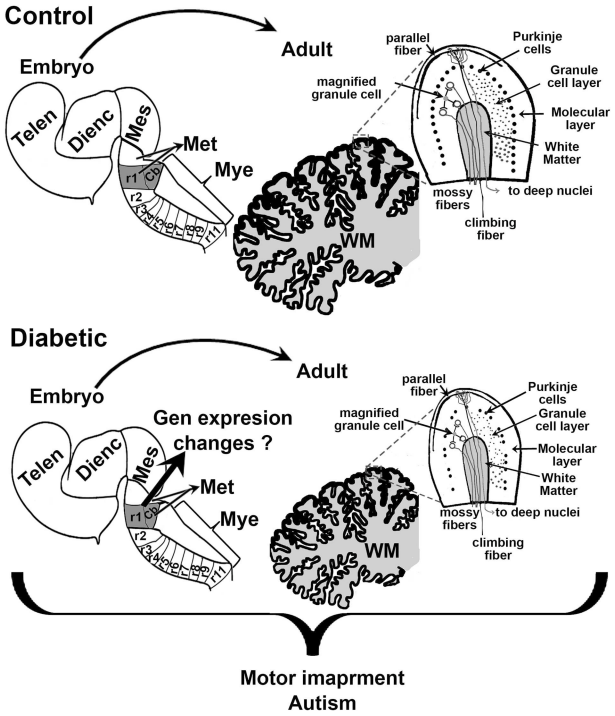

FIGURE 3 | Schematic proposal of the effect of maternal diabetes on embryo and offsprings cerebellum. Sagittal views of the embryonic neural tube and adult cerebellum in control **(top)** and diabetic **(bottom).** r1 indicates the rhombomere (rb) from which the cerebellum (Cb) rise. The scheme shows that embryos from diabetic animals present a smaller r1 and that this may reduce the size of the Cb, and the number of Purkinje and granular cells in the adult. Telen, telencephalon; Dienc, diencephalon; Mes, mesencephalon; Met, Metencephalon; Mye, Myelencephalon; WM, white matter.

could explain the postnatal changes in the cerebellum during fetal development.

PSYCHIATRIC DISORDERS

Autism and schizophrenia have been related to maternal diabetes. Autism spectrum disorder (ASD) is a neurodevelopmental disorder with an estimated prevalence of 1 in 68 children in 2016 (81). ASD is characterized by stereotyped behavior, severe social dysfunction, restricted attention and language impairments (82–86). Although ASD has a highly heritable factor (86), stressful environment *in utero* has also been implicated (83, 84, 86–91). In 2009, a two-fold increase in the risk of ASD in infants from GDM mothers was reported. However, authors have concluded that infections during pregnancy, maternal age, hypertension, and preeclampsia contribute to the incidence of the disease (92). This is supported by other studies that reported no relationship between GDM and risk of ASD (93, 94). However, a cohort study of 66,445 pregnancies examined the relationship of obstetric complications with ASD, finding that GDM presented a significant risk increment in the pathology of 1.2% of the children (89). A case-control study also reported that children from GDM mothers had a high incidence of ASD with expressive language deficits (84). Discrepancies among studies may be due to differences in the study design and ethnic populations.

Alterations in the cerebellum have also been related to autism (95–97). One of the most consistently abnormal findings in the postmortem brains of autistic individuals, regardless of age, sex, and cognitive ability is the significant decrease in the number of Purkinje cells in the posterolateral-neocerebellar and the adjacent archicerebellar cortices (98, 99). Both Yamano et al. and Razi et al. have shown that *in utero* hyperglycemia reduces the size of the cerebellum due to a reduction in the number of Purkinje and granular cells (78, 79). Although changes in cerebellum architecture in autism have been observed, the physiological relevance of these relationships remains unsolved, probably due to the clinical heterogeneity within the broad behavioral phenotype (100).

Genetic studies have revealed three promising ASD-implicated genes: engrailed homeobox 2 (EN2), gamma-aminobutyric acid type A receptor beta3 subunit (GABRB3), and MET proto-oncogene, receptor tyrosine kinase (MET). All have specific roles in cerebellar development (101, 102). The expression of En2 is restricted to midbrain and cerebellum. The loss of its function causes abnormal cerebellar foliation, with deficits in motor and social behavior (102). Interestingly, in embryos from diabetic mice a decrease in the expression of En2 has been reported (56, 103).

Several studies have shown a positive association between oxidative stress during fetal development and ASD, suggesting that oxidative damage is an important factor in the etiology of ASD (104, 105) and schizophrenia (106, 107). The release of reactive oxygen species (ROS) and the generation of oxidative stress under GDM is a critical aspect that may contribute.

On the other hand, schizophrenia is a chronic and severe psychiatric incapacitating disorder that affects a wide range of cognitive, emotional, and motor functions that have been associated with GDM. The symptoms include hallucinations or paranoid delusions, disorganized speech, and socialization, cognition, and memory impairment (108). The etiology of this disorder is unknown. However, epidemiological evidence suggests prenatal and perinatal complications as antecedents (109–112).

In a Swedish case-control cohort study, an increased susceptibility of developing schizophrenia in females from GDM mothers was reported, suggesting a gender susceptibility (113). A meta-analysis study reported that among the obstetric complications for schizophrenia, GDM was found to have a significant participation (114). Other studies described that there is no evidence of the association between GDM and the disease (109, 115). This inconsistency could be explained by the fact that most studies used questionnaires or scales where GDM or a history of maternal diabetes were not included.

On the other hand, it has been suggested that an increased level in plasma of pro-inflammatory cytokines such as IL-8 (interleukin-8) and tumor necrosis factor-α (TNF-α) during pregnancy could be related to offspring schizophrenia in adulthood (116). Thus, a hyperglycemic condition increases the expression of inflammatory cytokines in macrophages

by decreasing H3K9me3 levels in inflammatory cytokines promoters, such as TNF-α, and chemoattractant protein-1 and IL-6 (117).

Changes in the DNA methylation pattern have been reported in term placenta and cord-blood samples from GDM. Interestingly, the analysis showed that 57 genes associated with schizophrenia or schizoaffective disorders were affected (118). Moreover, several lines of evidence have shown that alterations in the degree of DNA and histone methylation are related to ASD and schizophrenia (119–122). All these data suggest that epigenetic changes during GDM may be related to offspring schizophrenia. A "two-hit" hypothesis has been proposed for this and other diseases, where the onset of disease cannot clearly be linked to a specific genetic or environmental insult. This is also applicable to ASD. For schizophrenia, it has been hypothesized that the first hit may affect neurogenic and cell specification pathways, while the second hit may have a greater effect on functional integration (123), two processes that have been suggested to be altered in embryos and early born diabetic animals (31, 38, 39, 56).

CONCLUSION

Morphological and functional alteration in the CNS and its relationship with changes in gene expression and maternal diabetes is complex. Particularly because other phenomena may be participating in the final function of a gene or group of genes, such as post-transcription, translation, or post-translational regulatory processes that define cell commitment, differentiation, migration, death, integration, and function during specific moments in development.

Although fetal programming due to maternal diabetes may be evident, and a lot of information regarding the intrinsic and extrinsic factors that participate in the development of several areas of the CNS has been generated and related to NTD, we have not developed proper experimental protocols to generate information related to how hyperglycemia affects the development of specific areas of the CNS during critical time windows. It is also clear that, depending on the timing of insult in relation to the stages of brain development, different cell populations may be selectively affected. Furthermore, it is likely that early defects may be exclusively due to hyperglycemia, while defects occurring in late development may be due to high glucose and/or hyperinsulinemia. Finally, it is important to bear in mind that the problem is complex, and that no single molecular mechanism can fully explain the effects of maternal diabetes on fetal programming during neurodevelopment. Global approaches are needed.

AUTHOR CONTRIBUTIONS

All the authors contributed in the same way for the realization of this review. Additionally, AM-H and ND reviewed and edited the final version.

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Controversies in Screening and Diagnostic Criteria for Gestational Diabetes in Early and Late Pregnancy

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This review serves to evaluate the screening and diagnostic strategies for gestational diabetes and overt diabetes in pregnancy. We focus on the different early screening and diagnostic approaches in first trimester including fasting plasma glucose, random plasma glucose, oral glucose tolerance test, hemoglobin A1c, risk prediction models and biomarkers. Early screening for gestational diabetes is currently not recommended since the potential benefits and harms of early detection and subsequent treatment need to be further evaluated in randomized controlled trials.

Keywords: gestational diabetes (GDM), screening, diagnostic criteria, pregnancy, early biomarkers

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INTRODUCTION

Gestational diabetes mellitus (GDM) is known to manifest in the second half of pregnancy in the setting of profound physiologic insulin resistance. Therefore, GDM is normally diagnosed after 24 weeks of gestation. The screening and diagnosis of GDM vary widely between medical specialties and among countries. Controversial areas surrounding screening for GDM include recommendations not to screen at all, a universal vs. a risk-based, selective approach, optimal timing of screening, the appropriate screening method [fasting plasma glucose (FPG), random plasma glucose (RPG), glucose challenge test (GCT)], or criteria for diagnosis (1 or 2 step, 75 vs. 100 g glucose load, whether 1 or 2 abnormal values are required for the diagnosis) and the appropriate cut-off values. Furthermore, there are debates concerning the relevance of treating additionally diagnosed, milder forms of GDM and about the cost effectiveness of different screening or diagnostic strategies. This article provides an update on screening and diagnostic strategies for GDM and overt diabetes. Furthermore, we will discuss the latest developments regarding early detection of GDM in the first trimester.

THE LONG-LASTING WAY OF DEVELOPING SCREENING AND DIAGNOSTIC CRITERIA

After almost six decades of research and tremendous effort to reach a consensus a globally and uniformly accepted guideline regarding how and when to screen and diagnose GDM is still not available. The original criteria were established based on the 3-h 100 g OGTT by O'Sullivan and Mahan in 1964 and predicted women who were most likely to develop type 2 diabetes mellitus (T2DM) later in life after pregnancy (1). But consecutive studies could show that even lesser degrees of hyperglycaemia were associated with an increased risk of adverse perinatal outcome, including large for gestational age fetuses, shoulder dystocia, neonatal hypoglycaemia, increased risk of cesarean section or hypertensive disorders (2–5). Subsequently, the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) study reported a linear continuous relationship between

maternal hyperglycaemia and perinatal adverse outcome, making it difficult to define clear diagnostic thresholds (6). Based on these results, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) developed a new guideline in 2010 recommending a universal one-step diagnostic test using the OGTT 75 g between 24 and 28 weeks of gestation with only one value to be considered as abnormal (7). Using the new criteria, the prevalence of GDM increased in the HAPO study to approximately 18%, but varied widely among the different population demographics. For example, the prevalence in the HAPO study ranged between 9.3 and 25.5% dependent on the participating center (8). The new diagnostic thresholds have significant impact on costs and on infrastructure capacity. But many experts justify the criteria and the increase in workload in the background of the globally mounting burden of T2DM (9). The IADPSG thresholds were accepted by many health care organizations such as the WHO in 2013 and are now referred to as the 2013 WHO criteria (10). But the debate about screening and diagnostic criteria still goes on. The American Diabetes Association (ADA), which endorsed the IADPSG criteria in 2011, amended their guideline in 2014 and now considers both approaches (the one-step 75 g OGTT and the two-step screening: GCT followed by a 100 g OGTT if abnormal) acceptable for GDM diagnosis (11). The ADA states that there are insufficient data to demonstrate the superiority of one screening and diagnostic approach over the other, as—using the 2013 WHO criteria—the impact on costs and short and long term outcome of mother and her offspring have not been adequately evaluated. In their updated guideline from 2018, the American College of Obstetricians and Gynecologists (ACOG) recommends the two-step screening approach using the Carpenter and Coustan or the National Diabetes Data Group criteria and states however that "individual practices and institutions may choose to use the IADPSG recommendations" (12) The International Federation of Gynecology and Obstetrics (FIGO) acknowledged the problems with varying resource settings in different regions in their guideline from 2015 (13). The 2013 WHO criteria are generally recommended but the performance of OGTT vary depending on local circumstances. Diagnosis should be based on results from venous serum or plasma, but the use of plasma-calibrated handheld glucometers may be acceptable in locations where laboratory support is unavailable. While the 2013 WHO criteria are becoming more widely accepted, the main diabetes and obstetric societies still struggle to find the ideal algorithm. Large-scale randomized controlled trials studying the impact of intervention on women who meet different GDM criteria and evaluating the cost effectiveness of changes in short- and long-term outcomes might help solve these problems.

SCREENING FOR OVERT DIABETES IN PREGNANCY AND RATIONALE FOR EARLY GDM SCREENING

Many health organizations recommend to test for overt diabetes in women at high risk at the first prenatal visit (7, 11). Women with overt diabetes in pregnancy suffer from a higher rate of vascular dysfunction. They have a higher risk of congenital malformation and a significantly increased risk of adverse pregnancy outcome (14). These women benefit the most from early treatment. However, early testing will also lead to the identification of hyperglycaemia under the threshold for overt diabetes in pregnancy. Like screening and diagnosis of GDM in late pregnancy, there is also no consensus on the diagnostic criteria for "early" GDM. Many experts do not recommend screening for GDM in the first trimester at all, as no valid data exists about the benefits and harms of diagnosing and treating GDM in early gestation (15). The aim of early testing would be mainly to identify women at low or high risk for GDM. This risk stratification would diminish the need for universal screening and diagnosis from 24 weeks onwards and would reduce workload and costs. The second goal would be to identify women who already have GDM and to start treatment as early as possible to adequately ameliorate the adverse short and long-term effects of prolonged intrauterine exposure to hyperglycaemia. Maternal hyperglycaemia occurring even before diagnosis of GDM after 24 weeks of gestation seems to already increase the rate of fetal growth (16) and—if treatment starts after 26 weeks—infant adiposity (17, 18). Recent studies suggest a long term risk for the offspring for T2DM and cardiovascular disease. GDM seems to influence DNA methylation involved in energy metabolism and anti-inflammatory processes. This "metabolic programming" might be modified by later intervention during pregnancy, but this needs to be elucidated in future studies. After assessment of GDM in first trimester—theoretically by now—early intervention would be of benefit for women who might be at highest risk for adverse pregnancy and long-term outcomes.

METHODS FOR EARLY GDM SCREENING

Many different methods have been evaluated for the screening of GDM in early pregnancy. There are direct glycaemic markers such as FPG, RPG, GCT, and/or OGTT, indirect methods like glycosylated hemoglobin A1c (HbA1c) or fructosamine and newer biochemical markers, many of which have been derived from proteomic or metabolomic analyses. We discuss the most promising approaches in detail below.

Fasting and Random Plasma Glucose in Early Pregnancy

Most health care organizations agree that screening for pre-conceptionally undiagnosed diabetes during pregnancy is recommendable, especially in high risk populations (19). Accordingly, the main advantage of FPG is its usefulness in diagnosing overt diabetes already at the first antenatal visit using standard diagnosis criteria [i.e., if FPG exceeds 125 mg/dl (6.9 mmol/l)] (7, 13, 20). Although the IADPSG consensus panel recommended in 2010 that GDM could be diagnosed by FPG concentrations between 92 and 125 mg/dl (5.1 and 6.9 mmol/l) at any time during gestation (including the first trimester), as well, this approach was criticized due to lack of evidence (20). First, the IADPSG thresholds considered diagnostic for GDM were derived from the HAPO study, where FPG and OGTT glucose

levels were assessed in the late second and early third trimester (6). Moreover, one study from Asia showed a continuous fall of FPG values during first trimester (21). Hence, the IADPSG cut-offs are not necessarily applicable at earlier gestational periods (9). Second, there are no randomized clinical trials available supporting any benefit of treating GDM diagnosed before 24 weeks of gestation (19). Due to these concerns, some authors suggested that the use of the IADPSG threshold for FPG is not justified in early pregnancy and IADPSG representatives issued a statement in 2016 to discontinue use of the FPG threshold (15). However, higher first trimester FPG levels might be regarded as an independent risk factor for later GDM development, comparable to pre-gestational BMI (22). Indeed, previous studies indicated an association between first trimester FPG and GDM manifestation between 24 and 28 weeks of gestation by using the IADPSG cut-offs, with concordance measures (area under the receiver operating characteristic curve, ROC-AUC) ranging between 0.614 (23) and 0.654 (21), respectively. Of note, one recent retrospective study suggested that RPG assessed between 12 and 16 weeks is able to predict GDM according to various diagnostic criteria with an ROC-AUC of 0.80 (24). This seems to be surprisingly high compared to the concordance measures observed for FPG. Although results are conflicting (previous studies reported a less optimistic ROC-AUC of 0.69 for RPG assessed between 24 and 28 weeks of gestation) and RPG might be affected by several pre-test conditions such as food intake, it has several advantages regarding time and cost effectiveness (24, 25). Thus, future research including prospective confirmatory studies are necessary.

OGTT in Early Pregnancy

An early OGTT (using an oral glucose load of 75 g glucose dissolved in 300 ml water) before 24 weeks of gestation could be also used for diagnosing overt diabetes if the 2-h plasma glucose level exceeds 199 mg/dl (11.1 mmol/l) according to FIGO and WHO guidelines (10, 13). Regarding diagnosis of GDM before 24 weeks of gestation the same concerns might be valid as discussed above for FPG. However, a recent study found that women meeting the IADPSG cut-offs already early in gestation showed impaired insulin sensitivity, which was partly explained by a higher degree of obesity in these patients (26). In accordance with these results, Lapolla et al. observed impaired insulin sensitivity in patients with early GDM diagnosis using the Carpenter-Coustan criteria (27).

Glycosylated Hemoglobin A1c in Early Pregnancy

HbA1c can be also used to detect overt diabetes at the first antenatal visit [≥6.5% (48 mmol/mol)] according to current guidelines. The test should be performed in a laboratory using a NGSP (National Glycohemoglobin Standardization Program) certified method standardized to the DCCT (Diabetes Control and Complication Trial) assay (20). Comparable to RPG, HbA1c has the advantage that it is inexpensive and does not require the fasting state. Fong and co-workers assessed its predictive performance for GDM progression in a retrospective cohort study which concluded that HbA1c levels between 5.7 and 6.4% (39–46 mmol/mol) could effectively identify patients at highest risk of developing GDM (28). A further study from New Zealand indicated that HbA1c ≥5.9% (41 mmol/mol) is highly predictive for pre-existing diabetes and adverse pregnancy outcomes (29). In addition, another study from Switzerland concluded that all pregnant women with first trimester HbA1c ≥6.0% (42 mmol/mol) developed GDM later in pregnancy, whereas those with HbA1c <4.5% (26 mmol/mol) did not (30). Conversely, Agarwal et al. found that the ROC-AUC of HbA1c assessed between 24 and 28 weeks was 0.54 and concluded that HbA1c remains a poor screening test for GDM using the WHO 1999 criteria (31). It might be of importance that HbA1c is subjected to pregnancy specific changes (32), requiring trimester specific reference values (33). Moreover, data from women after pregnancy with GDM indicated that HbA1c in the pre-diabetic range is a weak surrogate for the underlying pathophysiological components of impaired glucose metabolism, including impaired insulin action and β-cell dysfunction (34). Hence, HbA1c might be inferior to other tests for detecting subtle alterations in glucose metabolism.

Other Biochemical Markers

The current "gold standard" OGTT has a low reproducibility, is time consuming, unpleasant for some patients, dependent on ethnicity and the amount of glucose is given without consideration of maternal BMI (35). Therefore, the search for a simple, non-fasting point-of-care test or a risk model incorporating biomarkers (for a summary of risk models incorporating biomarkers ± maternal factors see **Table 1**) seems to be a logical consequence. Many biochemical markers in the first trimester have been evaluated (**Figure 1**), but often only in small case-control observations without further prospective validation. Some biomarkers such as fasting insulin, inflammatory markers such as C-reactive protein (CRP) or tumor necrosis factor-α (TNF-α) or soluble (pro)renin receptor failed to provide additional information about GDM risk beyond the risk assessment by clinical risk factors such as maternal BMI (47, 49, 50).

The currently available marker, placenta growth factor (PlGF), is highly expressed by the placenta. Low PlGF levels in early pregnancy as a sign of poor placentation were observed in women with preeclampsia and/or intrauterine growth restriction (IUGR) and PlGF is now widely used for predicting preeclampsia at the time of the aneuploidy screening in 11–14 weeks of gestation (51). In a small case-control study, Eleftheriades et al. could show an increase of PlGF in early pregnancy in women with GDM compared to unaffected pregnant women (37). A risk prediction model with maternal factors alone could be improved from an AUC of 0.73 to 0.77 by the addition of PlGF but not pregnancy associated plasma protein-A (PAPP-A). On the other hand, a large prospective cohort study from the UK with over 31,000 recruited women showed only little advantage in incorporating PlGF and PAPP-A to a risk model which included maternal factors (AUC 0.84) (36). Conflicting results also exist for PAPP-A. Some studies report that PAPP-A levels are decreased in early pregnancy in women who subsequently developed GDM (52–55), others show no

TABLE 1 | Summary of biomarkers tested in a multivariate model for later development of gestational diabetes.

Biomarker models	Gestational age (weeks)	Source	Population		Study design	Diagnostic criteria for GDM	Test performance measures	References
			GDM	No GDM				
MF, PAPP-A, PlGF	11–13	Serum	787	30.438	Prospective cohort	WHO 1999	AUC 0.841, DR 58% at FPR 10%, (PAPPA and PlFG added no advantage)	Syngelaki et al. (36)
MF, PlGF, PAPP-A	11–14	Serum and Plasma	40	94	Case-control	WHO 2013	AUC 0.77, DR 34.3% at FPR of 10% (no difference of PAPP-A in both groups)	Eleftheriades et al. (37)
MF, PAPP-A, free β-HCG	11–13	Serum	248	732	Case-control	ADIPS 1998	AUC 0.90, DR 73.8% at FPR 10%	Sweeting et al. (38)
MF, triglycerides, lipocalin-2, PAPP-A, Leptin, Adiponectin, PAI-2	11–13	Serum	248	732	Case-control	ADIPS 1998	AUC 0.91, DR 76.8% at FPR 10% (Leptin, Adiponectin, and PAI-2 added no advantage)	Sweeting at al. (39)
MF, PAPP-A, adiponectin, PP13, endoglin	11–13	Serum	12	60	Case-control	WHO 2013	DR 63.6% at FPR of 10%, + BMI increased DR to 73% (no differences of PP13 or endoglin in both groups)	Farina et al. (40)
CRP, 1.5 AG, adiponectin, SHBG	6–15	Serum	46	178	Prospective high-risk cohort	WHO 2013	AUC of 1.5 AG 0.61; adiponectin (<8.9 ug/ml) OR (3.3 95%CI 1.65–9.67), SHBG had no link to GDM when corrected for BMI, ethnicity, or family history	Corcoran et al. (41)
MF, adiponectin, leptin	6–14	Serum	107	2483	Prospective cohort	OGTT 7 g 2 h >9 mmol/l	AUC 0.81 DR 44% at FPR of 10%	Thagaard et al. (42)
MF, adiponectin, SHBG, follistatin-like 3	11–13	Serum	80	300	Case-control	WHO 1999	AUC 0.84, DR 58.6% at FPR 10%, (no difference of follistatin like-3 in both groups)	Nanda et al. (43)
GlyFn, adiponectin, CRP, placental lactogen, SHBG	11–13	Serum	90	92	Case-control	WHO 1999	AUC 0.92, no association between SHBG and GDM, glyFn alone had a high AUC of 0.91 (Sens 81%, Spec 90%)	Rasanen et al. (44)
AG 1.5	13–23	Serum	50	50	Case-control	WHO 2013	AUC 0.951 (Sens 87%, Spec 94.1%)	Boritza et al. (45)
SHBG, hsCRP	6–15	Serum	27	242	Prospective observational study	Caprenter-Coustan	AUC 0.756, Sens 74.1% und Spec 75.6%	Maged et al. (46)
MF, hsCRP, TNF-α	11–13	Serum	200	800	Case-control	WHO 1999	AUC 0.82, DR 52% at FPR 10% using MF alone (hsCRP and TNF-alpha added no advantage)	Syngelaki et al. (47)
Apolipoprotein E, coagulation factor IX, fibrinogen alpha chain, IGFBP-5	12–16	Serum	30	30	Case-control	WHO 2013	AUC 0.985 (95%CI 0.958–1.012), sens 80% and spec 98%, Cave: Proteomic analysis, results have not validated yet in independent cohort!	Zhao et al. (48)

AUC, area under the curve; BMI, body mass index; CRP, c-reactive protein; DR, detection rate; FPR, false positive rate; Free β-HCG, free β-human chorionic gonadotropin; GDM, gestational diabetes mellitus; GlyFn; glycosylated fibronectin; hsCRP, high sensitive c-reactive protein; IGFBP-5, insulin like growth factor binding protein-5; MF, maternal factors; OGTT, oral glucose tolerance test; PAI-2, plasminogen activator inhibitor-2; PP13, placental protein 13; PAPP-A, pregnancy associated plasma protein-A; PlGF, Placental growth factor; sens, sensitivity; SHBG, sexual hormone binding globulin; spec, specificity; TNF-α, tumor necrosis factor-α; WHO, world health organization; 1.5 AG, 1.5 Anhydroglucitol.

differences in comparison to normal women (56, 57). Sweeting et al. reported a lower PAPP-A level especially in women of South Asian ethnicity and in multiparous women (38). The addition of aneuploidy markers increased the predictive value with an AUC of 0.88% by maternal factors alone to 0.90% with a detection rate (DR) of 73.8% at a false positive rate (FPR) of 10%.

Adipocytokines such as adiponectin and leptin are hormones secreted by adipose tissue. Adiponectin plays an important role in glucose regulation and seems to be a good marker for whole body insulin sensitivity (58). A recent meta-analysis incorporating eight studies using early pregnancy adiponectin levels suggested moderate predictive ability of adiponectin in the prediction of GDM with an AUC of 0.79, a sensitivity of 60.3% (95% CI

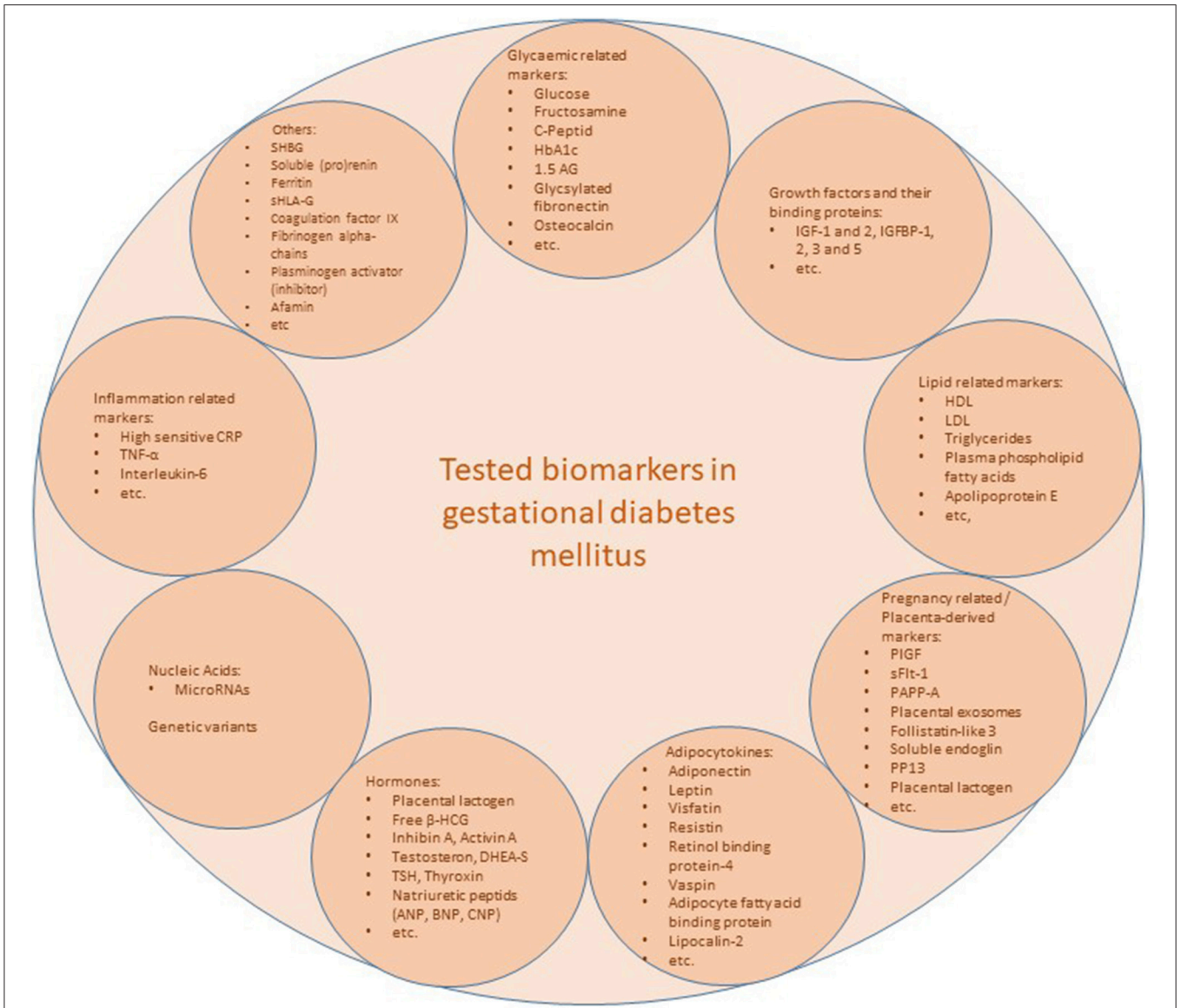

FIGURE 1 | Overview of tested biomarkers. CRP, c-reactive protein; Free β-HCG, free β-human chorionic gonadotropin; GlyFn; glycosylated fibronectin; HbA1c, hemoglobin A1c; HDL, high density lipoprotein; hsCRP, high sensitive c-reactive protein; IGF, insulin like growth factor; IGFBP, insulin like growth factor binding protein; LDL, low density lipoprotein; MF, maternal factors; PAI-2, plasminogen activator inhibitor-2; PP13, placental protein 13; PAPP-A, pregnancy associated plasma protein-A; PlGF, Placental growth factor; microRNAs, micro ribonucleic acids; sFlt-1, soluble Fms-like tyrosine kinase-1; SHBG, sexual hormone binding globulin; sHLA-G, soluble human leucocyte antigen-G; TNF-α, tumor necrosis factor-α; TSH, thyroid-stimulating hormone; 1.5 AG, 1.5 Anhydroglucitol.

46,0%, 73.1%) and a specificity of 81.3% (95% CI 71.6%, 88.3%) (59). Leptin regulates energy intake and expenditure and its levels correlate with the amount of visceral fat in first trimester (60). Qiu et al. found that each 10 ng/ml increase in leptin level was associated with a 20% higher risk of GDM (61), but others reported no alterations in leptin levels in women who subsequently developed GDM (62) or only an association in severely obese women (42). The latter study reported an AUC of 0.82 with a DR of 42% at a FPR of 10% in normal weight and moderately obese women when adiponectin and leptin were included in the maternal factor-based risk model. In a moderate sized case-control study (44), first trimester adiponectin and a newly introduced biomarker glycosylated fibronectin (GlyFn) were independently associated with later GDM development after adjustment for maternal clinical parameters (but not maternal BMI) with an AUC of 0.91 for GlyFn and an AUC of 0.63 for adiponectin. The glycated protein GlyFn still needs to be further validated in large prospective studies. Another marker of short-term glycaemic control is 1.5 Anhydroglucitol (1.5 AG). 1.5 AG is the 1-deoxy form of glucose and is a marker for short term (prior 24–72 h) glycaemic control and variances. Boritza et al. (45) could show that 1.5 AG could discriminate women with

GDM and normal women before 20 weeks of gestation in a small case-control study. 1.5 AG had a high predictive value with an AUC of 0.951, a sensitivity of 87% and a specificity of 94.1% with a cut-off of $\leq$ 60.3 umol/l.

Nonetheless, new biomarkers will need several years to evaluate and large-scale prospective observational studies to prove their clinical utility, as well as, to assess their cost effectiveness in comparison to later GDM screening and diagnosis are necessary. Additionally, it is unlikely that only one biomarker will have high enough sensitivity and specificity to assess early maternal hyperglycaemia. It is more likely that the combination of multiple parameters including baseline maternal characteristics such as BMI will achieve adequate predictive performances, as is the case with first trimester screening for aneuploidy or more recently for preeclampsia. Additionally, the fast-developing field of "omics," in particular proteomic and metabolomic analyses, will provide deeper insights into the pathophysiology of the different phenotypes of gestational diabetes. A specific protein or metabolite pattern will help to decipher biological processes in a more holistic way. These metabolic "fingerprints" of different body fluid sources could then be used for predictive purposes as has already been demonstrated in small case-control studies (48, 63).

Risk Factor-Based Screening

Clinical risk factors for GDM such as higher maternal age, obesity, GDM in a previous pregnancy, family history of diabetes, glycosuria, and ethnic background could be used in combination to identify women with increased risk of developing GDM. However, the proposed clinical risk indicators have shown limited diagnostic accuracy when used separately (64, 65). Therefore, some authors suggested that sensitivity and specificity for GDM screening with risk factors could be considerably improved by using clinical risk prediction models that include statistical combinations of several risk indicators (43, 65–70), which might be additionally combined with FPG (71, 72). Moreover, other biochemical markers might be included for improved prediction (73). However, the design of sufficient risk scores requires an adequate number of cases and healthy controls. As another limitation, the association of different risk factors (e.g., BMI) varies between different ethnic groups (74). Moreover, external validation in clinical practice is necessary (but often pending). A large number of risk estimation models for GDM can be found in the current literature, whereby most of them are based on different diagnostic criteria (65–68, 71). While they might be applicable even in early gestation to identify women at particularly high risk, their clinical significance has not been examined, or compared in independent populations. Furthermore, there is strong evidence from large epidemiological studies (75) that adherence to a healthy life-style (physical activity, healthy diet, non-smoker) prior to gestation is strongly associated with a lower risk for GDM. However, data on life-style factors is missing in published risk scoring algorithms, indicating the need for further research on this topic.

MATERNAL CHARACTERISTICS OF EARLY VS. LATE GDM

An overlap between the categorization of GDM and overt diabetes can be present if overt diabetes had not already been diagnosed before pregnancy. This in mind, pregnant women diagnosed with GDM in early pregnancy seem to be associated with worse pregnancy outcomes approximating those seen in overt diabetes (18). The data from Sweeting et al. suggests a heterogeneous "early" GDM phenotype with a continuous risk from overt diabetes in pregnancy to early GDM, with GDM diagnosed from 24 weeks of gestation onwards being the lowest risk condition. Additionally, maternal adiposity and a more insulin-resistant phenotype might also play a role in the heterogeneity of "early" GDM (26).

CONCLUSION AND RECOMMENDATIONS

GDM is currently diagnosed during the second or third trimester, when unfavorable metabolic dysfunctions might have already affected the mother and the fetus. The combination of maternal risk factors and the insulin resistance preceding biomarkers might improve early detection of a high risk GDM cohort. Future studies should evaluate whether early GDM screening and diagnosis can be improved with the addition of novel biomarkers implicated in the pathophysiology of GDM, whether earlier detection and intervention strategies can improve short and long term adverse outcome and whether the combined biomarker and maternal factor screening models are cost-effective.

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Assessing the Impact of Excessive Gestational Weight Gain Among Women With Type 1 Diabetes on Overweight/Obesity in Their Adolescent and Young Adult Offspring: A Pilot Study

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Aims/hypothesis: We sought to determine the impact of intrauterine exposure to excessive gestational weight gain (GWG) on overweight/obesity in adolescent/young adult offspring of women with type 1 diabetes mellitus (TIDM).

Methods: In 2008, a pilot study was conducted among 19 randomly-selected adolescent and adult offspring of mothers with TIDM who participated in the Diabetes in Pregnancy Program Project (DiP) between 1978 and 1995. Body mass index (BMI)-specific Institute of Medicine (IOM) guidelines for gestational weight gain (GWG) were defined as: 12.5–18.0 kilograms (kg) GWG; 11.5–16.0 kg GWG: 7.0–11.5 kg GWG; 5.0–9.0 kg GWG, for women classed as underweight, normal, overweight and obese according to pre-pregnancy BMI, respectively. Generalized estimating equations were used to estimate adjusted odds ratios (aOR, [95% confidence intervals, CI]) for overweight/obesity among offspring, related to IOM adherence, adjusting for pre-pregnancy BMI and mean maternal daily insulin units/kg body weight.

Results: Mean age of offspring at follow-up was 20.3 ± 3.3 years, 12(63%) were male, 4(21%) Black and 12(63%) overweight/obese. There were 9(82%) overweight/obese offspring among the 11 mothers who exceeded IOM guidelines for GWG compared with 3(38%) overweight/obese offspring among the 8 mothers with GWG within guidelines. Exceeding vs. adhering to IOM guidelines (OR = 7.50, [95%CI: 0.92–61.0]) and GWG per kilogram (OR = 1.39, [95%CI: 0.98–1.97]) were associated with offspring overweight/obesity at follow-up.

Conclusions/interpretation: Our pilot study suggests potential long-term implications of excessive GWG on metabolic health in offspring of mothers with TIDM, warranting future research examining the health impact of GWG in this population.

Keywords: Type 1 diabetes mellitus, gestational weight gain, overweight, obesity, offspring

INTRODUCTION

Perturbations in the fetal environment, such as excessive gestational weight gain (GWG) among women with type 1 diabetes mellitus (TIDM), may increase the offspring's risk of becoming overweight or obese (1). Women with TIDM are at higher risk of obstetric and perinatal complications, such as cesarean section, perinatal mortality and major malformations, compared to those without diabetes (2). Fetuses exposed to both maternal overnutrition and a hyperglycemic intrauterine environment tend to have higher insulin secretion compared with fetuses without these exposures, which may lead to alterations in metabolic programming (3, 4). The resulting state of fetal hyperinsulinemia is hypothesized to be involved in the development of overweight and obesity in offspring (1). Few studies have serially collected clinical maternal measures during pregnancy that allow for the longitudinal examination of time-specific exposure to GWG in women with TIDM as well as the long-term metabolic outcomes in their young adult offspring (5). Therefore, our aim was to investigate the impact of GWG, pre-pregnancy body mass index (BMI) and maternal insulin requirements over the gestational period on the body mass index (BMI) of offspring at approximately 20 years after birth.

MATERIALS AND METHODS

A pilot study was conducted in 2008 to investigate the feasibility of a larger follow-up study on health outcomes among the offspring of women who participated in the Diabetes in Pregnancy Program Project (DiP), a randomized clinical trial between 1978 and 1995. There were 336 singleton pregnancies complicated by T1DM, surviving beyond 28 days of life with no known infant deaths, over the course of the study. TIDM in the mothers had been identified in the DiP according to physician confirmation of ketoacidosis, and or c-peptide levels. To identify potential participants for the pilot study, a random list of these aforementioned pregnancies followed in the DiP was created. Contact was attempted for the first 44 on the list, in groups of 8, 32 were located, however one was deceased. Women were asked if a clinic visit could be scheduled for their offspring (if <18 years old) or for permission to directly contact their offspring (if ≥18 years) in order to schedule a clinic visit. Of the 31, 5 refused and 20 were scheduled for a clinic visit, 19 of whom participated. Lack of funding prevented any further recruitment. During this visit each study subject completed questionnaires regarding medical history, health status, diet and physical activity. Institute of Medicine (IOM) guideline adherence for GWG was the primary exposure of interest. GWG was defined as the difference between weight at delivery and self-reported pre-pregnancy weight, GWG adherence to 2009 IOM guidelines was based on specific body mass index (BMI) categories for maternal pre-pregnancy BMI. Women considered within IOM guidelines for GWG consisted of: (1) underweight [BMI-<18.5 kg/m^2]: 12.5–18.0 kg GWG; (2) normal weight [BMI-≥18.5–<25 kg/m^2]: 11.5–16.0 kg GWG; (3) overweight [BMI-≤25-<30 kg/m^2]: 7.0–11.5 kg GWG; 4) obese [BMI-≥30 kg/m^2]: 5.0–9.0 kg GWG. Due to sample size and study objective, GWG under (below lower limit of recommended weight gain for the specific BMI categories) and within guidelines were combined and compared to those over guidelines (exceeding upper limit of recommended weight gain for the specific BMI category). The primary outcome of interest was overweight/obese in the offspring at follow-up. Pre-pregnancy BMI in the woman and overweight/obese in the offspring at follow-up was categorized according to BMI as defined above. Maternal glycemic control was characterized using three different measures: mean glycohemoglobin A_1 [HbA_1, (%)] per trimester, mean serum pre-prandial and mean 90-minute post-prandial glucose concentrations (mg/dL) collected at clinic visits (6). Concentrations of HbA_1 were measured at study entry and every 4 weeks using Isolab column chromatography [interassay coefficient of variation 7.2%; normal range (5.5–8.5%)] (7). Insulin per kilogram (units/kg), which included insulin taken per day by injection or base pump and bolus, was calculated using the weight obtained at the same clinic visit. Institutional review board (IRB) approval was obtained from both Cincinnati Children's Hospital Medical Center and the University of Cincinnati.

Statistical Analysis

Under and within IOM guidelines and offspring overweight and obesity were combined for statistical analyses due to the small sample sizes in each category for this pilot study. Continuous variables are represented with mean ± standard deviation (SD) or least squares mean ± standard error of the mean and categorical variables as n (%). Fisher's exact test was used to compare normal and overweight/obese groups for maternal baseline characteristics and offspring outcomes for the categorical variables, due to sample size. *T*-tests were used for normally—and Kruskall–Wallis for non-normally-distributed continuous variables.

Inherent correlation between repeat pregnancies was accounted for by using Generalized Estimating Equations (GEE) when examining odds ratios for exposure to maternal adherence to IOM guidelines and overall maternal weight gain and offspring overweight/obesity.

A $p < 0.05$ was considered statistically significant. Statistical analyses were completed using SAS® software version 9.4 (SAS Institute, Cary NC).

RESULTS

Our study included 15 mothers and 19 offspring, with 4 mothers having two offspring in the study. Mean ± SD maternal age at delivery was 26.5 ± 4.1 years and 5 (26%) entered pregnancy as overweight/obese (**Table 1**). In 11 (58%) pregnancies, mothers of the 19 offspring exceeded IOM guidelines for GWG during pregnancy. Mean insulin dose over gestation was higher for mothers who exceeded IOM guidelines (90.1 ± 44.6 mg/dL) compared to mothers under or within guidelines (69.6 ± 23.2, mg/dL), $p = 0.25$.

Among the 19 offspring, mean age at follow-up was 20.3 ± 3.3 years and 12 (63%) were male (**Table 1**). There were 7 (37%) normal weight offspring, and 12 (63%) overweight/obese offspring at follow-up. All mothers of offspring who were

TABLE 1 | Maternal characteristics during pregnancy of women with type 1 diabetes in the DiP (1978–1995) and offspring characteristics at follow-up (2009) overall and by maternal adherence to GWG IOM guidelines, $N = 19$.

	GWG under/within IOM guidelines[a]	GWG over IOM guidelines[a]		All
Maternal characteristics	8 (42)	11 (58)	*p*	$n = 19$
Age at delivery (y)	27.6 ± 3.5	25.6 ± 4.5	0.32	26.5 ± 4.1
Married	8 (100)	6 (55)	0.04	14 (74)
Black	1 (13)	3 (27)	0.44	4 (21)
Primaparous	4 (50)	6 (55)	0.84	10 (53)
Pre-pregnancy BMI (kg/m^2)	21.7 ± 1.9	24.7 ± 5.1	0.10	23.4 ± 4.2
Pre-pregnancy BMI classification			0.08	
Normal (18.5 kg/m^2 to <25 kg/m^2)	8 (100)	6 (55)		14 (74)
Overweight (≥25 kg/m^2 to <30 kg/m^2)	0 (0.0)	4 (36)		4 (21)
Obese (≥30 kg/m^2)	0 (0.0)	1 (9)		1 (5)
Gestational Weight Gain (kilogram) during 1st 20 weeks	4.3 ± 1.7	8.3 ± 4.4	0.04	6.4 ± 3.9
Gestational Weight Gain (kilogram)	12.5 ± 2.4	19.1 ± 3.9	0.0005	16.3 ± 4.6
Preeclampsia	1 (13)	2 (14)	0.74	3 (16)
Previous c-section	2 (25)	2 (14)	0.72	4 (21)
Cesarean section	6 (75)	6 (55)	0.36	12 (63)
Pre-term delivery (prior to 37 weeks' gestation)	2 (25)	2 (18)	0.72	4 (21)
Maternal diabetes and glucose management				
Age at diagnosis Insulin-Dependent Diabetes (years)	15.9 ± 6.7	15.8 ± 5.0	0.98	15.8 ± 5.6
White classification			0.37	
B	3 (38)	3 (27)		6 (32)
C	1 (13)	5 (45)		6 (32)
D	3 (38)	1 (9)		4 (21)
R (Retinopathy)	1 (13)	2 (18)		1 (5)
RF (Retinopathy and Nephropathy)	1 (13)	1 (9)		2 (11)
Mean Glycohemoglobin A_1 (HbA_1) (%)				
First trimester HbA_1	9.3 ± 1.6	10.5 ± 3.3	0.41	10.0 ± 2.7
Second trimester HbA_1	7.9 ± 1.0	8.2 ± 1.8	0.71	8.1 ± 1.4
Third trimester HbA_1	7.3 ± 0.8	7.8 ± 1.6	0.41	7.6 ± 1.3
Mean pre-prandial glucose over gestation[b]	124.3 ± 54.8	135.2 ± 48.9	0.68	131.4 ± 49.7
Mean post-prandial glucose over gestation[b]	156.5 ± 48.0	173.1 ± 32.2	0.38	166.1 ± 39.3
Mean insulin dose over gestation[c]	69.6 ± 23.2	90.1 ± 44.6	0.25	81.5 ± 37.7
Mean insulin dose per kilogram weight over gestation[d]	1.1 ± 0.4	1.1 ± 0.5	0.56	1.1 ± 0.43
Mean Arterial Pressure (MAP) 1st 20 weeks of gestation	84.2 ± 11.9	85.9 ± 7.4	0.74	85.1 ± 9.6
Offspring characteristics				
Age of offspring at follow-up (years)	20.8 ± 3.3	19.9 ± 3.4	0.54	20.3 ± 3.3
Male	5 (63)	7 (64)	0.96	12 (63)
Race				
Black	1 (13)	3 (27)	0.44	4 (21)
Birthweight (grams)	3,737 ± 916	3,854 ± 508	0.72	3,805 ± 688
Large for gestational age[e]	5 (63)	7 (64)	0.96	12 (63)
Gestational age at delivery (weeks)	38.0 ± 1.5	38.6 ± 1.7	0.66	38.3 ± 1.6
BMI (kg/m^2)	24.0 ± 2.7	30.1 ± 8.6	0.39	27.5 ± 7.3
BMI classification			0.07	
Normal (18.5 kg/m^2 to <25 kg/m^2)	5 (63)	2 (18)		7 (37)
Overweight (≥25 kg/m^2 to <30 kg/m^2)	3 (37)	5 (45)		8 (42)

(Continued)

TABLE 1 | Continued

	GWG Under/Within IOM guidelines[a]	GWG Over IOM guidelines[a]		All
Obese (≥30 kg/m^2)	0 (0)	4 (36)		4 (21)
Offspring waist circumference (cm) over NHLBI guidelines[f]	1 (13)	5 (45)	0.13	6 (32)
Paternal history of diabetes	1 (13)	2 (18)	0.45	3 (16)
Hypertension[g]	1 (13)	0 (0)	0.42	1 (5)

[a]*BMI-specific 2009 IOM guidelines for adherence were defined as: underweight by BMI: 12.5–18.0 kg GWG; normal by BMI: 11.5–16.0 kg; overweight by BMI: 7.0–11.5 kg GWG; obese by BMI (all classes): 5.0–9.0 kg GWG.*

[b]*Means were calculated from measurements taken in each trimester.*

[c]*Mean insulin dose over gestation was calculated from measurements of insulin dose taken at approximate weekly visits over full gestation, including at entry to delivery.*

[d]*Mean insulin dose per kilogram weight over gestation was calculated by dividing insulin dose taken at approximate weekly measurements over full gestation by participants' weight (in kg) at that visit.*

[e]*Large for gestational age was defined as infants with a birthweight >90th percentile, according to gestational age, sex and race.*

[f]*Waist circumference measurements for offspring >88 cm for females and >102 cm for males was defined as exceeding guidelines per NHLBI.*

[g]*Hypertension was defined as systolic blood pressure ≥130 mm Hg, diastolic blood pressure ≥ 80 mm Hg or physician-prescribed antihypertensive meds.*

GWG,gestational weight gain; IOM,Institute of Medicine; BMI,body mass index; cm,centimeter; NHLBI,National Heart, Lung and Blood Institute.

Missing values: marital status, n = 1 (5); gestational weight gain in 1st 20 weeks, n = 4 (21); mean HbA1 in 1st trimester, n = 5 (26%); mean HbA1 in 2nd trimester, n = 2 (11%); mean preprandial glucose over gestation, n = 2 (11%); mean MAP, n = 3 (16%).

Data are expressed as mean ± standard deviation for continuous variables and n (%) for categorical variables.

TABLE 2 | Odds Ratios (OR) and adjusted Odds Ratios (aOR) for overweight/obesity among offspring at follow-up of women with type 1 diabetes mellitus in the DiP (1978-1995), using separate models of Gestational Weight Gain Adherence and Total Gestational Weight Gain.

	Model I		Model II		Model III	
***N*=19**	**OR (95% CI)**	***p***	**aOR (95% CI)**	***p***	**aOR (95% CI)**	***p***
IOM adherence[a]						
Under/Within IOM guidelines	Reference		Reference		Reference	
Over IOM guidelines	7.50 (0.92-61.0)	0.06	4.49 (0.45-44.5)	0.20	Not estimable	
Total gestational weight gain (kilograms)[b]	1.39 (0.98–1.97)	0.07	1.25 (0.86–1.81)	0.23	1.51 (0.96–2.39)	0.07

Model I—unadjusted

Model II—Model I and pre-pregnancy BMI (kg/m^2) as continuous

Model III—Model I and mean insulin (mg/dL) dose per kilogram weight over total gestation [a]*BMI-specific 2009 IOM guidelines for adherence were defined as: underweight by BMI: 12.5–18.0 kg GWG; normal by BMI: 11.5–16.0 kg; overweight by BMI: 7.0–11.5 kg GWG; obese by BMI (all classes): 5.0-9.0 kg GWG*

[b]*Total gestational weight gain (kg) was calculated as (weight at delivery)—(weight at LMP).*

Participant's mean insulin dose per kilogram weight over gestation was calculated by dividing insulin dose (mg/dL) taken at approximate weekly measurements over full gestation by participant's weight (in kg) at that visit.

CI, 95% confidence interval; IOM, Institute of Medicine; BMI, body mass index (kg/m^2).

normal weight at follow-up were themselves normal weight upon entering pregnancy, while for offspring who were overweight/obese at follow-up 58% of mothers were normal weight upon entering pregnancy (**Supplementary Table 1**). Mean maternal HbA_1 of overweight/obese offspring was significantly higher than maternal HbA_1 of normal weight offspring at the 1st ($p = 0.01$), 2nd ($p = 0.04$), and 3rd ($p = 0.02$) trimesters (**Supplementary Table 1**).

Exposure to the intrauterine environment of mothers who exceeded IOM guidelines for GWG compared to exposure to the intrauterine environment of mothers who adhered to guidelines conferred a non-significant but over 7-fold increased odds (OR = 7.50, [95%CI: 0.92–61.0], $p = 0.06$) of overweight/obesity at follow-up among offspring (**Table 2**). After adjusting for pre-pregnancy BMI the association was slightly attenuated (aOR = 4.49, [95%CI: 0.45–44.5], $p = 0.20$).

Examining the actual weight over pregnancy rather than adherence to IOM guidelines showed a similar association. The unadjusted odds for exposure to increasing GWG (per kg) and overweight/obesity among offspring at follow-up was OR = 1.39, [95%CI: 0.98–1.97], $p = 0.07$ (**Table 2**). After adjusting for pre-pregnancy BMI, the relationship between total GWG (per kg) and offspring BMI was slightly attenuated (aOR = 1.25, [95%CI: 0.86–1.81], $p = 0.23$). Adjusting for mean insulin dose per maternal body weight (kg) over gestation, total GWG per kg was associated with non-statistically significant increased odds (aOR = 1.51, [95%CI: 0.96–2.39], $p = 0.07$) for overweight/obesity among offspring at follow-up.

DISCUSSION

In this pilot study of 19 offspring, we identified a high prevalence of mothers exceeding IOM guidelines for GWG, in addition, these mothers were more likely to have overweight/obese offspring at follow-up compared to mothers staying under/within IOM guidelines. Nine of the 12 overweight/obese offspring had mothers who exceeded IOM guidelines. All five mothers who entered pregnancy as overweight/obese according to BMI had

overweight/obese offspring at follow-up, while all offspring who were normal weight had mothers who entered pregnancy with a normal BMI. Even with a small sample size, we observed an association between mothers exceeding IOM guidelines for GWG and overweight/obesity outcomes among young adult offspring in this population, albeit with borderline statistical significance. Prior studies have established associations between excessive GWG and LGA at birth (8, 9). In addition, there is an increased risk of adult overweight and glucose intolerance among offspring of women with diabetes who are born overweight (5). Secher et al. found a positive association between higher GWG and increased offspring birth weight, independent of glycemic control and prepregnancy BMI (9). In our study, nearly 60% of offspring who were overweight/obese at follow-up were born LGA, while only 42% of normal weight offspring were born LGA. Although we did not adjust for LGA or maternal glucose control, we observed a slight increase in the odds of overweight/obesity among offspring with exposure to maternal weight gain per kg after adjusting for maternal insulin per kg during pregnancy. However, given the observed significant differences in glucose control throughout gestation among mothers of normal weight offspring compared to overweight/obese offspring, our results suggest important clinical implications if, indeed, we are observing a true association. Exposure to excessive GWG, reported by some as particularly important in early pregnancy, (10) during which time metabolic programming of the fetus begins, may lead to long-term effects on the metabolic health of offspring (3–5). Appropriate GWG and glucose management is paramount for this population, particularly for women entering pregnancy as overweight/obese. As our results show these women are also more likely to have higher HbA_1 throughout gestation, exceed IOM guidelines for total GWG and have higher GWG during early pregnancy.

Limitations of our study include the small sample size, restricting our ability to perform statistical analysis to examine fully adjusted associations and possible interactions. Although data were available, it was also difficult to account for potential modifiers, including maternal and offspring smoking status, breast feeding as an infant, paternal history of diabetes, offspring dietary intake, and reduced offspring physical activity. Although our sample included only four black offspring, this proportion is representative of the T1DM population and this was a randomly selected group. Despite the limitations, an important strength of this study was in the comprehensive, longitudinal data from the original cohort, with repeated clinical measures over pregnancy and comprehensive follow-up data on offspring. This follow-up study aimed to demonstrate feasibility of a larger study and successful recruitment of adult offspring of women in the DiP cohort.

Our pilot study suggests that there are long-term implications of increasing maternal GWG on overweight/obese outcomes in offspring of mothers with TIDM. Our data also demonstrate the importance of identifying modifiable behaviors, including maternal adherence to IOM guidelines for GWG and adequate glucose control throughout pregnancy, to reduce metabolic adversities in adult offspring. Future directions include following up on a larger number of participants in this cohort to evaluate the long-term impact of exposure to the intrauterine TIDM environment.

DATA SHARING STATEMENT

The datasets for this manuscript are not publicly available because there is an ongoing funded R01 for follow-up of 250 offspring. Requests to access the datasets should be directed to JK (jane.khoury@cchmc.org).

AUTHOR CONTRIBUTIONS

KM and JK study concept and design; JK acquisition of data; KM statistical analysis; KM, KB, LD, RD, CJ, and JK interpretation of data; KM drafting of the manuscript; KM, KB, LD, RD, CJ, and JK critical revision of the manuscript for important intellectual content; CJ and JK administrative, technical, and material support.

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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.2018.00713/full#supplementary-material

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ORIGINAL RESEARCH
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Association Between Maternal Hyperglycemia and Composite Maternal-Birth Outcomes

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Objective: The overall impact of maternal hyperglycemia on maternal and birth outcomes is largely underestimated, therefore quantifying the true burden of hyperglycemia in a whole population it is a challenging task. This study aims at examining the association between blood glucose concentration during pregnancy and a composite score of adverse maternal-birth outcomes in a large-scale prospective cohort study in China.

Methods: Pregnant women within "the Born in Guangzhou Cohort Study" China who underwent a standard 75-g oral-glucose-tolerance-test (OGTT) between 22 and 28 gestational weeks were included. A composite score of stillbirth, duration of pregnancy, birth weight, preeclampsia, and cesarean section was developed based on a published maternal-fetal outcomes scale, weighed by the relative severity of the outcomes. Multiple linear regression models were used to assess the associations between OGTT glucose measurements and log composite score. Logistic regression models were used to assess relations with outcome as a categorical variable (0, 1– < 3, and ≥3).

Findings: Among 12,129 pregnancies, the composite score ranged from 0 to 100 with a median of 2.5 for non-zero values. Elevated fasting glucose level was associated with higher composite score (adjusted coefficients 0.03 [95% CI, 0.02–0.04] for 1-SD increase). For 1-SD increase in fasting glucose, the risk of having a composite score 1– <3 and ≥3 rises by 13% (95% CI, 8–17%) and 15% (95% CI, 7–23%), respectively. Similar association and increase in risk was found for 1 and 2-h glucose.

Conclusion: Elevated fasting, 1 and 2-h glucose levels are associated with a range of adverse maternal-birth outcomes. The composite score model can be applied to the risk assessment for individual pregnant women and to evaluate the benefits for controlling glucose levels in the population.

Keywords: hyperglycemia, pregnancy, stillbirth, birth weight, duration of pregnancy, preeclampsia, cesarean section, composite outcome

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INTRODUCTION

Gestational diabetes mellitus (GDM) is one of the most prevalent major complications during pregnancy worldwide (1–3). Previous studies have linked maternal hyperglycemia to increased risks of various adverse perinatal outcomes, including macrosomia, large for gestational age (LGA), cesarean section, preterm birth, gestational hypertension and hyperbilirubinemia (3, 4). However, all such studies have only investigated the perinatal outcomes in isolation without taking into account the complex inter-relationship between maternal and fetal outcomes, and among the perinatal outcomes themselves; examples include macrosomia and cesarean section (5), gestational hypertension and preterm birth (6, 7). In addition, the varying strengths of association with these outcomes, which have different levels of severity and consequences, have not been fully appreciated (3, 4, 8). This has led to difficulties in assessing the influence of maternal hyperglycemia on both maternal and birth outcomes, and developing optimal management of maternal hyperglycemia, as well as evaluating the effectiveness of glucose-lowering interventions in the general pregnant women population. A comprehensive consideration of multiple adverse outcomes, both in pregnant women and in newborns (9), is therefore needed.

Indeed, some previous studies have attempted to assess multiple perinatal outcomes (8–12). For example, to evaluate the need of care during pregnancy and delivery, Novicoff et al. created an outcome score that sums the points of all maternal and fetal outcomes based on relative desirability and frequency of occurrence (10). Verma et al. developed a morbidity assessment index for newborns (called MAIN score), capturing the entire severity spectrum of morbidity at birth to estimate the effectiveness of obstetric interventions on neonatal morbidity (9, 12). However, none of these studies have evaluated the influence of maternal hyperglycemia on composite maternal and birth outcomes. A comprehensive measure focusing on capturing GDM-related morbidity in both mother and child should add an important dimension to current understanding of GDM risk and management.

The present study aims to examine the association between blood glucose concentrations during pregnancy, and risk-adjusted adverse maternal-birth outcomes represented by a composite score in a large-scale prospective cohort study in China.

MATERIALS AND METHODS

Study Design and Population

The data used in the present study is part of the Born in Guangzhou Cohort Study (BIGCS), which is a birth cohort study conducted in the Guangzhou Women and Children's Medical Center (GWCMC), China. Study participants were recruited from pregnant women attending their first routine antenatal exam at GWCMC. Inclusion criteria were: residents in Guangzhou, <20 weeks gestation, intending to deliver at GWCMC, and planning to remain in Guangzhou with the child for at least 3 years after delivery. A detailed description of the BIGCS protocol has been published elsewhere (13). For this analysis, we excluded participants with pre-pregnancy diabetes or chronic hypertension, according to the self-reported medical history. Women with missing blood glucose and delivery data were also excluded (**Figure 1**).

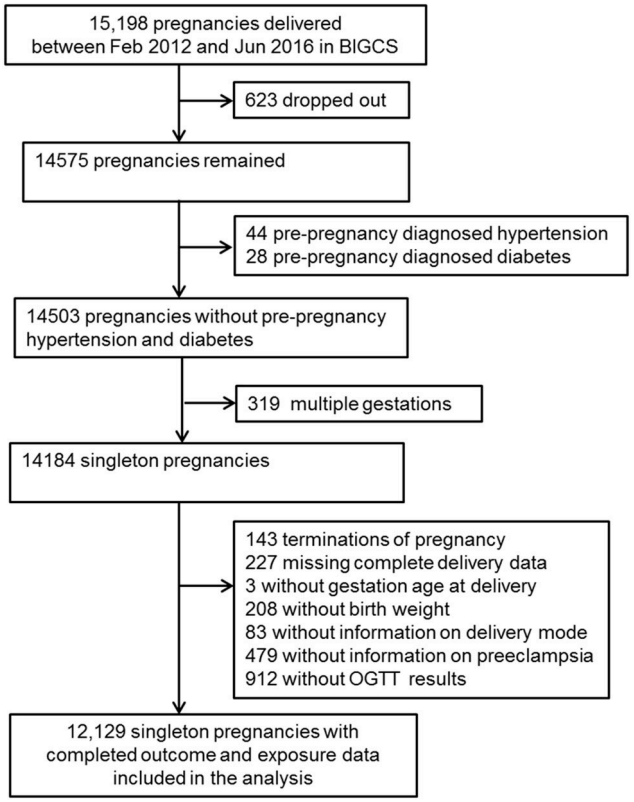

FIGURE 1 | The flowchart of the study.

At enrollment participants underwent an interviewer-administered questionnaire, which collected a wide range of information including socio-demographic data, exposures at home and at workplace, personal lifestyle, medical histories, and health status before pregnancy. The study protocol was approved by GWCMC Ethics Approval Board. Written informed consent was obtained from all participants.

Oral Glucose-Tolerance Test

Between 22 and 28 weeks of gestation, all pregnant women underwent a standard oral glucose tolerance test (OGTT), during which blood samples (2 mL) were collected at fasting, 1 and 2 h after a 75 g glucose load with NaF/EDTA tubes. Detailed procedures of the OGTT test were presented elsewhere (14). Women with GDM diagnosis [based on the International Association of Diabetes and Pregnancy Study Groups [IADPSG] criteria (15)] received routine consultation on diet and exercise. They were then asked to self-monitor their preprandial blood glucose levels after having dietary management for 3–5 days. Those with fasting blood glucose ≥5.3 mmol/L or 2 h

postprandial glucose ≥6.7 mmol/L after dietary control were prescribed insulin in addition to a diet and exercise regime (16).

Maternal and Birth Outcomes

Information about pregnancy complications, mode of delivery, gestational age, birth weight, parity, and gender of newborns were obtained from GWCMC's electronic medical records. Gestational age was confirmed by ultrasound examination in the first- or second-trimester. Birth weight was measured by midwives immediately after delivery. Birthweight z scores were calculated using a local population-based birth weight reference (17). LGA was defined as a birthweight larger than the 90th percentile for gestational age by gender and small for gestational age (SGA) was defined as a birthweight lower than the 10th percentile for gestational age by gender, based on the same birth weight reference (17). Preterm birth was defined as birth before 37 weeks of gestation. Spontaneous preterm birth was the birth following spontaneous preterm labor and/or preterm premature rupture of the membranes before 37 weeks of gestation, irrespective of the mode of delivery (vaginal, cesarean section) (18); all this information was obtained from medical records, which was also independently confirmed by two pediatricians. Gestational hypertension was defined as blood pressure ≥140/90 mmHg on at least two occasions separated by at least 4 h after 20th week of gestation without the presence of protein in the urine and returned to normal within 12 weeks postpartum (19). Preeclampsia was defined as gestational hypertension combined with proteinuria [protein level in the urine ≥300 mg/24 h or ≥(+) by a dipstick test on at least two occasions separated by at least 6 h] (19). Stillbirth cases were identified from the medical records with the 10th revision of the International Classification of Diseases (ICD10) codes of O36.401 and P95.

We developed a composite outcome score based on the adverse maternal and infant outcomes related to GDM as suggested by the literature (3, 4); maternal-infant outcomes included stillbirth, duration of pregnancy, birth weight, preeclampsia, and cesarean section. A score was assigned to each outcome according to a published unified maternal-fetal outcome risk assessment model, ranging from 0 (perfect outcome) to 100 (maternal or infant death) (10). Each mother-newborn pair was scored based on the existence and severity of the five outcomes as shown in **Table 1**. A comprehensive outcome score was calculated by summing all above-mentioned outcomes scores for each mother-newborn pair. Higher scores indicated the presence of worse maternal and perinatal outcomes.

Potential Confounders

The following maternal risk factors were selected a priori as potential confounders: age, maternal education level, maternal income, pre-pregnancy body mass index (BMI), maternal smoking status, secondhand smoking (smoke exposure during pregnancy), use of assisted reproduction technology, parity, and gestational age at the time of OGTT. Except for gestational age at OGTT and parity, all information was derived from the self-reported questionnaire at enrollment.

TABLE 1 | Maternal-birth outcomes scoring scale.

Outcomes	Score*
Stillbirth	100
Preeclampsia	5
Cesarean delivery	2.5
Gestational age ≤24 wk	11
Gestational age 25–26 wk	7
Gestational age 27–28 wk	6
Gestational age 29–30 wk	4
Gestational age 31–33 wk	3
Gestational age 34–36 wk	2
Gestational age ≥37 wk and <42 wk	0
Gestational age >42 wk	1
Birth weight <750 g	10
Birth weight 750–1,000 g	7
Birth weight 1,000–1,500 g	5
Birth weight 1,500–2,500 g	3
Birth weight 2,500–4,000 g	0
Birth weight >4,000 g	1

**The score assigned to each outcome was referred to a published unified maternal-fetal outcome risk assessment model, ranging from 0 (perfect outcome) to 100 (maternal or infant death) (10).*

Statistical Analysis

Descriptive statistics of the characteristics and outcomes were reported for the whole sample and across categories of the composite scores. To assess the relationship between OGTT glucose measurements and maternal and fetal outcomes as represented by the composite score, we analyzed the data in two ways. First, the composite score was treated as a continuous variable and was log (base 10) transformed [log (score+1)]. Multiple linear regression models were used to assess the association between the OGTT glucose measurements and log composite score. Regression coefficients were calculated, representing the change in log composite score for 1-SD change in each fasting, 1 and 2 h glucose measurement. Second, we categorized the composite scores (without transformation) into three groups based on the median of non-zero scores (2.5): 0, low (including scores 1, 2, and 2.5) and high (≥3). Logistic regression models were used to evaluate the relationships of the OGTT glucose measurements with composite outcomes score categories, with "0" as reference. Odds ratios (ORs) were calculated for a 1-SD increase in each OGTT measurement. The linear and logistic regression models were adjusted for pre-specified potential confounders, including maternal age (continuous), maternal education (middle school or below, college, undergraduate or postgraduate), maternal income (<1,500, 1,500–4,500, 4,501–9,000, ≥9,001 Yuan), maternal pre-pregnancy body mass index (BMI, continuous), parity (0, ≥1), smoking status (yes, no), second-hand smoking exposure during pregnancy (yes, no), use of assisted reproduction technology

TABLE 2 | Characteristics of the 12,129 study participants and their newborns by different groups of composite score.

Characteristic	All participants	Composite score			
		0	1– <3	≥3	*p*-value
n	12,129	7,296	3,935	898	
Age (years), mean ± SD	29.1 ± 3.4	28.6 ± 3.1	29.9 ± 3.7	29.5 ± 3.6	<0.0001
PLASMA GLUCOSE (mmol/L), MEAN ± SD					
Fasting	4.3 ± 0.4	4.3 ± 0.4	4.3 ± 0.4	4.3 ± 0.5	<0.0001
1-h	7.7 ± 1.7	7.6 ± 1.6	7.8 ± 1.7	7.9 ± 1.8	<0.0001
2-h	6.6 ± 1.3	6.5 ± 1.3	6.8 ± 1.4	6.8 ± 1.5	<0.0001
Length of gestation at time of OGTT (wk), mean ± SD	24.5 ± 1.6	24.5 ± 1.6	24.5 ± 1.6	24.4 ± 1.6	0.441
Income (Yuan), *n* (%)					<0.0001
<1,500	1,115 (9.6)	674 (9.7)	363 (9.7)	78 (9.3)	
1,500–4,500	3,460 (29.9)	2,207 (31.6)	1,001 (26.7)	255 (30.4)	
4,501–9,000	4,947 (42.8)	2,933 (42.0)	1,641 (43.8)	373 (44.5)	
≥9,001	2,040 (17.7)	1,166 (16.7)	741 (19.8)	133 (15.9)	
Education, *n* (%)					0.0051
Middle school or below	1,112 (9.2)	645 (8.8)	377 (9.6)	90 (10.0)	
College	3,068 (25.3)	1,847 (25.3)	969 (24.6)	252 (28.1)	
Undergraduate	6,472 (53.4)	3,929 (53.9)	2,067 (52.5)	476 (53.0)	
Postgraduate	1,471 (12.2)	875 (12.0)	522 (13.3)	80 (8.9)	
Pre-pregnancy body mass index (kg/m^2), mean ± SD and *n* (%)	20.4 ± 2.7	20.1 ± 2.5	20.8 ± 2.8	20.7 ± 3.0	<0.0001
≤18.5	2,967 (25.0)	1,994 (27.9)	768 (20.0)	205 (23.6)	<0.0001
18.5–23.9	7,785 (65.6)	4,634 (64.8)	2,598 (67.5)	553 (63.6)	
24.0–27.9	939 (7.9)	450 (6.3)	398 (10.3)	91 (10.5)	
≥28.0	181 (1.5)	74 (1.0)	86 (2.2)	21 (2.4)	
Smoking during pregnancy, *n* (%)	59 (0.5)	34 (0.5)	16 (0.4)	9 (1.0)	0.0584
Passive smoking during pregnancy, *n* (%)	3,494 (29.6)	2,166 (30.4)	1,082 (28.3)	246 (28.5)	0.0467
Parity at enrollment ≥1, *n* (%)	1,613 (13.4)	845 (11.7)	654 (16.8)	114 (12.8)	<0.001
Use of assisted reproduction technology, *n* (%)	396 (3.4)	182 (2.6)	168 (4.4)	46 (5.3)	<0.0001

OGTT, oral glucose tolerance test.

(yes, no), and gestational age when OGTT was performed (continuous).

All analyses were performed using SAS 9.3 software (SAS Institute, Cary, NC, USA). $P < 0.05$ were considered to indicate statistical significance.

RESULTS

A total of 15,198 pregnant women in BIGCS delivered between February 2012 and January 2016. We excluded women with multiple births, those who dropped out before delivery or terminated their pregnancy, had pre-pregnancy diagnosed hypertension and diabetes, or whose delivery data and OGTT results were missing, resulting in 12,129 mothers and their singleton births in this analysis (**Figure 1**).

Characteristics of mothers and newborns, and by composite score groups are shown in **Table 2**. Pregnant women had a mean age of 29.1 years (SD 3.4). The majority of them had attained college or above qualifications and over half of them had a monthly income more than 4,500 Yuan. Few women smoked but 30% were exposed to secondhand smoke during pregnancy. Not unexpectedly, the vast majority (86.6%) of the pregnant women were primipara. Only 3.4% women undertook assisted reproduction technology. About 10% of the participants were overweight or obese before pregnancy BMI ≥24.0 kg/m^2, according to the Chinese guidelines (20). The mean plasma glucose levels were 4.3 mmol/L (SD 0.4) at fasting, 7.7 mmol/L (SD 1.7) at 1-h, and 6.6 mmol/L (SD 1.3) at 2-h post 75 g glucose load, respectively. Using the IADPSG criteria, there were 1,662 (1,662/1,662, 100%) women diagnosed with GDM who received a subsequent diet and exercise advice. Ten (10/1,662, 0.6%) women went on to receive insulin therapy. Around 60% of the pregnancies ($n = 7,296$) resulted in optimal outcome (with a score of 0), one-third ($n = 3,935$) having scores between 1 and <3, and 7% ($n = 898$) had a composite score ≥3 (20% being 3). There were statistically significant differences in most of the maternal characteristics measured across the three composite score groups.

Overall, the prevalence of preeclampsia (0.7%) and stillbirth (0.1%) was low, although one-third of deliveries were by cesarean section. Infants were born weighing on average 3191.5 g (SD 423.9) after a mean gestational length of 38.8 weeks (SD 1.4), with 3.5% of the babies having spontaneous preterm birth (<37 weeks).

TABLE 3 | Obstetrical and newborn outcomes of the 12,129 study participants.

Characteristic or outcome	All participants	Composite score		
		0	1– < 3	≥3
n	12,129	7,296	3,935	898
OBSTETRICAL OUTCOMES				
Hypertension, *n* (%)	295 (2.4)	82 (1.1)	100 (2.5)	113 (12.8)
Gestational hypertension	214 (1.8)	82 (1.1)	100 (2.5)	32 (3.6)
Preeclampsia	81 (0.7)	–	–	81 (9.15)
Cesarean delivery, *n* (%)	4,161 (34.3)	–	3,639 (92.5)	522 (59.0)
NEWBORN OUTCOMES				
Stillbirth, *n* (%)	13 (0.1)	–	–	13 (1.4)
Gestational age at delivery (week), mean ± SD	38.8 ± 1.4	39.0 ± 1.0	38.9 ± 1.2	36.7 ± 2.7
Preterm birth, *n* (%)	579 (4.8)	–	188 (4.8)	391 (44.5)
Spontaneous preterm birth, n (%)	419 (3.5)	–	169 (4.3)	250 (33.9)
Birth weight (g), mean ± SD	3191.5 ± 423.9	3194.5 ± 316.8	3292.0 ± 373.8	2720.5 ± 863.5
Birth weight Z-score, mean ± SD	0.1 ± 1.0	0.0 ± 0.8	0.3 ± 0.9	−0.2 ± 1.8
BIRTH WEIGHT FOR GESTATIONAL AGE, *N* (%)				
SGA	875 (7.3)	431 (5.9)	122 (3.1)	322 (36.7)
AGA	9,917 (82.3)	6,354 (87.6)	3,210 (81.9)	353 (40.2)
LGA	1,254 (10.4)	465 (6.4)	586 (15.0)	203 (23.1)

SGA, small for gestational age, AGA, appropriate for gestational age, LGA, large for gestational age.

TABLE 4 | Adjusted coefficients and odds ratios for associations between maternal glycemia and composite maternal-birth outcomes.

Composite score	Plasma glucose level						
		Fasting		At 1-h		At 2-h	
	n	Crude	Adjusted[a]	Crude	Adjusted[a]	Crude	Adjusted[a]
Continuous, coefficients (95% CI)[b]	12,129	0.05 (0.04, 0.07)	0.03 (0.02, 0.04)	0.05 (0.04, 0.06)	0.02 (0.01, 0.04)	0.06 (0.05, 0.07)	0.03 (0.02, 0.04)
CATEGORICAL, OR (95% CI)							
0	7,296	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
1- < 3	3,935	1.20 (1.15, 1.25)	1.13 (1.08, 1.17)	1.16 (1.12, 1.21)	1.06 (1.02, 1.11)	1.19 (1.15, 1.24)	1.09 (1.05, 1.14)
≥3	898	1.21 (1.13, 1.29)	1.15 (1.07, 1.23)	1.22 (1.14, 1.30)	1.15 (1.07, 1.24)	1.22 (1.14, 1.30)	1.14 (1.06, 1.22)

CI, confidence interval, OR, odd ratio.

[a] *Regression coefficients or odds ratios were for an increase in the glucose level of 1 SD (0.42 mmol/L for the fasting glucose level, 1.66 mmol /L for the 1 h glucose level, and 1.35 mmol/L for the 2 h glucose level.). Adjusted for age, income, educational level, smoking during pregnancy, second hand smoking exposure during pregnancy, pre-pregnancy BMI, parity, assisted reproductive technology, gestational age at the OGTT.*

[b] *Composite score was log transformed as log(score+1).*

Among those who had a non-zero composite score, 4,138 (84.2%) had one outcome, 620 (12.6%) had two, 155 (3.2%) had three or more. Cesarean delivery was the most common outcome, occurring in 4,161 (85.0%) of the women with non-zero score.

Table 3 summarizes selected pregnancy-related and birth outcomes by composite score groups. The prevalence of gestational hypertension, preeclampsia, cesarean delivery, preterm birth, spontaneous preterm birth, LGA, SGA, and stillbirth increased significantly with higher composite score categories.

Table 4 shows the associations between fasting, 1 and 2-h plasma glucose and composite perinatal outcomes score after adjusted for confounders. Elevated fasting glucose values was significantly associated with higher log composite score as a continuous variable (coefficient 0.03 [95% CI, 0.02–0.04] for 1-SD increase). For 1-SD increase in fasting glucose, the likelihoods of having composite outcomes score 1– <3 and ≥3 increased by 13% (95% CI, 8–17%) and 15% (95% CI, 7–23%) in the logistic regression model, respectively. Similar associations were found for 1 and 2-h glucose values.

DISCUSSION

In the present study, a composite maternal-birth outcome scoring scale based on five perinatal outcomes that are most related to gestational diabetes was adapted from a risk model covering maternal and birth outcomes. Significant associations

between increased fasting, 1 and 2-h post-load plasma glucose levels and increased composite maternal and birth outcomes score were found.

A number of studies have investigated the associations between maternal glucose levels and individual perinatal outcomes, such as preterm birth or LGA (3, 4). However, none has considered the multiple adverse maternal and birth outcomes holistically in the same study. Individual outcomes, e.g., LGA, cesarean delivery, preterm birth, preeclampsia, when considered separately, are unable to capture the overall impact of maternal hyperglycemia in mothers and their newborns, hampering the risk assessment of maternal hyperglycemia and the evaluation of effectiveness and benefit for glucose level management in the general pregnant women population. In the present study, the composite outcome score included birth weight and gestational age, both of which have been widely used as outcome measures of effectiveness of national health policies and interventions for pregnant women, especially in developing countries (17, 18), and have been reported to be strongly associated with GDM (via abnormal birthweight and preterm birth) (4, 21). We also included preeclampsia and cesarean section—both associated with elevated glucose concentrations during pregnancy (4, 21). Hence, the composite score combining complications in both mother and newborn is an interpretable measure of the actual morbidity that are most related to maternal hyperglycemia during pregnancy.

In the present analysis, we found that the risk of having a higher composite score (between 1 and <3, ≥3) decreased for 1-SD lower in fasting, 1 and 2-h glucose levels. This may help to assess the risk of developing adverse maternal and birth outcomes for individual pregnant women and recognize specific needs for hyperglycemic women, thus facilitating resource-allocation and care optimization for the high-risk, high-cost patient encounter (8, 9). For example, among the women with hyperglycemia, only those who have high risk of overall adverse outcomes need more intensive care and intervention. On the other hand, the linear association between maternal glucose level and the comprehensive outcome score highlights the importance of controlling maternal plasma glucose at low levels, rather than aiming at the level just below the diagnostic cut-off. While there is evidence of substantial benefits from intervention for women with GDM and their newborns, previous intervention studies reported improvement in targeted morbidities separately (22, 23) rather than across a spectrum of major morbidities to assess overall health improvement. As a result, clinicians may opt to implement different treatment and management strategies depending on the particular outcome they focus on, which may not necessarily be the best option for the patients. The results also can be applied to evaluate the overall effectiveness and benefit of controlling glucose level in the whole pregnant women population. According to our findings, if the fasting glucose level increases by 1-SD (0.42 mmol/L) in pregnant women, the risk of having a composite adverse outcomes score 1-<3 and ≥3 would increase by 13 and 15%, respectively. Consider China where there is a large number of pregnant women, ∼18 million per year, an excess of 0.76 million (13% × 32.4% [prevalence of women with composite score 1–<3] × 18 million) and 0.20 million (15% × 7.4% [prevalence of women with composite score ≥3] × 18 million) women per year would suffer from mild and relative severe adverse maternal and birth outcomes if fasting glucose would increase by 1-SD in the population. Adequate control of fasting glucose concentration is therefore necessary in both individual and population levels.

The strengths of our study include the use of high-quality outcome data extracted from medical records and that a wide range of potential confounders have been considered in the analysis. This study had some limitations though. First, we did not adjust for the dietary intake of the women during pregnancy, which could potentially affect fetal growth and other maternal-birth outcomes. Second, we recognize that the weights we applied for the five outcomes in the present study, adapted from the maternal and infant scoring system developed by Novicoff et al. (10), may not accurately reflect the relative severity of the outcomes. However, there has been no universally agreed standard values and ranking for the outcomes of interest. Once a more appropriate standardized scale for each outcome is developed, studies like ours will be required to confirm the findings. Third, neonatal hypoglycaemia is also an important outcome related to gestational diabetes. Unfortunately, the scale we used did not include hypoglycaemia as an outcome and we also did not collect data about this outcome.

In conclusion, using a composite scale that ranks the relative severity of multiple maternal and infant outcomes, we assessed the effects of elevated fasting, 1 and 2-h post-load plasma glucose and confirmed the potential benefits of adequate control of maternal glucose level in improving maternal-birth outcomes as a whole. This could be applied to the risk assessment for individual pregnant women as well as to the evaluation of effectiveness and benefit for controlling glucose level in the population.

AUTHOR CONTRIBUTIONS

XQ and H-MX designed the study and directed its implementation. S-YS, J-RH, J-HL, N-NC, W-QX, and M-YY were involved in study design, questionnaires development, data collection, and follow-up of participants. S-YS participated in the data analysis and drafted the manuscript. L-FZ carried out the data analysis, contributed to the writing of the paper. J-HL managed the data. KL and XQ revised the manuscript. All authors critically revised the manuscript, and approved the final version.

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Exposure to Bisphenol a Substitutes and Gestational Diabetes Mellitus: A Prospective Cohort Study in China

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Background: The association of bisphenol A (BPA) and gestational diabetes mellitus (GDM) has been investigated in only a small number of studies, and research on the associations between BPA substitutes and GDM is scarce.

Objective: We aimed to investigate the associations of four bisphenols [bisphenol A (BPA), bisphenol S (BPS), bisphenol F (BPF), and bisphenol AF (BPAF)] levels in urine sample with the risk of gestational diabetes mellitus (GDM) and plasma glucose levels.

Methods: A total of 1,841 pregnant women from a cohort study were recruited at their first prenatal examination between 2013 and 2015 in Wuhan, China. Concentrations of four bisphenols (BPA, BPS, BPF, BPAF) were measured in first-trimester urine samples using Ultra-high performance liquid chromatography system coupled to a Triple Quadrupole mass spectrometer (UHPLC-TQMS). An oral glucose tolerance test (OGTT) was performed at 24–28 gestational weeks and GDM was diagnosed *post hoc* using International Association of Diabetes and Pregnancy Study Groups criteria. We used multivariable logistic regression models to examine the associations of urinary bisphenols with the risk of GDM, and multiple linear regression models to determine the associations between bisphenols exposure and plasma glucose levels.

Results: Urinary BPAF was associated with increased odds of GDM among women with normal pre-pregnancy BMI [adjusted odds ratio (aOR) = 1.70 (95% CI: 1.08, 2.67) for the highest group compared to the lowest group], and the association remained significant after additional adjustment for other bisphenols [aOR = 1.68 (95% CI: 1.03, 2.72)]. No significant associations were observed for other bisphenols and GDM. Consistent with the result of GDM, women in the highest BPAF category had a mean of 0.05 mmol/L (95% CI: 0.01, 0.09) higher fasting plasma glucose (FPG) levels than women in the lowest category. For BPA and plasma glucose, non-linear associations were observed between urinary BPA and FPG and the sum of the PG *z*-score among women who were overweight (p for non-linear association <0.05). We also found that the per-unit increase in natural log transformed specific gravity adjusted BPS [ln (SG-adj BPS)] was associated with a 0.03 mmol/L (95% CI: 0.01, 0.04) increase in FPG levels and the associations might

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be modified by fetal sex (p for interaction <0.05). Among women with female fetus, a per-unit increase in ln (SG-adj BPS) was associated with a 0.04 mmol/L (95% CI: 0.02, 0.06) increase in FPG, a 0.11 mmol/L (95% CI: 0.04, 0.17) increase in 1 h-PG and a 0.19 mmol/L (95% CI: 0.08, 0.30) increase in the sum of PG z-score.

Conclusions: Our results provide evidence that BPAF and BPS might be potential risk factors of GDM, which require to be studied further.

Keywords: gestational diabetes, bisphenol A, bisphenol S, bisphenol F, bisphenol AF, plasma glucose, endocrine disrupting chemicals

INTRODUCTION

Gestational diabetes mellitus (GDM) is a common complication during pregnancy and is defined as "any degree of glucose intolerance with onset or first recognition during pregnancy" (1, 2). GDM and hyperglycemia during pregnancy have been reported to be associated with adverse maternal, neonatal, and postnatal outcomes; thus, it is important to find potential risk factors for GDM. Except for the common-known risk factors (a high maternal age, being overweight before pregnancy, a family history of type 2 diabetes, a history of diabetes before pregnancy, etc.), concerns are increasingly being raised on the environmental factors for developing GDM, especially for some environmental chemicals that have endocrine-disrupting effects (3–5).

Bisphenol A (BPA, 2,2-bis(4-hydroxyphenyl)-propane), a typical endocrine disruptor, is widely used in the production of polycarbonate plastics and epoxy resins used in numerous consumer products, to which human beings are widely exposed to in daily life. Increasing evidence has indicated that BPA may be harmful to human health, especially with regard to endocrine metabolism (6–9). Evidence from animal studies has suggested that BPA exposure may disrupt glucose homeostasis and contribute to metabolic disorders; thus, BPA may be a risk factor for the development of diabetes (10–12). Epidemiological studies also suggested that BPA was associated with type 2 diabetes among the general population (13–16). In terms of pregnant women, as far as we were aware, only five epidemiological studies addressed the associations of urinary BPA concentration with blood glucose levels or GDM, but the conclusions were inconsistent (17–21).

Due to public concern on the potential harmful effects of BPA exposure, bisphenol analogs were used to substitute BPA in the manufacture of certain plastics and epoxy resins (22, 23). Three widespread and commercially used bisphenol analogs are bisphenol S (BPS, 4,4′-sulfonyldiphenol), bisphenol F (BPF, 4,4′-dihydroxydiphenylmethane), and bisphenol AF (BPAF, 2,2-bis(4-hydroxyphenyl)-hexafluoropropane) (23, 24). According to previous studies, BPA, BPS, BPF, and BPAF were detected in indoor dust samples and in food and beverages (25, 26), which indicates an ubiquitous exposure to bisphenols of human beings.

Considering the structural similarity to BPA, the analogs (BPS, BPF, BPAF) may have similar endocrine-disrupting effects to those of BPA (23, 27, 28). Studies in zebrafish have reported the disrupting effects on steroid hormones of BPS (29), BPF (30), and BPAF (31). However, the potential harmful effects on glucose homeostasis of these BPA substitutes, which were used more and more widely and frequently in our daily life, are still unknown.

Since findings on the associations between BPA exposure and the risk of GDM were inconsistent, and little was known on the endocrine-disrupting effects on human metabolism of BPA substitutes, we conducted the prospective cohort study to investigate the potential disrupting effects of bisphenols exposure on glucose metabolism. In this prospective study, we examined concentrations of four typical and widely used bisphenols (BPA, BPS, BPF, BPAF) in first-trimester urine samples of pregnant women and estimated the associations of the four bisphenols with GDM and plasma glucose levels in a population of pregnant women in central China. Meanwhile, it was reported that women with a different BMI before pregnancy or who were carrying a fetus of a different sex have different endocrine environments (32–34), so we carried out a further stratified analysis by pre-pregnancy BMI and fetal sex.

METHODS

Study Population

We conducted this prospective study and recruited pregnant women at the Wuhan Maternal and Child Healthcare Hospital (WMCHH) in Wuhan, China. A total of 2,145 eligible pregnant women with donated urine samples were recruited at WMCHH between October 2013 and April 2015. Eligibility criteria included singleton pregnancy, gestational age < 16 weeks at enrollment, and a willingness to give birth at the study hospital.

Among 2,145 pregnant women, we first excluded those who did not undertake the GDM screening during pregnancy ($n = 301$). Women with reported a family history of diabetes or reported a history of diabetes before pregnancy were also excluded ($n = 3$). Eventually, 1,841 pregnant women were included in the final analysis.

This study was approved by the ethics committees of Tongji Medical College, Huazhong University of Science and Technology [No. (2012)07], and Wuhan Maternal and Child Healthcare Hospital (No. 2012003). All participants agreed and signed informed consent.

Urine Samples Collection and Bisphenols Measurements

Urine samples were collected at 13 wk of gestation on average (ranging from 8 to 18 wk), and stored in polypropylene containers at −20°C until further analysis.

Urinary bisphenols concentrations were quantified using an Ultimate 3000 Ultra-high performance liquid chromatography system (Dionex, Sunnyvale, CA, USA) coupled to a Thermo Scientific™ TSQ Quantiva™ Triple Quadrupole mass spectrometer (Thermo Scientific, San Jose, CA) (UHPLC-TQMS) with the isotope labeled internal standards purchased from Sigma-Aldrich (St. Louis, U.S.A), which was described in our previous study (35). Briefly, 1 mL of urine sample was incubated with β-glucuronidase at 37°C overnight mixed with the internal standard solution (with the final concentration of 20 ng/mL). After enzymatic hydrolysis, the solution was extracted 3 times with a 3 mL solvent [methyl tert-butyl ether/ethyl acetate (5/1, v/v)] each time. The supernatants were combined and evaporated under nitrogen gas flow, and then reconstituted in 200 μL acetonitrile/water (6/4, v/v). Chromatographic separation was achieved on Thermo Scientific Betasil C18 column (2.1 mm × 100 mm, 3 μm) using a mobile phase gradient with water and acetonitrile. The compounds were detected by negative-ion electrospray ionization mass spectrometry and multiple reaction monitoring mode. The blanks and quality control samples were incorporated into each batch of samples. As reported before, the limit of detection (LOD), defined as the concentrations producing a signal-to-noise ratio equal to 3, was 0.2 μg/L for BPA and BPS, 0.1 μg/L for BPF and BPAF (35).

Considering the individual variation of urine dilution, we adjusted the bisphenols concentration by urine specific gravity (SG), which was measured by a handheld digital refractometer (Atago, Tokyo, Japan). The following formula was used to adjust urinary concentrations of bisphenols:

SG-adjusted Bisphenols (μg/L) = unadjusted Bisphenols (μg/L) × $[(SG_m\text{-}1)/(SG_i\text{-}1)]$

where SG_m is the median SG ($SG_m = 1.014$) of all the samples ($n = 1{,}841$), and SG_i is the observed SG for the individual urine sample.

GDM Diagnosis and Plasma Glucose Measurements

All pregnant women were routinely required to undertake the one-step GDM screening—a 2 h 75 g oral glucose tolerance test (OGTT) at 24–28 weeks of gestation in the study hospital. The diagnosis of GDM was according to the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria: fasting plasma glucose (FPG) ≥ 5.1 mmol/L (≥ 92 mg/dL), or 1 h plasma glucose (1 h-PG) ≥ 10.0 mmol/L (≥ 180 mg/dL), or 2 h plasma glucose (2 h-PG) ≥ 8.5 mmol/L (≥ 153 mg/dL) (2). We extracted glucose laboratory data of OGTT from the medical information system of the hospital, which recorded FPG ($n = 1{,}841$), 1 h-PG ($n = 1{,}830$), 2 h-PG ($n = 1826$) measured values for pregnant women in this study.

Covariates

For each participant, a face-to-face interview was conducted within 3 days before or after delivery by specially trained nurses to collect a variety of information, including demographic and socioeconomic characteristics (e.g., maternal age, occupation, and education levels) and lifestyle factors during pregnancy (e.g., smoking, passive smoking, and alcohol consumption). Pre-pregnancy body mass index (BMI, kg/m^2) was calculated using self-reported pre-pregnancy weight, which was extracted from the records of the first prenatal visit, and height was measured at the hospital. Information on family history of diabetes, diabetes history, and the infant's sex were retrieved from the medical information system mentioned above.

Statistical Analysis

Descriptive statistics were conducted to summarize the characteristics of the GDM group and non-GDM group in our study population. For measured bisphenol concentrations below the LOD, we assigned a value equal to the LOD divided by the square root of 2 in the analysis (36). Due to the skewed distribution of SG-adjusted bisphenols concentrations, we used the natural log-transformed values for further analysis, and the natural log-transformed SG-adjusted BPA concentration was abbreviated as ln (SG-adj BPA). We performed a Spearman correlation analysis to assess the correlations between urinary bisphenols [ln (SG-adj bisphenols)].

We selected the covariates included in the final models based on either their biologic plausibility (regardless of statistical significance) or the association with GDM in bivariate analysis ($p < 0.10$). According to these criteria, pre-pregnancy BMI (<18.5 kg/m^2, 18.5–23.0 kg/m^2, ≥ 23 kg/m^2), maternal age at delivery (years) and educational levels (high school or lower, college, university or above) were selected based on bivariate analysis ($p < 0.10$), and parity (nulliparous, multiparous), passive smoking during pregnancy (yes, no) and fetal sex (male, female) were selected based on biologic plausibility reported by previous studies.

We categorized participants into tertiles based on the distribution of SG-adjusted urinary BPA, BPS, and BPF concentrations, and BPAF concentration was categorized into a binary variable using the 66.6th percentile as the cut-point because 1,057 (57.41%) objects have a concentration value lower than LOD. We first used the multivariable logistic regression model to assess the association of bisphenols levels and GDM. Two multivariable regression models were conducted—model 1 was designed to investigate the effect of single bisphenol exposure and odds ratios for GDM with an adjustment for the covariates mentioned above, and model 2 was aimed to explore the co-exposure effects of multi-bisphenols by considering other bisphenols additionally in one model. We further conducted stratified analysis of bisphenols and GDM among women with normal weight (18.5 kg/m^2 q pre-pregnancy BMI < 22.9 kg/m^2) and women who were overweight (23.0 kg/m^2 q pre-pregnancy BMI < 28.0 kg/m^2). We selected the BMI cut-off point of 23.0 kg/m^2 for overweight subjects, according to a reported optimal cut-off value of BMI for urban Chinese female adults (37). A BMI of 28.0 kg/m^2 was used to discriminate between overweight and obesity according to the Working Group on Obesity in China (WGOC). Since few had met the criteria of being obese in our population ($n = 41$) and considering the potential confounding effects of a disrupted endocrine environment due to extreme

TABLE 1 | Characteristics of 1,841 participants in this study.

Characteristics	All participants (n = 1,841)	GDM (n = 167)	Non-GDM (n = 1,674)	P-Value[a]
Maternal age (years)	28.58 ± 3.27	30.07 ± 4.11	28.44 ± 3.14	<0.01
Pre-pregnancy BMI (kg/m²)	20.90 ± 2.87	22.32 ± 3.03	20.76 ± 2.81	<0.01
Pre-pregnancy BMI categories (kg/m²)				<0.01
<18.5	336 (18.25)	12 (7.19)	324 (19.35)	
18.5–22.99	1,162 (63.12)	84 (50.30)	1,078 (64.40)	
≥23	343 (18.63)	71 (42.51)	272 (16.25)	
Maternal education				0.01
High school or lower	355 (19.28)	47 (28.14)	308 (18.40)	
Some college	560 (30.42)	50 (29.94)	510 (30.47)	
University or above	926 (50.30)	70 (41.92)	856 (51.14)	
Passive smoking during pregnancy				0.30
No	1,246 (67.68)	107 (64.07)	1,139 (68.04)	
Yes	595 (32.32)	60 (35.93)	535 (31.96)	
Parity				0.01
0	1,615 (87.72)	136 (81.44)	1,479 (88.35)	
≥1	226 (12.28)	31 (18.56)	195 (11.65)	
Fetal sex				0.57
Male	976 (53.01)	85 (50.90)	891 (53.23)	
Female	865 (46.99)	82 (49.10)	783 (46.77)	

BMI, body mass index; GDM, gestational diabetes mellitus; Data were presented as N (%) and Mean ± SD.

[a]*p-Values estimates were based on Student's t-test for continuous variables expressed as Mean ± SD, and Pearson chi-squared test for categorical variables expressed as N (%).*

body weight status, we excluded those women with obesity in the BMI-stratified analysis.

To examine the associations between urinary bisphenol concentrations and plasma glucose (PG) levels, we performed multiple linear regression models for the continuous variables of glucose measurements, and bisphenol concentrations were treated as categorical variables and continuous variables, respectively. For continuous variables of bisphenols, we calculated the results with per-unit increases in ln (SG-adj bisphenols). We calculated z-scores for FPG, 1 h-PG, and 2 h-PG by subtracting the mean from each woman's glucose measurement in this study and dividing it by the corresponding standard deviation; the sum of the three resulting z-scores for each woman was used as an outcome variable (38, 39).

Pre-pregnancy BMI and the fetal sex were evaluated as potential effect modifiers, and stratified analyses were performed. In the BMI-stratified analysis, we restricted our analysis to the women with normal and overweight BMI group. We calculated the p-value for trend in analysis using the median values of each category of bisphenol and set it as a continuous variable in the statistical model. We calculated the p-value for interaction in the stratified analysis using likelihood ratio tests to examine the significance of interaction terms between continuous bisphenol concentration and the stratified variable. In the tertile analysis of BPA and glucose levels in overweight group, we observed that BPAs in the middle tertile were associated with decreased plasma glucose levels. To verify whether there were non-linear relationships between bisphenols and glucose levels or GDM, we conducted a restricted cubic spline (RCS) analysis for bisphenols among overweight participants.

All statistics were performed using SAS version 9.4 (SAS institute, Cary, NC). A two-sided $p < 0.05$ was considered as statistically significant.

RESULTS

The characteristics of 1,841 participants in this study are shown in **Table 1**. Among 1,841 participants, 167 (9.07%) women were diagnosed with GDM. Compared to women without GDM, women with GDM were older (30.07 vs. 28.44, years), had greater pre-pregnancy BMI (22.32 vs. 20.76, kg/m^2), had lower educational levels (the proportion of high school or lower was 28.14% vs. 18.40%), and were more likely to be multiparous (18.56% vs. 11.65%). No significant differences were observed in passive smoking and fetal sex for women with GDM vs. non-GDM (**Table 1**).

Table 2 shows the distributions of urinary bisphenols concentrations, and plasma glucose levels at 24–28 weeks of gestation. BPF had the highest detection rate (>LOD) (94.72%), followed by BPS (90.06%), BPA (79.25%), and BPAF (42.53%). Similarly, BPF had the highest geometric mean (GM) (1.74 μg/L for un-adjusted and 2.01 μg/L for SG-adjusted) and BPAF had the lowest GM (0.025 μg/L for un-adjusted and 0.030 μg/L for SG-adjusted). The high detection rates of urinary bisphenols concentration suggested that the participants in this study were widely and frequently exposed to BPA substitutes. Urinary bisphenols showed weakly pairwise correlations, with Spearman correlation coefficients lower than 0.3 (**Table S1**). The arithmetic mean (AM) of fasting plasma glucose (FPG) was 4.35

TABLE 2 | Distributions of first-trimester urinary bisphenols concentrations and plasma glucose levels.

Bisphenols	*N*	>LOD (%)	GM (95% CI) or AM ± SD	P25	P50	P75	P95
UN-ADJUSTED (μg/L)							
BPA	1,841	79.25	0.72 (0.66, 0.79)	0.27	1.11	2.66	10.05
BPS	1,841	90.06	0.30 (0.28, 0.32)	0.12	0.25	0.64	4.43
BPAF	1,841	42.59	0.025 (0.024, 0.026)	<LOD	<LOD	0.033	0.13
BPF	7,76[a]	94.72	1.74 (1.51, 1.99)	0.60	1.28	8.05	42.14
SG-ADJUSTED (μg/L)							
BPA	1,841	79.25	0.87 (0.79, 0.96)	0.34	1.41	3.13	14.71
BPS	1,841	90.06	0.36 (0.33, 0.38)	0.14	0.31	0.81	5.51
BPAF	1,841	42.59	0.030 (0.028, 0.031)	<LOD	<LOD	0.049	0.21
BPF	776[a]	94.72	2.01 (1.75, 2.32)	0.60	1.74	8.72	44.70
PLASMA GLUCOSE LEVELS (mmol/L)							
FPG	1,841	100%	4.35 ± 0.48[d]	4.07	4.31	4.57	5.04
1 h-PG	1,830[b]	100%	6.99 ± 1.57[d]	5.89	6.83	7.93	9.80
2 h-PG	1,826[c]	100%	6.31 ± 1.29[d]	5.49	6.19	6.98	8.49
Sum of PG *z*-score	1,820[d]	NA	−0.01 ± 2.42[d]	−1.53	−0.29	1.10	3.97

LOD, limit of detection; GM, geometric mean; AM, arithmetic mean; SD, standard deviation; SG, specific gravity; FPG, fasting plasma glucose; PG, plasma glucose; NA, not available.
[a] *For BPF, 776 urine samples were examined.*
[b] *Among 1,841 subjects, 11 didn't have the 1 h-PG measures in medical records.*
[c] *Among 1,841 subjects, 15 didn't have the 2 h-PG measures in medical records.*
[d] *Among 1,841 subjects, 1,820 (98.86%) has complete 3 time points OGTT measures.*

mmol/L, the AM of plasma glucose after 1 h (1 h-PG) was 6.99 mmol/L, and the AM of plasma glucose after 2 h (2 h-PG) was 6.31 mmol/L. Their median values and selected percentiles are presented in **Table 2**.

Table 3 shows the associations of SG-adjusted urinary bisphenol concentrations in tertiles (BPAF was categorized to concentration ≥66.6 percentage (0.036 μg/L) or below) with GDM. We did not observe any significant associations between the urinary levels of bisphenols and GDM among all participants. However, in stratified analysis, the highest category of BPAF was significantly associated with increased odds of GDM among women with normal weight ($18.5 \leq$ BMI < 23 kg/m^2, $n = 1{,}162$) compared to the lowest category [odds ratio (OR) = 1.70 (95% CI: 1.08, 2.67) after adjustment for maternal age, educational levels, parity, passive smoking, and fetal sex]. The association remained significant after further adjustments for urinary BPA and BPS levels. No significant associations of levels of BPA, BPS, and BPF were found with GDM (**Table 3**).

Table 4 presents the associations of urinary bisphenol levels and plasma glucose levels among all participants. Compared to the lowest category of BPAF, women in the highest category had a mean of 0.05 mmol/L (95% CI: 0.01, 0.09) higher fasting glucose concentration. We also observed that BPS was associated with increased FPG levels [β = 0.03 (95% CI: 0.01, 0.04)] and an increased sum of PG *z*-score [β = 0.07 (95% CI: −0.00, 0.14)] with per-unit increases in ln (SG-adj BPS). We further performed a stratified analysis to investigate the associations of urinary bisphenols with glucose levels among women with normal weight and overweight, respectively. The results showed that BPAF was significantly associated with increased FPG [β = 0.07 (95% CI: 0.02, 0.12)] and the sum of the PG *z*-score [β = 0.26 (95% CI: 0.01, 0.50)] for the highest category compared to the lowest one after adjustment for potential confounders among women with normal pre-pregnancy BMI (**Table S2**). BPA in the second tertile was found to be associated with decreased FPG [β = −0.19 (95% CI: −0.36, −0.02)] and 2 h-PG levels [β = −0.43 (95% CI: −0.84, −0.03)] and the sum of the PG *z*-score [β = −0.95 (95% CI: −1.77, −0.13)] compared to the first tertile among overweight group, but there was no significance in trend analysis (**Table S3**). No other significant associations of bisphenols and GDM or plasma glucose levels were observed in pre-pregnancy BMI and fetal sex stratified analysis (**Tables S4**, **S5**, and **S7**).

Figure 1 showed the restricted cubic spline analysis for the associations between BPA and glucose levels among women who were overweight. Non-linear associations were observed among overweight women in terms of fasting plasma glucose levels and *z*-score of plasma glucose (p for non-linear association <0.05). The dose-response relationships between BPA and plasma glucose levels also indicated a "U-shaped" association between BPA exposure and fasting plasma glucose levels (**Figure 1**). However, no significant non-linear association was observed for GDM among overweight women (**Figure S1**).

Additionally, we found that the associations between BPS and plasma glucose might be modified by fetal sex (p for interaction <0.01 for sum of PG *z*-score and 1 h-PG, <0.05 for FPG) (**Table 5**). Specifically, significant results were only observed among women with female fetus and the per-unit increase in ln (SG-adj BPS) was associated with higher FPG [β = 0.04 (95% CI: 0.02, 0.06)], 1 h-PG [β = 0.11 (95% CI: 0.04, 0.17)], and the sum of the PG *z*-score [β = 0.19 (95% CI: 0.08, 0.30)].

DISCUSSIONS

While the relationship between BPA and GDM has been investigated in some studies, reports on the effects of its analogs

TABLE 3 | Associations of urinary concentrations of bisphenols and GDM.

Bisphenols	All participants (n = 1,841)			18.5 q BMI < 23 kg/m^2 (n = 1,162)			23 q BMI < 28 kg/m^2 (n = 302)		
	GDM/Total	Model 1	Model 2	GDM/Total	Model 1[a]	Model 2[a]	GDM/Total	Model 1[a]	Model 2[a]
		OR (95% CI)	OR (95% CI)		OR (95% CI)	OR (95% CI)		OR (95% CI)	OR (95% CI)
BPA									
Low	61/613	Reference	Reference	24/387	Reference	Reference	30103	Reference	Reference
Medium	51/613	0.84 (0.56, 1.26)	0.84 (0.56, 1.26)	26/381	1.14 (0.64, 2.03)	1.13 (0.63, 2.03)	18/105	0.53 (0.26, 1.06)	0.53 (0.26, 1.07)
High	55/615	0.94 (0.63, 1.40)	0.90 (0.60, 1.37)	34/394	1.38 (0.80, 2.39)	1.22 (0.69, 2.16)	18/94	0.59 (0.29, 1.19)	0.61 (0.28, 1.31)
p for trend		0.90	0.71		0.24	0.51		0.21	0.27
BPS									
Low	58/614	Reference	Reference	26/366	Reference	Reference	25/113	Reference	Reference
Medium	58/612	1.06 (0.71, 1.58)	1.07 (0.72, 1.60)	27/394	0.94 (0.53, 1.65)	0.92 (0.52, 1.62)	25/95	1.59 (0.81, 3.15)	1.66 (0.83, 3.30)
High	51/615	0.89 (0.59, 1.34)	0.84 (0.55, 1.28)	31/402	1.05 (0.61, 1.82)	0.88 (0.50, 1.55)	16/94	0.79 (0.38, 1.64)	0.83 (0.38, 1.81)
p for trend		0.45	0.30		0.76	0.70		0.26	0.37
BPAF									
Low	109/1,227	Reference	Reference	46/769	Reference	Reference	50/223	Reference	Reference
High	58/614	1.24 (0.87, 1.76)	1.33 (0.91, 1.93)	38/393	1.70 (1.08, 2.67)*	1.68 (1.03, 2.72)*	16/79	0.97 (0.49, 1.91)	1.22 (0.57, 2.60)
p for trend		0.23	0.14		0.02*	0.04*		0.93	0.61
BPF (n = 776)[b]									
Low	21/258	Reference	Reference	9/164	Reference	Reference	10/38	Reference	Reference
Medium	24/258	1.24 (0.65, 2.36)	1.25 (0.65, 2.42)	14/158	1.67 (0.69, 4.09)	1.55 (0.62, 3.90)	7/40	0.73 (0.23, 2.29)	0.85 (0.25, 2.85)
High	30/260	1.29 (0.69, 2.41)	1.25 (0.66, 2.38)	17/157	2.06 (0.86, 4.93)	1.87 (0.75, 4.65)	12/65	0.70 (0.25, 1.96)	0.93 (0.31, 2.81)
p for trend		0.56	0.66		0.18	0.28		0.62	0.99

BMI, body mass index; GDM, gestational diabetes mellitus. Model 1: Adjusted for maternal age, pre-pregnancy BMI, educational levels, parity, passive smoking and fetal sex. Model 2: Additionally adjusted for other bisphenols based on model 1, for BPA, BPS, BPAF, adjustments was except for BPF due to the differences in sample size.

[a] *Adjusted for confounders above except for pre-pregnancy BMI.*

[b] *Urine samples of 776 participants were measured for BPF. Among them, 479 women's pre-pregnancy BMI were between 18.5 and 22.9, and 143 participants' pre-pregnancy BMI were between 23 and 27.9 (kg/m^2).*

**Significant p-value.*

(BPS, BPF, and BPAF) on glucose homeostasis of pregnant women are rather limited. To our knowledge, this is the first study to examine the associations between exposures to BPA substitutes and the risk of GDM and plasma glucose levels. In this study, we found that BPAF was associated with an increased risk of GDM and increased plasma glucose levels among pregnant women with normal pre-pregnancy weight. In addition, we observed fetal sex specific effects of BPS on glucose metabolism, which indicated that women carrying a female fetus might be more sensitive and vulnerable to BPS exposure than those carrying a male fetus.

We were aware of five epidemiology studies that had investigated the effects of BPA exposure on glucose metabolism among pregnant women (17–21). Three of them addressed the associations between BPA and GDM, two of which reported null associations and one retrospective study from China reported that urinary BPA levels at the third trimester were associated with a decreased risk of GDM and lower plasma glucose levels (20). Another two studies found positive associations of urinary BPA concentrations with glucose levels during pregnancy. In this study, we did not find associations between BPA and GDM, but we observed non-linear associations between BPA and glucose levels among women who were overweight before pregnancy. The retrospective study from China assessed BPA levels in urine samples just before delivery, while in the present study we used urine samples in early pregnancy, which may contribute to the inconsistence. We observed that moderate BPA exposure were associated with decreased plasma glucose among women who were overweight, which seemed to be opposite to the findings of Bellavia et al.'s (21) study. There are several reasons we assumed this. First, the concentrations of urinary BPA in this study were lower than that reported in Bellavia et al.'s (21) study [geometric means (GM): 0.72 vs. 1.23 for un-adjusted and 0.87 vs. 1.3 for SG-adjusted concentration, ug/L], which may explain the different findings. Second, Bellavia et al. (21) did not exclude women with obesity in their analysis, which may contribute to some confounding effects in the results. Moreover, there are some significant differences in our study population and that of Bellavia et al.'s (21) study, such as races (Chinese vs. American) and the rate of being overweight/obese [18.63% in our study population compared to 44.96% in Bellavia et al.'s (21) study]. Also, Bellavia et al. (21) did not report non-linear associations between BPA exposure and glucose levels. Thus, we believe that our results of BPA and glucose levels among overweight women are not in conflict with Bellavia et al.'s (21), and those could be supplementary to the evidence of disrupting effects of BPA exposure on glucose levels of pregnant women.

The non-monotonic dose-response (NMDR) endocrine-disrupting effects of BPA on glycemia metabolism have been

TABLE 4 | Associations of urinary bisphenols and glucose levels among all participants ($n = 1,841$)[a].

Bisphenols	FPG		1 h-PG		2 h-PG		Sum of PG *z*-scores	
	b (95% CI)	*p*	b (95% CI)	*p*	b (95% CI)	*p*	b (95% CI)	*p*
BPA								
Per-unit increase in ln (SG-adj BPA)	0.00 (−0.01, 0.01)	0.95	−0.02 (−0.05, 0.02)	0.32	−0.01 (−0.04, 0.02)	0.43	−0.02 (−0.07, 0.03)	0.42
Low	Reference		Reference		Reference		Reference	
Medium	−0.04 (−0.09, 0.01)	0.12	−0.05 (−0.22, 0.11)	0.53	−0.01 (−0.14, 0.13)	0.94	−0.13 (−0.38, 0.13)	0.34
High	0.01 (−0.04, 0.06)	0.68	−0.05 (−0.22, 0.12)	0.55	0.00 (−0.14, 0.14)	0.99	−0.02 (−0.28, 0.24)	0.88
p for trend		0.39		0.62		0.98		0.94
BPS								
Per–unit increase in ln (SG–adj BPS)	0.03 (0.01, 0.04)	<0.01*	0.04 (−0.01, 0.09)	0.09	0.01 (−0.03, 0.05)	0.54	0.07 (−0.00, 0.14)	0.05
Low	Reference		Reference		Reference		Reference	
Medium	0.03 (−0.02, 0.09)	0.20	0.13 (−0.04, 0.30)	0.14	−0.03 (−0.17, 0.11)	0.70	0.12 (−0.14, 0.37)	0.37
High	0.05 (−0.00, 0.10)	0.06	0.14 (−0.03, 0.31)	0.10	0.01 (−0.12, 0.15)	0.84	0.18 (−0.07, 0.44)	0.16
p for trend		0.11		0.21		0.70		0.22
BPAF								
Per–unit increase in ln (SG–adj BPAF)	0.01 (−0.00, 0.03)	0.13	0.01 (−0.05, 0.08)	0.66	0.00 (−0.05, 0.05)	0.99	0.03 (−0.06, 0.12)	0.53
Low	Reference		Reference		Reference		Reference	
High	0.05 (0.01, 0.09)	0.03*	0.04 (−0.11, 0.18)	0.64	0.05 (−0.07, 0.17)	0.39	0.15 (−0.07, 0.37)	0.19
p for trend		0.03*		0.64		0.39		0.19
BPF ($n = 776$)								
Per–unit increase in ln (SG-adj BPF)	0.01 (−0.01, 0.02)	0.51	0.02 (−0.03, 0.08)	0.45	−0.02 (−0.06, 0.03)	0.42	0.01 (−0.08, 0.09)	0.87
Low	Reference		Reference		Reference		Reference	
Medium	0.01 (−0.08, 0.09)	0.90	−0.08 (−0.35, 0.20)	0.58	−0.18 (−0.40, 0.04)	0.12	−0.20 (−0.60, 0.21)	0.34
High	0.01 (−0.08, 0.10)	0.89	−0.07 (−0.34, 0.21)	0.64	−0.20 (−0.42, 0.02)	0.08	−0.18 (−0.59, 0.23)	0.38
p for trend		0.93		0.80		0.22		0.60

SG, specific gravity; FPG, fasting plasma glucose; PG, plasma glucose.

[a]*Adjusted for maternal age, pre-pregnancy BMI, educational levels, parity, passive smoking and fetal sex.*

**Significant p-value.*

reported in both *in vivo* and *in vitro* studies (9, 40–42). Alonso-Magdalena et al. demonstrated an inverted U-shaped relationship between BPA in environmentally relevant doses and insulin content measured after 48 h (43). Several *in vivo* experiments have reported the great disrupting effects of low-dose BPA on blood glucose homeostasis and pancreatic β-cell function (10, 11, 44, 45). The administration of low-dose BPA exposure (10 μg/kg) among adult mice led to a rise of plasma insulin and induced a rapid decrease in glycemia (44). The U-shaped relationship between BPA and fasting plasma glucose levels found in this study, though only observed among pregnant overweight women, indicates an NMDR effect of BPA exposure on human glucose metabolism, which needs to be verified and investigated in future studies.

Due to the wide and frequent use of BPA substitutes, we also assessed the relationships of BPA substitutes and GDM in this study. We found that urinary BPAF was associated with GDM among women with normal weight. Evidence from cyto-experiments and animal studies suggested that BPAF could be a rather toxic substance (46, 47). Furthermore, our results were generally consistent with a recent case-control study which reported that BPAF and BPS were associated with type 2 diabetes among general population in China (48). We only observed positive associations among women with normal weight, which account for the majority of the participants in this study (63.05%), and the potential explanations might be: (1) overweight women overweight are at a high risk of GDM and the adverse effects of BPAF exposure may be covered; (2) before-pregnancy adiposity status of pregnant women might have interaction effects with BPAF exposure and may lead to different results between women with normal weight and women who are overweight. However, BPAF had the lowest detection rate (42.53%) among the four bisphenols, though we had tried to restrict our analysis in a subgroup of BPAF-detectable women and the results were largely consistent (**Table S6**). Meanwhile, the number of

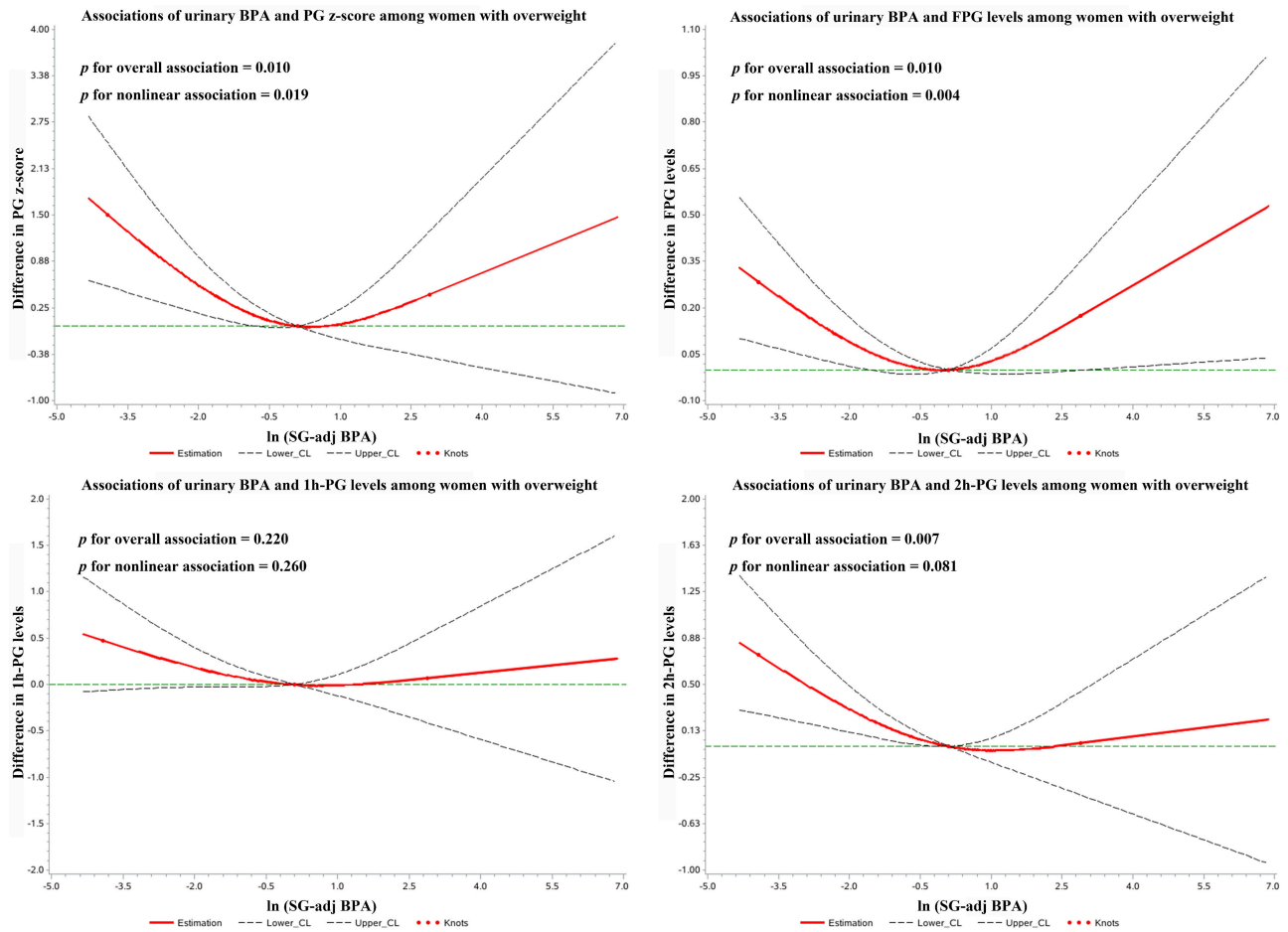

FIGURE 1 | Restricted cubic spline for the associations between urinary BPA and plasma glucose levels among women with overweight. The red lines represent differences in glucose levels for natural log transformed specific gravity adjusted urinary BPA concentration with adjustment for maternal age, educational levels, parity, passive smoking, and fetal sex. Knots were set at the 5th, 50th, 95th percentiles and the reference value was set to median of urinary BPA distribution among women with overweight. Dashed lines represent 95% CI.

GDM cases was small (n = 16) in the high BPAF category of the overweight group, which may cause a high variance in analysis. Thus, the results should be interpreted with caution and further investigations are required to confirm our findings.

We also found that BPAF and BPS were associated with higher glucose levels among all participants, and fetal sex specific effects were observed for BPS exposure. The disrupting effects on glucose metabolism of BPAF and BPS were reported in animal studies (47). *In vitro* experiments indicated that the potential mechanism of endocrine-disrupting effects of BPAF and BPS might be involved in the stimulation of estrogen receptors (49–51). Consistent with the results of GDM, in the further stratified analysis, we found that BPAF was associated with increased glucose levels among women with normal weight. A possible explanation is that higher adiposity levels imply higher levels of circulating estrogen in women who are overweight, higher circulating estrogen levels could efficiently compete for receptors with BPAF and the disrupting effect of BPAF can be partially eliminated. In this study, we only observed glucose-disrupting effects of BPS among women carrying a female fetus in stratified analysis by fetal sex. We speculate that the fetal sex-difference effects may be attributed to different sex-hormone levels in maternal circulations. Since sex-difference effects of BPS were also reported in another study (52), the underlying mechanism needs to be further studied in detail.

We did not observe any associations between urinary BPF and GDM or glucose levels, though it was reported to be associated with increased 17b-estrodiol (E2), both in animal studies and cell experiments (34, 43). However, it should be noted that BPF was measured in a population with a relatively smaller sample size compared to other bisphenols in this study. However, BPF has the highest detection rate (94.72%) in urine samples of pregnant women, which indicates a ubiquitous exposure to this BPA substitute among the study population. Therefore, more studies with a larger sample size is needed to clarify the potential health effects in human population.

TABLE 5 | Associations of BPS concentrations with glucose levels, stratified by fetal sex[a].

BPS	FPG		1 h PG		2 h-PG		Sum of PG *z*-scores	
	b (95% CI)	*p*	b (95% CI)	*p*	b (95% CI)	*p*	b (95% CI)	*p*
WOMEN WITH FEMALE FETUS (*n* = 865)								
Per-unit increase in ln (SG-adj BPS)	0.04 (0.02, 0.06)	<0.01*	0.11 (0.04, 0.17)	<0.01*	0.04 (−0.01, 0.10)	0.13	0.19 (0.08, 0.30)	<0.01*
Low	Reference		Reference		Reference		Reference	
Medium	0.04 (−0.04, 0.12)	0.31	0.07 (−0.18, 0.31)	0.60	0.00 (−0.21, 0.21)	1.00	0.11 (−0.27, 0.50)	0.56
High	0.11 (0.03, 0.19)	<0.01*	0.28 (0.04, 0.52)	0.02*	0.08 (−0.13, 0.28)	0.46	0.45 (0.07, 0.84)	0.02*
p for trend		<0.01*		0.02*		0.40		0.02*
WOMEN WITH MALE FETUS (*n* = 976)								
Per-unit increase in ln (SG-adj BPS)	0.01 (−0.01, 0.03)	0.15	−0.01 (−0.08, 0.05)	0.66	−0.01 (−0.06, 0.04)	0.59	−0.03 (−0.12, 0.06)	0.56
Low	Reference		Reference		Reference		Reference	
Medium	0.03 (−0.04, 0.10)	0.41	0.20 (−0.03, 0.44)	0.09	−0.04 (−0.22, 0.15)	0.70	0.14 (−0.20, 0.48)	0.42
High	0.00 (−0.07, 0.07)	0.99	0.03 (−0.21, 0.26)	0.82	−0.05 (−0.23, 0.14)	0.63	−0.05 (−0.39, 0.29)	0.78
p for trend		0.73		0.66		0.7		0.51
p for interaction[b]		0.03*		<0.01*		0.11		<0.01*

SG, specific gravity; FPG, fasting plasma glucose; PG, plasma glucose.

[a]*Adjusted for maternal age, pre-pregnancy BMI, educational levels, parity, passive smoking.*

[b]*p for interaction was calculated using the interaction term of ln (SG-adj BPS)*sex*

**Significant p-value.*

From the aspect of molecular composition, though four bisphenols are similar in chemical structure, a fact that should not be ignored is that BPA and BPF only contains carbon and hydrogen atoms while BPS additionally contains sulfur atom and BPAF additionally contains fluorine atoms which may contribute to different biological effects. A recent animal study also suggested that bisphenols may disrupt the endocrine system in different manners, whereas BPS and BPAF exposure, compared to BPA and BPF exposure, significantly disrupted glucose homeostasis, as reported in this study. Moreover, since BPA was substituted by its analogs, the exposure dose of BPA was lower, while that of BPA substitutes was higher, which may lead to more evident findings in BPAF and BPS (48).

One strength of our study is that we used a cohort-based prospective study design to investigate the associations of urinary bisphenols with GDM and blood glucose levels with adjustment for potential confounders. We also performed a model adjusted to other bisphenols and tested the co-exposure effects. Another strength is that we used the OGTT data of each participant obtained from the medical records system and the diagnosis of GDM was based on the criteria from IADPSG by professional physicians. Moreover, we further analyzed the data stratified by fetal sex and pre-pregnant BMI to investigate the potential modification effects.

In this study, urinary bisphenols levels were used since phenols are mainly excreted into urine, and bisphenols concentrations in urine samples are widely accepted biomarkers of the recent exposures to bisphenols (22, 53, 54). A limitation of this study is that we measured bisphenols in only one spot urine sample for each woman and this may have led to a misclassification of the women's bisphenols exposure. Considering the short biological half-life of bisphenols (BPA < 6 h, BPS < 7 h) (55, 56), one spot urine may be insufficient for an accurate evaluation of BPA exposure. However, according to previous studies, a single spot urine sample is able to predict a subject's tertile categorization which was used for the analyses in this study (57, 58). In addition, we did not collect the information on the source of exposure and we were unable to verify whether human exposure to BPA, BPS, BPF, and BPAF were from the same source. However, this lack of information had no impact on our main results and conclusion of this study. Also, we did not have information on baseline glucose levels and energy intake; thus, it cannot be adjusted in the analysis models. Moreover, the limited sample size in stratified analysis has restricted the power to make a robust conclusion, which should be improved in future studies. Finally, due to the potential differences between women's races, exposure patterns, and random effects, more prospective studies with more accurate exposure evaluations are needed to confirm the findings of our study.

CONCLUSION

In this prospective cohort study, we found that BPAF was associated with an increased risk of GDM among pregnant women of normal weight. Additionally, a disrupting effect on plasma glucose was observed for BPS and the effect might be modified by fetal sex. In conclusion, we observed the endocrine-disrupting effects of BPA substitutes (BPS and BPAF) on blood glucose metabolism among Chinese pregnant women, which might constitute potential risk factors of GDM.

ETHICS STATEMENT

This study was approved by the ethics committees of Tongji Medical College, Huazhong University of Science and Technology [No. (2012)07], and Wuhan Maternal and Child Healthcare Hospital (No. 2012003). All participants agreed and signed informed consent.

AUTHOR CONTRIBUTIONS

WZ analyzed the data and wrote this manuscript. Valuable suggestions in data analysis and paper writing were gained from WX and SX. WL, XL, and JH helped for urine samples collection and baseline data obtaining. BZ helped a lot in our work conducted in the study hospital. YZ and JL were responsible for urine samples measurements. ZC and YL were in charge of this study and paid a lot of time in revising this manuscript.

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SYSTEMATIC REVIEW
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Insulin-Like Growth Factor Axis Biomarkers and Gestational Diabetes Mellitus: A Systematic Review and Meta-Analysis

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The insulin-like growth factor (IGF) axis has been implicated in glucose homeostasis. It is plausible to hypothesize that the IGF axis is involved in the development of gestational diabetes mellitus (GDM). In a systematic review of the evidence on IGF axis biomarkers in relation to GDM, we searched the PubMed and EMBASE for publications up to May 31, 2018, on the associations of circulating IGF axis biomarkers with GDM. Eligible studies must meet the pre-specified quality assessment criteria. Meta-analyses were conducted where there were at least three studies on the same biomarker at the same gestational age window—early (<20 weeks), mid (20–29 weeks), or late (30+ weeks) gestation. Twelve studies were included (484 GDM, 1755 euglycemic pregnancies). Meta-analyses showed that GDM was consistently associated with higher IGF-I concentrations in mid-gestation (six studies) and late gestation (six studies). There were only two studies on IGF-I in early gestation and GDM with inconsistent findings. GDM was associated with lower IGFBP-2 concentrations in early, mid-, or late gestation, according to data from one or two studies. GDM was associated with higher IGFBP-3 concentrations in late gestation according to a meta-analysis of five studies. There was no association with GDM for IGFBP-3 in early or mid-gestation, according to data from one study. Other IGF axis biomarkers (IGF-II, IGFBP-1,−4,−5−6, and −7) showed no or inconsistent associations, and the data at early gestation were scanty or absent. Available evidence is suggestive but inconclusive concerning whether the IGF axis is involved in the development of GDM. More studies on IGF axis biomarkers in early gestation are warranted. If a specific IGF axis molecule is proven to be involved in the development of GDM, this may point to a new molecular target for designing interventions to reduce the incidence of GDM.

Keywords: insulin-like growth factor, insulin-like growth factor binding protein, gestational diabetes mellitus, systematic review, meta-analysis

INTRODUCTION

Gestational diabetes mellitus (GDM), commonly defined as impaired glucose tolerance with onset or first recognition during pregnancy, affects 5–15% of pregnant women (1, 2). The etiology of GDM remains incompletely understood, but pancreatic β-cell function insufficiency in compensating for pregnancy-induced insulin resistance is thought to be important, resulting in hyperglycemia in the second half of pregnancy (3). GDM develops when the maternal insulin supply is insufficient to maintain euglycemia during pregnancy. GDM increases the risk of maternal complications (gestational hypertension and preeclampsia) and fetal and neonatal complications (congenital malformations, macrosomia, preterm birth, and shoulder dystocia) (4). GDM may also "program" long-term adverse consequences such as the metabolic syndrome, type 2 diabetes, and cardiovascular disease in the offspring (5).

Traditionally linked to the regulation of cellular growth and differentiation, the insulin-like growth factor (IGF) axis is a signal transduction complex consisting of (1) the growth factors (IGF-I and IGF-II), (2) IGF binding proteins (IGFBPs) that may regulate their bio-available fractions, and (3) membrane receptors through which they act (6). Given the structural similarities of IGFs with insulin, IGFs and other components of the IGF axis have been implicated in glucose homeostasis (7, 8). Therefore, it is plausible to hypothesize that the IGF axis is involved in the development of GDM.

The metabolic effects of IGF-I are to provide a signal to cells that adequate nutrients are available to avoid apoptosis and enhance cellular protein synthesis enabling cells to undergo hypertrophy in response to an appropriate stimulus and stimulating cell division. Studies have shown that IGF-I can promote glucose uptake in peripheral tissues (9, 10) and suppress hepatic glucose production (11, 12). A significant positive correlation between insulin sensitivity and endogenous IGF-I concentration in patients with glucose intolerance has been reported (13). We are unaware of any data on whether IGF-II is related to insulin sensitivity.

The IGFBPs may also play a role in glucose metabolism. IGFBP-1 may regulate glucose levels through its impact on free IGF-I level (14). IGFBP-2 has been associated with an anti-diabetic effect in mice (15). IGFBP-3 is the most abundant IGFBP in circulation, and its metabolic effects are largely opposite to IGF-I; IGFBP-3 inhibits the biological activity of IGF-I by sequestrating IGF-I into a circulating reservoir, thereby reducing free IGF-I levels in circulation, and has been positively associated with the risk of diabetes (16).

Given the suggested roles of the IGF axis in glucose homeostasis, it is plausible that maternal circulating concentrations of IGF axis biomarkers may be associated with GDM. To our knowledge, there is no systematic review on the relationships between maternal IGF axis biomarkers and GDM. We thus conducted a systematic review and meta-analysis of the literature on circulating IGF axis biomarkers in relation to GDM, following the MOOSE Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies (17).

Abbreviations: IGF, insulin-like growth factor; IGFBP, IGF binding protein.

METHODS

This was a systematic review and meta-analysis. Before the review, we conducted an initial literature screen in PubMed to affirm that the topic of interest has not yet been systematically reviewed. The review protocol was not registered in any registry.

Data Sources

We searched the PubMed and EMBASE for publications up to May 31, 2018 (date last searched) on IGF axis biomarkers in relation to GDM using the following keywords: (gestational diabetes mellitus or gestational diabetes or GDM) and (insulin-like growth factor or IGF-I or IGF-II or IGF-1 or IGF-2 or IGF1 or IGF2 or insulin-like growth factor binding protein or IGF binding protein or IGFBP). There was no language restriction on publications. We did not use the keyword IGF receptor since exploratory searches did not find any report on circulating IGF receptors in GDM (probably undetectable in circulation). A total of 282 article titles were retrieved. Two reviewers (XRW, a PhD candidate in pediatrics; and WJW, a PhD candidate in perinatal epidemiology) independently screened all titles and abstracts for relevance (i.e., whether the study addresses the associations of maternal circulating IGF axis biomarkers with GDM). Discrepancies were resolved through discussions with a senior reviewer (XY, professor in pediatrics; XH, associate professor in obstetrics; FO, professor in pediatric epidemiology; Z-CL, scientist in perinatal epidemiology). Review articles were considered relevant in this initial literature screening. A total of 72 abstracts were deemed relevant, and the full-length articles were obtained for further assessment of eligibility. Bibliographies of retrieved articles were cross-referenced to identify additional studies. This review did not cover unpublished studies, which might be of uncertain quality.

Study Selection

Eligible studies must meet all of the following criteria: (1) studies must contain original data on maternal IGF axis biomarkers in relation to GDM in humans, (2) observational studies (cross-sectional, case–control, or cohort studies), and (3) plasma or serum concentrations of IGF axis biomarkers available. We excluded review articles (9), studies measuring IGF axis biomarkers from inappropriate blood samples (following stimulation or collected in the non-pregnancy period, $n = 21$), studies that did not separate GDM from chronic diabetes ($n = 13$), and studies with cases only ($n = 5$), leaving 24 articles for study quality assessment.

The primary studies were assessed using pre-defined quality assessment criteria for non-randomized observational studies adapted from Duckitt and Harrington (18) with some modifications to match the needs of the present systematic review. The assessment items included the representativeness of study participants, comparability of groups, definition of outcome, ascertainment of outcome, sample size, and study design (**Table 1**). Two reviewers independently conducted the

TABLE 1 | Quality assessment of non-randomized observational studies.

1. Selection of participants (1/0)

Cohort studies (1/0)
Selected cohort was representative of the general population (population-based studies) or target catchment (hospital-based studies) population (1)
Cohort was a selected unrepresentative group or the selection of the group was not defined (0)
Case–control studies (1/0)
Cases and controls drawn from the same population (1)
Cases and controls drawn from different sources or the selection of groups was not described (0)

2. Comparability of groups (2/0)

No significant differences between the groups reported in terms of age and pre-existing medical conditions were explicitly reported, or these differences were adjusted for in the analyses (2).
Differences between groups were not examined (1).
Groups differed and no adjustment results provided (0)

3. Definition of outcomes (2/0)

Definition of outcomes (gestational diabetes)
Referenced definition or explicit specified commonly accepted definition (2)
Explicit modified definition, but according to commonly accepted definition (1)
Unspecified or unacceptable definition (0)

4. Ascertainment of outcomes (2/0)

How the diagnosis was made
Prospectively diagnosed or review of notes/hospital discharge records (2)
ICD or database coding (1)
Process not described (0)

5. Sample size (1/0)

≥300 participants in a cohort study, or ≥20 in each study group in a case control study (1)
<300 participants in a cohort study, or <20 in either study group in a case control study (0)

6. Study design (2/0)

Prospective (2)
Cross-sectional or retrospective (1)
Not described or poorly designed (0)

Exclusion: score zero in any item (1 to 6) or a total score <7 out of 10 maximal points

quality assessment, and any differences were resolved through discussions with a senior third reviewer (Z-CL, XH, or FO).

We excluded studies that scored zero in any of the six quality assessment categories or with a total score <7 out of 10 maximal points. A total of 12 original studies were retained in the final systematic review (19–30); their quality scores are presented in **Table 2**. Among these studies, 7 studies scored 10, 3 studies scored 9, and 2 studies scored 8. The flowchart in the selection of studies is presented in **Figure 1**.

Data Extraction, Tabulation, and Analysis

Two reviewers independently extracted relevant study data from the original articles, and discrepancies were resolved through discussions with a senior reviewer (XY, XH, FO, or Z-CL). The following information was extracted into an Excel spreadsheet: the first author, country, year of publication, maternal race/ethnicity, age, body mass index (BMI), definition of GDM, method in the ascertainment of GDM, study design, sample size, comparability of groups, gestational age at blood sampling, type of maternal blood specimen (plasma or serum), and mean and standard deviation (SD) of the reported plasma or serum concentrations of IGF axis biomarkers (IGF-I, IGF-II, and IGFBPs). Where the SD was unavailable but the standard error (SE) available, the SD was calculated from the SE (SD = SE multiplied by the square root of sample size). Where the required data are unclear or unavailable, we contacted the corresponding author through email for clarification in at least two attempts 2 weeks apart.

Data were summarized for IGF axis biomarkers in early (≤19 weeks, before the GDM diagnosis), middle (20–29 weeks, around the time of GDM diagnosis; GDM is routinely screened at 24–28 weeks of gestation, although some high-risk patients may be screened earlier), and late pregnancy (30+ weeks, after the diagnosis and/or treatment) separately. Summary statistics were calculated using Review Manager 5.3 (Cochrane Collaboration, Oxford, United Kingdom). The inverse variance method was adopted in the meta-analysis to calculate the weighted mean difference (WMD) and 95% confidence intervals (CI) comparing GDM vs. euglycemic (control) women. The heterogeneity between studies was indicated intuitively by the I^2 statistics. If $I^2 > 50\%$, a random effects model was used in the pooled data analysis; otherwise, a fixed effects model was applied. When there were less than three study data lines in a comparison, we did not conduct pooled data meta-analysis, but described the key results in individual studies.

Where the odds ratios (ORs) or relative risks (RRs) were reported, we described them in individual studies. Because the ORs or RRs were often unavailable, and were calculated according to different approaches (e.g., the RRs comparing the highest vs. lowest quartiles, or the highest vs. lowest tertiles), we did not calculate the pooled OR or RR.

Patient and Public Involvement

There is no patient and public involvement in this systematic review.

RESULTS

Characteristics of Included Studies

Of the 12 studies included in the systematic review (total: 484 GDM and 1,755 euglycemic/control pregnancies), 4 studies were from North America, 2 from Australia, and 6 from other countries (**Table 3**). Caucasian was the most commonly studied race. There were four prospective cohort studies, three nested case–control studies, and five case control studies. There were eight studies measuring IGF axis biomarkers in maternal serum and four studies in maternal plasma.

IGF Axis Biomarkers

IGF-I was reported in all the 12 studies, while IGF-II was reported in only 3 studies (**Table 4**). IGFBP-1,−2, and −3 were reported in five, two, and six studies, respectively (**Table 5**), while there was only one study on IGFBP-4,−5,−6, and −7 (25). We did not

TABLE 2 | Quality assessment scores of 12 studies included in the systematic review of circulating IGF axis biomarkers and gestational diabetes mellitus (GDM).

References	Selection participants	Comparability of groups	Outcome		Sample size	Study design	Score
			Definition	Ascertainment			
Luo et al. (22)	1	2	2	2	1	2	10
Qian et al. (23)	1	1	2	2	1	2	9
Hayati et al. (24)	1	1	2	2	1	2	9
Matuszek et al. (19)	1	2	2	2	1	2	10
O'Leary and Longley (26)	1	2	1	1	1	2	8
Ramirez et al. (20)	1	2	2	2	1	2	10
Zhu et al. (27)	1	2	2	2	1	2	10
Grissa et al. (21)	1	2	2	2	1	2	10
Lappas (25)	1	2	2	2	1	1	9
Hughes et al. (29)	1	2	1	2	1	1	8
Qiu et al. (28)	1	2	2	2	1	1	10
Liao et al. (30)	1	1	2	2	1	1	10

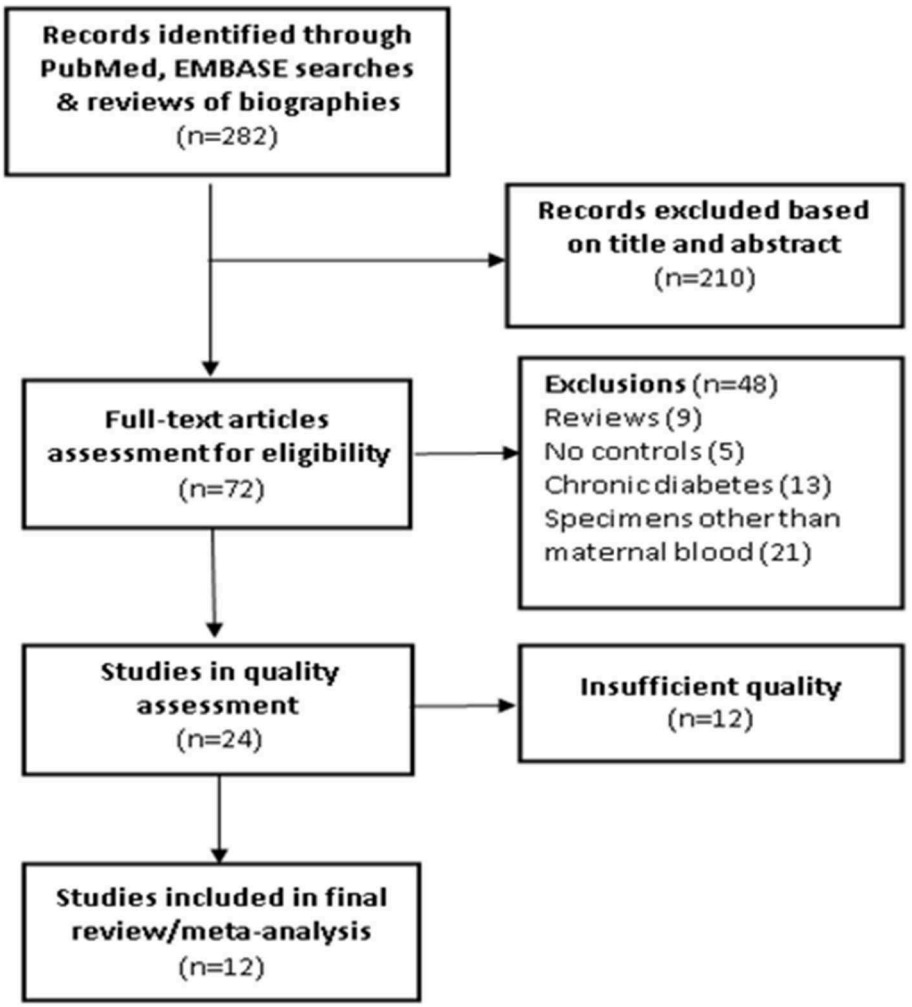

FIGURE 1 | Flowchart in the selection of studies in a systematic review of maternal circulating IGF axis biomarkers and gestational diabetes mellitus (GDM).

TABLE 3 | Characteristics of included studies in a systematic review of insulin-like growth factor (IGF) axis biomarkers and GDM.

References	Country	Study type*	GDM/ Control	Ethnicity	Population	Age (years)	BMI (kg/m^2)	Definition of GDM	GA (weeks) at blood sampling	Specimen
Ramirez et al. (20)	USA	PC	30/42	Hispanic (92%)	Obese	GDM 30.9 ± 4.7 NGT 28.1 ± 5.4	GDM 34.1 ± 4.5 NGT 34.4 ± 4.7	ADA 100 g OGTT	24–28	Serum
Matuszek et al. (19)	Poland	CC	46/21	Caucasian	General	GDM 29 (28-32) NGT 29 (26-33)	GDM 23.2 NGT 21.6	PDA 75 g OGTT	24–28	Serum
Zhu et al. (27)	USA	NCC	107/214	Multi-ethnic	General	GDM 30.5 ± 5.7 NGT 30.4 ± 5.4	GDM 28.2 ± 6.4 NGT25.6 ± 5.3	ACOG 100 g OGTT	10–14, 15–26, 32–39	Plasma
Lappas (25)	Australia	CC	44/30	Caucasian	Non-obese	GDM 34.8 ± 5.3 NGT 33.3 ± 4.4	GDM 25.0 ± 3.3 NGT 24.3 ± 3.8	ADPS	At delivery	Plasma
	Australia	CC	26/36	Caucasian	Obese	GDM 33.8 ± 4.6 NGT 32.3 ± 4.2	GDM 36.9 ± 6.6 NGT 37.8 ± 6.0	75 g OGTT		
Luo et al. (22)	Canada	PC	27/280	Caucasian	General	GDM 31 ± 4.7 NGT 30.8 ± 4.7	GDM 25.3 ± 5.9 NGT 23.4 ± 4.6	ADA 75 g OGTT	24–28; 32–35	Plasma
Grissa et al. (21)	Tunisia	CC	30/30	Tunisian	General	19–42	GDM 24.9 ± 2.9 NGT 23.2 ± 2.3	FBG≥5.5	At delivery	Serum
Hayati et al. (24)	Malaysia	CC	25/50	Asian	General	NA	NA	WHO 75 g OGTT	28, 36	Serum
O'Leary and Longley (26)	Australia	NCC	34/200	Caucasian	General	NA	NA	ADA 75 g OGTT	28–35	Serum
Qian et al. (23)	China	CC	20/38	Asian	General	22–34	NA	75 g OGTT	At delivery	Serum
Qiu et al. (28)	USA	PC	47/757	White	General	≥35: 27.9%	≥30: 8.7%	ADA 100 g OGTT	13	Plasma
Hughes et al. (29)	Britain	PC	20/29	NA	General	GDM 30 (19-42) NGT 26 (20-38)	NA	WHO 75 g OGTT	31–40	Serum
Liao et al. (30)	New Zealand	NCC	28/28	Caucasian	General	GDM 31.4 ± 4.8 NGT 31.3 ± 4.2	GDM 27.2 ± 4.8 NGT 26.2 ± 4.2	IADPSG 75 g OGTT	20	Serum

**Study type: PC, prospective cohort study; NCC, nested case–control study; CC, case–control study. GA, gestational age; GDM, gestational diabetes mellitus; NGT, normal glucose tolerance; FBG, fasting blood glucose; ADA, American Diabetes Association; ACOG, American College of Obstetricians and Gynecologists; ADPS, Australia Diabetes in Pregnancy Society; IADPSG, International Association of Diabetes and Pregnancy Study Groups; PDA, Polish Diabetes Association; WHO, World Health Organization; NA, not available.*

conduct country- or race-specific subgroup analyses due to the small number of studies.

Publication bias was assessed by Funnel plot when there were at least three studies on the same biomarker. There was no evidence of publication biases in all reported IGF axis biomarkers (data not shown).

Early Gestation (<20 Weeks)

There were only two studies on IGF axis biomarkers in early gestation in relation to subsequent development of GDM. Zhu and colleagues reported a nested case–control study on total IGF-I, IGFBP-2, IGFBP-3, and IGF-I/IGFBP-3 ratio in 107 GDM and 214 euglycemic control women (27). IGF-I concentration at 10–14 weeks of gestation was positively associated with subsequent development of GDM. Compared to the highest vs. lowest quartiles in IGF-I concentration, there was a 2.87-fold increased risk of GDM after adjusting for major risk factors ($RR = 2.87$, 95% CI 1.28–6.42, $P = 0.02$). A similar association was observed for the molar ratio of IGF-I to IGFBP-3. However, IGFBP-3 itself was not associated with GDM. A strong negative association was observed between IGFBP-2 and GDM; the highest quartile at 10–14 weeks was associated with a 95% reduced risk of GDM (RR = 0.05, 95% CI 0.02–0.16, $P < 0.001$).

Qiu et al. studied 804 women in a prospective cohort to analyze plasma concentrations of free IGF-I and IGFBP-1 at 13 weeks of gestation in relation to subsequent development of GDM (28). They found that both free IGF-I and IGFBP-1 were inversely associated with GDM. Women with free IGF-I in the highest tertile (≥1.08 ng/ml) experienced a 69% reduced risk of GDM ($RR = 0.31$, 95% CI 0.12–0.75, $P = 0.01$) compared to women with concentrations in the lowest tertile (<0.80 ng/ml). There was a 57% decreased risk of GDM among women with IGFBP-1 in the highest tertile (≥68.64 ng/ml) ($RR = 0.43$, 95% CI 0.18–1.05), but the association was marginal ($P = 0.059$).

Mid-gestation (20–29 Weeks)

IGF-I

IGF-I concentrations in mid-gestation were elevated in GDM vs. euglycemic pregnancies, with na WMD of 42.1 ng/ml (95% CI 28.9–55.4, $P < 0.0001$), according to data from six studies (**Table 4**; **Figure 2**).

IGF-II

There were two studies on IGF-II in mid-gestation and GDM. Luo et al. reported similar plasma total IGF-II concentrations at 24–28 weeks of gestation in GDM and euglycemic women (22). Similarly, Liao and colleagues reported no difference in serum IGF-II concentrations between GDM and control women (30).

TABLE 4 | Summary data in studies on circulating IGF-I and IGF-II levels[a] in GDM and control (euglycemic) pregnancies.

Study type*	References	Country	Specimen	Assay	GA.	GDM (ng/ml)[a]			Controls (ng/ml)[a]			GDM vs. control difference (95% CI)[c]
				Method	Weeks	*N*	Mean	*SD*	*N*	Mean	*SD*	
IGF-I												
PC	Luo et al. (22)	Canada	Plasma	ELCA	24–28	27	285.6	109.5	280	200.4	80.4	**85.20 (42.84, 127.56)**
PC	Luo et al. (22)	Canada	Plasma	ELCA	32–35	27	403.6	171.8	279	307.0	123.6	**96.60 (30.19, 163.01)**
PC	Hayati et al. (24)	Malaysia	Serum	ELISA	28	25	300	90	50	254	127	**46.00 (2.85, 89.15)**
PC	Hayati et al. (24)	Malaysia	Serum	ELISA	36	25	389	85	50	302	106	**87.00 (42.58, 131.42)**
PC	Matuszek et al. (19)	Poland	Serum	ELISA	24–28	46	152.6	87.5	21	120.8	58.8	31.75 (−3.91, 67.41)
PC	Hughes et al. (29)	Britain	Serum	RIA	31–40	20	416	92	29	296	83	**120.00 (69.62, 170.38)**
PC	Ramirez et al. (20)	USA	Serum	ELISA	26	30	173.96	65.04	42	197.0	191.1	−23.04 (−85.34, 39.26)
NCC	Zhu et al. (27)	USA	Plasma	ELISA	10–14	107	180.2	64.1	214	164.9	57.4	**15.27 (0.90, 29.64)**
NCC	Zhu et al. (27)	USA	Plasma	ELISA	15–26	107	217.5	92.2	214	181.9	70.3	**35.61 (15.76, 55.46)**
NCC	Zhu et al. (27)	USA	Plasma	ELISA	32–35	38	335.8	579.1	45	293.1	144.8	42.78 (−146.13, 231.69)
NCC	Zhu et al. (27)	USA	Plasma	ELISA	37–39	51	326.5	302.0	58	297.1	216.3	29.41 (−70.43, 129.25)
PC	Grissa et al. (21)	Tunisia	Serum	ELISA	Delivery	30	650.9	168.0	30	414.3	100.6	**236.66 (166.59, 306.73)**
CC	Lappas (25)	Australia	Plasma[&]	ELISA	Delivery	44	58.0	35.8	30	57.4	25.7	0.60 (−13.42, 14.62)
CC	Lappas (25)	Australia	Plasma[&]	ELISA	Delivery	26	55.1	40.8	36	51.8	31.2	3.30 (−15.40, 22.00)
CC	Qian et al. (23)	China	Serum	RIA	Delivery	20	239.85	68.9	38	201.5	52.4	38.35 (3.86,72.84)
NCC	Liao et al. (30)	New Zealand	Serum	ELISA	20	28	275.7	60.9	28	218.5	58.7	50.20 (25.87, 88.53)
PC	Qiu et al. (28)	USA	Plasma	ELISA	13	47	NA	NA	757	NA	NA	Categorical data only
Meta-Analysis[b]												
GA	<20 weeks	2 studies										<3 studies
	20–29	6 studies	$P < 0.001$									**42.12 (28.87, 55.37)**
	30+	6 studies	$P < 0.001$									**98.07 (47.23, 148.90)**
IGF-II												
PC	Luo et al. (22)	Canada	Plasma	ELCA	24–28	27	864.6	164.3	280	905.0	143.2	−40.40 (−104.6, 23.80)
PC	Luo et al. (22)	Canada	Plasma	ELCA	32–35	27	983.5	244.8	279	995.7	180.4	−12.20 (−106.93, 82.53)
CC	Lappas (25)	Australia	Plasma[&]	ELISA	Delivery	44	293.7	189.0	30	269.5	175.8	24.20 (−67.43, 115.83)
CC	Lappas (25)	Australia	Plasma[&]	ELISA	Delivery	26	309.9	245.8	36	267.8	210.0	42.10 (−86.66, 170 86)
NCC	Liao et al. (30)	New Zealand	Serum	ELISA	20	28	814.7	131.8	28	836.4	100.5	−21.70 (−83.09, 39.69)
Meta-Analysis[b]												<3 studies

*Some studies reported maternal IGF-I and/or IGF-II data at multiple gestational age windows, and thus occupied multiple data lines in the table. *Study type: PC, Prospective Cohort study; NCC, Nested Case Control study; CC, Case-Control study; NA, not available. GA, gestational age (weeks); ELISA, enzyme-linked immunosorbent assay; ELCA, enzyme-labeled chemiluminescent assay; RIA, radioimmunoassay.*

[a] Conversion factor: IGF-I, 1 nmol/L = 7.649 μg/L or ng/ml; IGF-II, 1 nmol/L = 7.469 μg/L or ng/ml.

[b] There was only one study on free IGF-I or free IGF-II (Lappas 2015 study); all other studies are on total IGF-I or total IGF-II; meta-analysis was conducted on total IGF-I or IGF-II in circumstances with ≥3 studies.

[c] The differences with 95% CIs excluding the zero are shown in bold.

[&] The study on free IGF-I or free IGF-II.

TABLE 5 | Summary data in studies on circulating IGFBP-1, IGFBP-2, and IGFBP-3 levels in GDM and control pregnancies.

Study type*	References	Pub year	Country	Specimen	Assay	GA	GDM (ng/ml)			Controls (ng/ml)			GDM vs. control difference (95% CI)[b]
					Method	Weeks	*N*	Mean	*SD*	*N*	Mean	*SD*	
IGFBP-1													
NCC	O'Leary and Longley (26)	1994	Australia	Serum	RIA	28–35	34	146	105	200	135	199	11.00 (−25.88, 47.88)
PC	Ramirez et al. (20)	2014	USA	Serum	ELISA	26	30	44.9	18.8	42	62.2	21.2	**−17.28 (−26.57, −7.99)**
NCC	Liao et al. (30)	2017	New Zealand	Serum	ELISA	20	28	41.04	18.1	28	67.58	32.6	−26.54 (−40.35, 12.73)
CC	Lappas (25)	2015	Australia	Plasma	ELISA	Delivery	44	54.4	33.8	30	72.3	41.1	−17.90 (−35.77,−0.03)
CC	Lappas (25)	2015	Australia	Plasma	ELISA	Delivery	26	56.2	39.3	36	41.1	31.8	15.10 (−3.23, 33.43)
PC	Qiu et al. (28)	2005	USA	Plasma	ELISA	13	47	NA	NA	757	NA	NA	Categorical data only
Meta-Analysis[a]													<3 studies
													In all the 3 GA periods
IGFBP-2													
CC	Lappas (25)	2015	Australia	Plasma	ELISA	Delivery	44	157.8	109.4	30	189.3	126.5	−31.50 (−87.12, 24.12)
CC	Lappas (25)	2015	Australia	Plasma	ELISA	Delivery	26	150.3	131.6	36	146.4	125.4	3.90 (−61.19, 68.99)
NCC	Zhu et al. (27)	2016	USA	Plasma	ELISA	10–14	107	91.61	50.44	214	116.99	47.67	**−25.38 (−36.87, −13.89)**
NCC	Zhu et al. (27)	2016	USA	Plasma	ELISA	15–26	107	82.26	27.90	214	103.82	41.16	**−21.56 (−29.20, −13.92)**
NCC	Zhu et al. (27)	2016	USA	Plasma	ELISA	32–35	38	78.57	29.36	45	88.92	29.36	−10.35 (−23.03, 2.33)
NCC	Zhu et al. (27)	2016	USA	Plasma	ELISA	36–39	51	87.44	15.53	58	91.87	15.53	−4.43 (−10.27, 1.41)
Meta-Analysis[a]													<3 studies
													In all the 3 GA periods
IGFBP-3													
NCC	Zhu et al. (27)	2016	USA	Plasma	ELISA	10–14	107	4638.4	892.7	214	4513.9	858.2	124.5 (−80.0, 329.0)
NCC	Zhu et al. (27)	2016	USA	Plasma	ELISA	15–26	107	4648.6	819.2	214	4561.2	872.1	87.4 (−106.9, 281.7)
NCC	Zhu et al. (27)	2016	USA	Plasma	ELISA	32–35	38	5034.3	1596.0	45	5120.0	2089.9	−85.7 (−879.6, 708.2)
NCC	Zhu et al. (27)	2016	USA	Plasma	ELISA	36–39	51	5085.7	1602.9	58	4965.7	1922.5	120.0 (−542.1, 782.1)
PC	Grissa et al. (21)	2010	Tunisia	Serum	ELISA	Delivery	30	1836.5	912.9	30	1302.6	912.8	**533.9 (71.9, 995.9)**
PC	Hughes et al. (29)	1995	Britain	Serum	RIA	31–40	20	6612.5	1918.7	29	5546.8	1403.5	**1065.7 (81.8, 2049.6)**
CC	Lappas (25)	2015	Australia	Plasma	ELISA	Delivery	44	3997.4	996.3	30	3693.7	1081.2	303.7 (−182.5, 789.9)
CC	Lappas (25)	2015	Australia	Plasma	ELISA	Delivery	26	3959.9	1433.3	36	4029.0	1487.4	−69.1 (−803.7, 665.5)
CC	Qian et al. (23)	2000	China	Serum	RIA	Delivery	20	5676	1628	38	5746	1512	−70.0 (−930.3, 790.3)
NCC	Liao (30)	2017	New Zealand	Serum	ELISA	20	28	5189.2	1783.8	28	5297.3	1270.3	−108.1 (−919.2, 703.0)
Meta-Analysis[a]													
GA	<20 weeks	1	Study										<3 studies
	20–29	2	Study										<3 studies
	30+	5	Studies	$P = 0.02$									**282.15 (39.78, 524.53)**

**Study type: PC, prospective cohort study; NCC, nested case–control study; CC, case–control study; NA, not available. GA, gestational age; ELISA, enzyme-linked immunosorbent assay; ELCA, enzyme-labeled chemiluminescent assay; RIA, radioimmunoassay.*

[a]Meta-analysis was conducted in circumstances with ≥3 studies.

[b]The differences with 95% CIs excluding the zero are shown in bold.

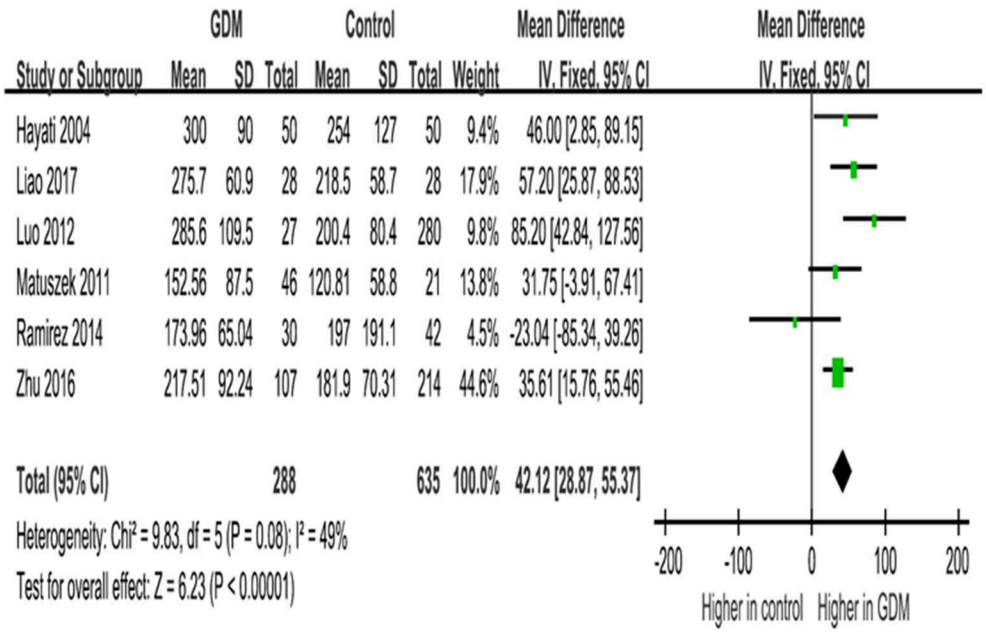

FIGURE 2 | Mean differences (95% CIs) of maternal circulating *total IGF-I concentrations (ng/ml) in mid-gestation (20–29 weeks)* comparing GDM vs. euglycemic (control) pregnancies in six studies. Positive values denote higher values in GDM patients; negative values denote higher values in control subjects.

IGFBPs

There were three studies on IGFBP-1, IGFBP-2, or IGFBP-3 concentrations in mid-pregnancy comparing GDM vs. euglycemic women. Ramirez and colleagues reported significantly lower IGFBP-1 concentrations in GDM vs. controls (mean: 44.9 ± 18.8 vs. 62.2 ± 21.2 ng/ml, $P = 0.0004$) (20). Zhu et al. reported a significant reduction in IGFBP-2 concentrations at 15–26 weeks of gestation in GDM vs. controls (mean: 82.3 ± 27.9 vs. 103.8 ± 41.2 ng/ml, $P < 0.0001$), but similar IGFBP-3 concentrations (mean: 4648.6 ± 819.2 vs. 4561.2 ± 872.1 ng/ml, $P = 0.38$) (27). Liao et al. reported a significant reduction in IGFBP-1 concentrations at 20 weeks of gestation in GDM vs. controls (mean: 41.0 ± 18.1 vs. 67.6 ± 32.6 ng/ml, $P < 0.001$) (30), but similar IGFBP-3 concentrations (mean: 5189.2 ± 1783.8 vs. 5297.3 ± 1270.3 ng/ml, $P = 0.35$) (30).

Late Gestation (30+ Weeks)

IGF-I

In late gestation, women with GDM had significantly higher IGF-I concentrations (WMD = 98.1 ng/ml, 95% CI 47.2–148.9, $P = 0.0002$), according to data on circulating IGF-I concentrations at ≥30 weeks of gestation in six studies (seven data lines, **Figure 3**).

IGF-II

There were two studies on IGF-II concentrations in late pregnancy and GDM; both did not find an association between IGF-II and GDM (22, 25).

IGFBP-1

There were two studies on maternal IGFBP-1 concentrations in late gestation (25, 26); both reported no significant differences in GDM vs. euglycemic pregnancies.

IGFBP-2

There were significantly lower IGFBP-2 concentrations in late gestation compared GDM to euglycemic pregnancies (WMD = −5.64 ng/ml, 95% CI −10.90 to −0.37, $P = 0.04$), according to data from two studies (four data lines, **Table 5**).

IGFBP-3

IGFBP-3 concentrations in late gestation were significantly higher in GDM vs. euglycemic pregnancies (WMD = 282.2 ng/ml, 95% CI 39.8–524.5, $P = 0.02$), according to data from five studies (seven data lines, **Figure 4**).

Other IGFBPs

We are aware of only one study on the associations of GDM with IGFBP-4,−5,−6, or−7, and no association was detected (25).

DISCUSSION

Main Findings

To our knowledge, this is the first systematic review on IGF axis biomarkers and GDM. Circulating levels of IGF axis biomarkers are gestational age dependent (22, 27). Thus, gestational age window-specific analyses are critical. This review shows that current research evidence is suggestive but insufficient concerning whether the IGF axis is involved in

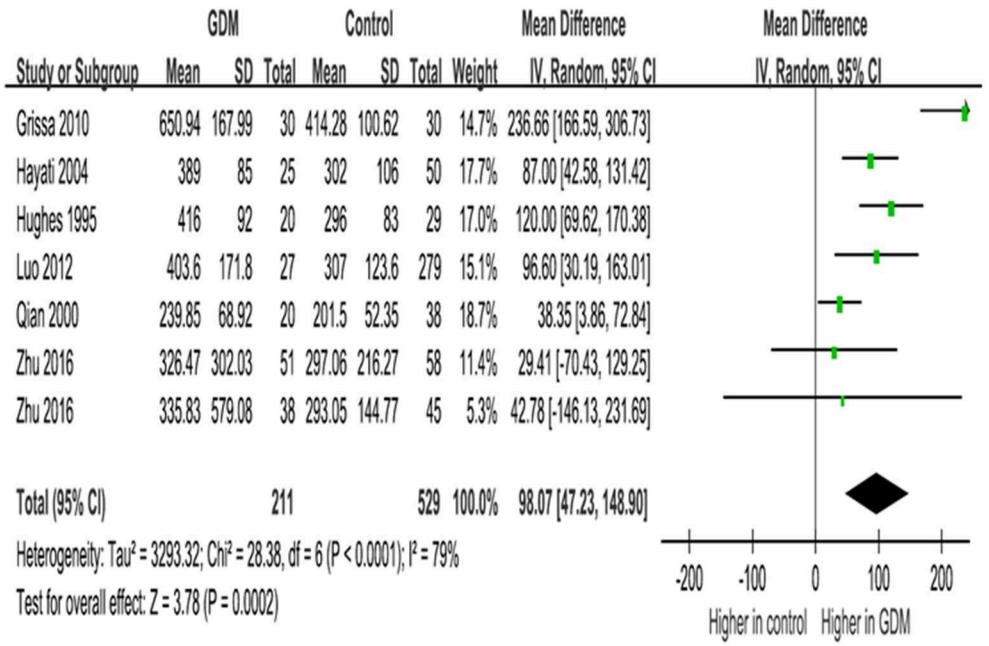

FIGURE 3 | Mean differences (95% CIs) of maternal circulating *total IGF-I concentrations (ng/ml) in late gestation (30+ weeks)* comparing GDM vs. euglycemic pregnancies in six studies (seven data lines). Positive values denote higher values in GDM patients; negative values denote higher values in control subjects.

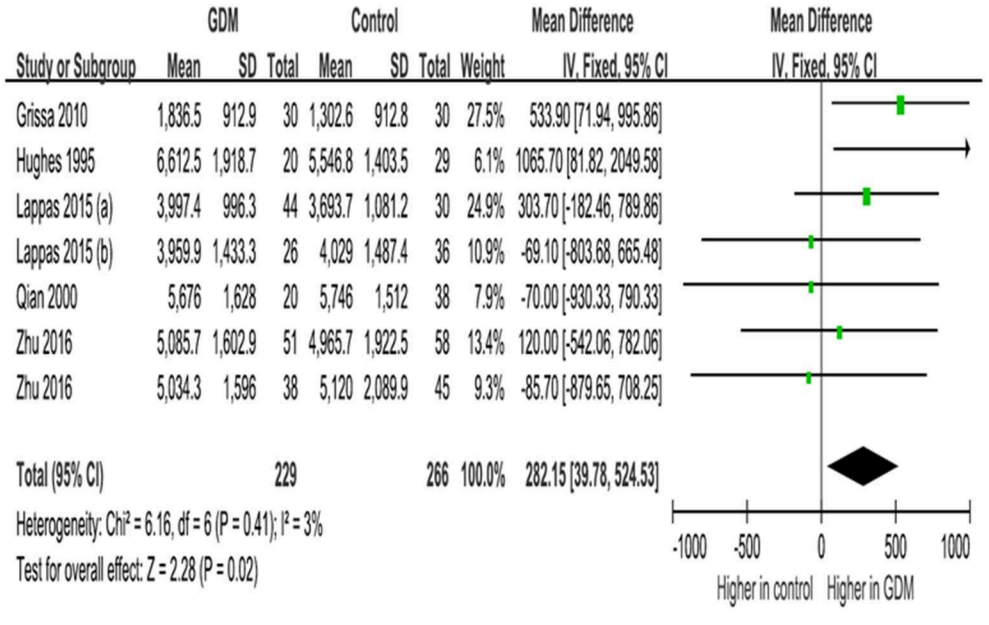

FIGURE 4 | Mean differences (95% CIs) of maternal circulating *IGFBP-3 concentrations (ng/ml) in late gestation (30+ weeks)* comparing GDM vs. euglycemic (control) women in five studies (seven data lines). Positive values denote higher values in GDM patients; negative values denote higher values in control subjects.

the development of GDM. GDM was consistently associated with higher IGF-I levels in mid- and late gestation, but there were only two studies in early gestation with inconsistent findings. IGFBP-2 was consistently negatively associated with GDM throughout gestation, but the findings were based on only one or two studies in early, mid-, or late gestation. IGFBP-3 in late gestation was positively associated with GDM, but there was no association for IGFBP-3 in early or mid-gestation according to data from a single study. Other IGF axis biomarkers have shown no or inconsistent associations with GDM, and the data in early gestation are absence or scanty.

The IGF axis has been implicated in glucose homeostasis (7, 8). It is a plausible hypothesis that the IGF axis is involved in the etiology of GDM. If the hypothesis is true, there should be significant alterations in the expression/circulating levels of IGF axis biomarkers before the clinical onset of the disease in early gestation that may or may not persist in mid- and late gestation. Changes in a biomarker can be either a cause or consequence of GDM. GDM is routinely diagnosed around 24–28 weeks of gestation. Only those changes before 20 weeks of gestation could be more confidently considered possibly causal in the development of GDM. Changes in circulating levels of IGF axis biomarkers in mid- and late gestation could be either a cause or consequence of GDM. The scarcity of studies on IGF axis biomarkers at <20 weeks of gestation forestalls a conclusive statement on the etiological role of IGF axis in the development of GDM.

IGF-I and IGF-II

Under normal physiological conditions, IGF-I has a hypoglycemic effect inhibiting insulin secretion and increasing insulin sensitivity (31). Glucose modulates the secretion of IGF-I through the release of insulin, while IGF-I may regulate insulin levels by a negative feedback (31, 32). Our review suggests that IGF-I levels may be elevated at the time around or after the diagnosis of GDM, but whether it may play a causal role remains uncertain since there were only two studies on IGF-I in early gestation with inconsistent findings (27, 28). The causes of the conflicting findings are unclear, and the solution to resolve the question may be through new and large (adequately powered) prospective pregnancy cohort studies with high-quality biomarker data. The higher IGF-I levels in GDM in mid- and late gestation could be attributable to elevated insulin secretion (33, 34), and/or enhanced secretion of placental growth hormone—the main driver of maternal IGF-I production in pregnancy (35). In contrast, IGF-II appears not to be related to GDM, although caution is warranted in data interpretation since there was only one study on IGF-II in mid-gestation and no study in early gestation.

IGFBPs

IGFBPs play an important role in insulin signaling, enhancing peripheral glucose uptake and decreasing hepatic glucose output (36). It remains unclear what the differences in the biological significance of various IGFBPs. This review showed that IGFBP-2 was consistently negatively associated with GDM throughout the pregnancy, but the finding was based on one or two studies in early, mid-, or late gestation, and requires confirmation in more independent studies. The negative association between IGFBP-2 and GDM is consistent with the negative association between IGFBP-2 and type 2 diabetes in adults (37). IGFBP-2 over-expression has been associated with reduced susceptibility to obesity and diabetes *via* inhibition of adipogenesis and stimulation of insulin sensitivity in mice (15, 38). The pleiotropic actions of IGFBP-2 suggest its potential as a critical molecule involved in the development of GDM. There is a lack of significant post-prandial fluctuations in IGFBP-2 concentrations (39), rendering IGFBP-2 as a promising early gestational biomarker in predicting the development of GDM, but confirmative studies are wanted.

Our review demonstrates that IGFBP-3 concentrations are elevated in late gestation in women with GDM. In contrast, there are no significant changes in IGFBP-3 concentrations in early or mid-gestation in women who later developed GDM, suggesting that the elevated IGFBP-3 levels may be a consequence, rather than the cause of GDM, but it should be cautioned that there was only one study on IGFBP-3 in early gestation.

There was only one study on IGFBP-1 in early gestation and there were only two studies in mid-gestation; all reported lower IGFBP-1 levels in GDM (20, 28, 30). This is consistent with the finding in adults that higher IGFBP-1 levels are correlated with better glucose tolerance and lower insulin resistance (40). This review also showed that the difference in IGFBP-1 levels disappeared in late gestation. The scanty data in early gestation and inconsistent data in late gestation suggest the need for more studies to clarify the association between IGFBP-1 and GDM.

We identified only one study on IGFBP-4, −5, −6, or −7 concentrations in late gestation in relation to GDM, and the study did not find any significant association (25). There was no study on these IGFBPs in early or mid-gestation.

Strengths and Weaknesses

The main strength is the coverage of all studies with high-quality original data on circulating IGF biomarkers and GDM; all included studies are of high quality (**Table 2**). The main weakness is the inability to review the data in the gray literature or unpublished studies, which may be of uncertain quality.

CONCLUSIONS

Current research evidence is suggestive, but limited and insufficient concerning whether the IGF axis is involved in the development of GDM. More studies on IGF axis biomarkers in early gestation and subsequent development of GDM are warranted.

AUTHOR CONTRIBUTIONS

Z-CL, XH, XY, and FO conceived the study. X-RW and W-JW conducted the literature search and data extraction. X-RW, W-JW, XH, XY, FO, and Z-CL contributed to the reviews of retrieved papers. X-RW and W-JW conducted the data meta-analysis and drafted the manuscript. All authors contributed to data interpretation, revising the article critically for important intellectual content, and approved the final version for publication.

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Improving Models of Care for Diabetes in Pregnancy: Experience of Current Practice in Far North Queensland, Australia

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Aims: To map health practitioners' experiences and describe knowledge regarding screening and management of Diabetes in Pregnancy (DIP) in Far North Queensland, Australia.

Methods: Mixed methods including a cross-sectional survey (101 respondents) and 8 focus groups with 61 health practitioners. All participants provided clinical care for women with DIP.

Results: A wide range of healthcare professionals participated; 96% worked with Indigenous women, and 63% were from regional or remote work settings. Universal screening for gestational diabetes at 24–28 weeks gestation was reported as routine with 87% using a 75 g Oral Glucose Tolerance Test. Early screening for DIP was reported by 61% although there was large variation in screening methods and who should be screened <24 weeks. Health practitioners were confident providing lifestyle advice (88%), dietary, and blood glucose monitoring education (67%, 81%) but only 50% were confident giving insulin education. Electronic medical records were used by 80% but 55% also used paper records. Dissatisfaction with information from hospitals was reported by 40%. In the focus groups improving communication and information technology systems were identified as key areas. Other barriers described were difficulties in care coordination and access for remote women.

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Conclusions: Communication, information technology systems, coordination of care, and education for health professionals are key areas that will be addressed by a complex health systems intervention being undertaken by the DIP Partnership in North Queensland.

Keywords: gestational diabetes–mellitus, diabetes in pregnancy, model of care, screening practices, diabetes management, care coordination, access to health care

INTRODUCTION

Effective management of diabetes in pregnancy (DIP) is increasingly a public health concern, as rates of this condition continue to rise in Australia and globally (1, 2). DIP includes both diabetes diagnosed during pregnancy termed Gestational Diabetes Mellitus (GDM) and pre-existing Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM). The introduction of the most recent World Health Organization (WHO) and Australasian Diabetes in Pregnancy Society (ADIPS) diagnostic criteria have contributed to an increase in the screening and diagnosis of GDM (3) as has the "epidemic" of T2DM in the general population. Early screening of women considered at high-risk has led to an increase in early diagnosis of GDM, more frequent antenatal appointments and busier clinics.

Australian population groups such as Aboriginal and Torres Strait Islander women and other high risk ethnic groups have higher rates of GDM and pre-existing T2DM compared to Caucasian women (4, 5). In Australia during the 2-year period from 2014 to 2015, 10% of all births recorded in the National Perinatal Data Collection were complicated by DIP. Of these 9% had GDM, and 1% had pre-existing diabetes (6, 7). In the Northern Territory, the rate of GDM in Aboriginal women is close to 16% and the rate of T2DM is 4% (8). Additionally, Indigenous babies are more likely to have pregnancy-related complications and increased care requirements, regardless of the mother's diabetes status (pre-existing diabetes, GDM, or no diabetes) (6).

These women require intensive multidisciplinary care during pregnancy, as well as pre- and post-partum. Well-recognized acute and chronic complications of DIP can be improved with individualized treatment (9), and by improving systems through measures such as implementation of screening practices (10) and standardized models of care (11, 12).

High quality care for women with DIP and their infants requires a range of services provided by multiple specialties including midwives, diabetes educators, dietitians, obstetricians, general practitioners, endocrinologists, pediatricians, and other health workers. Coordination of the care can be challenging, particularly for women in rural and remote regions, and the increasing number of women diagnosed with DIP threatens to stretch health resources (13).

In the Northern Territory (NT) of Australia, a National Health and Medical Research Council funded Diabetes in Pregnancy Partnership commenced in 2012. The project was designed to address the complex issue of optimizing management of women with DIP and to reduce gaps between evidence and practice (14). The NT Partnership is a collaboration between clinicians, researchers, health care services and policy makers. It has established strong relationships with communities and health services, formed an active Clinical Reference Group, developed enhanced models of care for DIP and successfully established the NT DIP Clinical Register in Darwin and Alice Springs regions (15, 16). Initial results indicate a significant increase in reporting of gestational diabetes and an increased awareness and understanding of the disease burden of DIP (15, 16). In 2016 the partnership was expanded to include Far North Queensland (FNQ) with the aim of establishing a DIP Clinical Register and development of enhanced models of care to augment health professionals' capacity for managing DIP (Diabetes In Pregnancy Partnership In North Queensland "DIPPINQ").

FNQ has a population of approximately 240,000 in the greater Cairns area and 20,000 in Cape York and the Torres Strait Islands. A large proportion of the region's population identify as Aboriginal or Torres Strait Islander: 10% of the Cairns population, 69% of the Torres Strait Islands' population and 52% of Cape York's total population (17). Cairns Hospital is the major referral center for the Cairns and Hinterland Health Service and the Torres and Cape York Hospital and Health Service which in total span ∼273 000 square kilometers, slightly larger than the United Kingdom (18). The rate of GDM in the region is 12–14%, much higher than the national average of 5–10% (19, 20). There are 2,500 deliveries per year at the Cairns Hospital. In 2016, when using the WHO criteria for diagnosis of GDM, 14.5% were complicated by hyperglycaemia: 12% of women had GDM, 2% of women had T2DM and 0.5% T1DM (local hospital data). Multiple service providers including Aboriginal Community Controlled Medical Services, the Royal Flying Doctor Service, private General Practice and Queensland Health primary to tertiary care are involved in care provision. Multiple, separate information systems are used by the various providers. The WHO and ADIPS guidelines for DIP have provided standards for the management of DIP in the region since 2015, and a comprehensive Queensland Health Clinical Guideline for GDM updated in 2015 is widely available. However, adherence to guidelines and provision of seamless care for a high-risk population is an ongoing challenge.

Here we describe findings from formative work of the DIPPINQ. The aims of our study were to 1. Describe knowledge among health practitioners regarding screening and management of women with DIP and 2. Map practitioners' experiences providing care for women with DIP to inform future interventions to improve models of care in Far North Queensland.

METHODS

This study used a mixed methods approach to map the knowledge and experiences of health practitioners who provide healthcare for women with DIP and their families in FNQ. Quantitative data from a survey of health practitioners were triangulated with qualitative data from focus groups conducted with health practitioners.

Health Practitioner Survey

A cross-sectional survey comprising 48 questions (see **Supplement 1**) was adapted to the FNQ context from earlier work conducted by the Partnership in the NT. The NT survey was informed by a series of regional workshops with stakeholders. Workshop participants identified issues associated with models of care for DIP. The main constructs underpinning the survey included: communication; information technology; care-coordination; logistics and access; knowledge, education, and guidelines (13).

In FNQ it was distributed electronically via a web based site (Survey Monkey) and hard copies were distributed at meetings and workshops between November 2016 and April 2017. One hundred and one health professionals involved in DIP care from all disciplines participated.

Participants were purposively recruited by advocates of the Partnership from a number of partner organizations (government and non-government, Aboriginal Controlled Community Health Organizations, primary to tertiary care providers). Snowball sampling was employed, whereby participants were asked to forward the survey link within their relevant networks.

Health Practitioner Focus Groups

A phenomenological methodology guided the qualitative aspect of this study. Participants were purposively recruited to participate in focus groups through Partnership networks (as above). No exclusion criteria were placed on eligibility. A total of eight focus groups with 61 health professionals took place between March and May 2017. Five face-to-face groups were held in conjunction with Partnership workshops in Cairns. The remaining three were arranged in outlying regions via teleconference. Each focus group comprised health professionals from the same organization or region (min = 3, max = 11). Participants worked in primary to tertiary health care settings in urban, regional and remote locations of FNQ. The average duration of focus groups was 58 min.

The focus groups were facilitated by members of the research team with expertise in community-based research (JB, SC, CW, RK). Participants provided informed consent and permission for audio-recording and transcription of discussions. Only one participant declined the invitation to participate.

Discussion in the groups was guided by one of two scenarios about a pregnant woman's journey with DIP. These were developed by the research team and considered relevant to different health care settings. Facilitators used an interview schedule to enquire about the method of coordinating appointments and travel, which providers the woman would see, what guidelines were used, how information was communicated and who was responsible for various aspects of the woman's care.

Data Analysis

Survey data were exported from Survey Monkey and analyzed using Stata version 14 (Stata Corporation, College Station, Texas). Basic frequencies and percentages were reported and comparisons were made between groups in selected answers using Chi-square tests. Open ended responses were thematically coded.

Focus group data were inductively analyzed in NVivo (QSR International Version 10, 2012) by three members of the research team (SC, JB, and RK). Coding structures were cross-checked for accuracy and interpretation of meaning. A second round of coding was undertaken by RK to ensure saturation was reached on main themes and that results reported on provide further insight into survey findings.

Ethics

Ethics approval for this study was obtained from the Far North Queensland Human Research Ethics Committee (HREC/16/QCH/15).

RESULTS

Health Professional Survey

There were 101 survey respondents (see **Figure 1**), including a range of health professionals (HP) from an even mix of urban, remote, and regional work settings, with midwives being the largest professional group. Ninety-six percent of participants worked with Indigenous women. Of these, 55% had been in their current position for 0–5 years, 18% for 5–10 years, and 28% more than 10 years. Practitioners were not routinely involved in pre-pregnancy counseling, but many were involved in patient care for some time post-partum (**Table 1**).

Current Screening Practice

The majority (85%) of respondents reported universally screening for DIP at 24–28 weeks gestation and 87% reported using a 75 g 2 h Oral Glucose Tolerance Test (OGTT) at this time. Routine screening for diabetes in early pregnancy (<24 weeks gestation) was reported by 61% of HP and there was variation in which screening test was used. Fifty two percent reported using a 75 g OGTT, 19% used a HbA1C and 19% a random Blood Glucose Level (BGL) in early pregnancy. Five percent were unsure and 5% did not answer. There was similar discordance in agreement regarding which risk factors would indicate screening was required in early pregnancy, the most common reasons being a previous history of GDM or glucose intolerance, obesity, and previous large baby. Other risk factors including ethnicity were deemed less important (**Figure 2**).

Current Screening Practice

Fifty-eight percent of HP were confident in managing women with DIP (6% reported not being confident and 35% neutral). Results revealed that most were confident providing lifestyle advice, dietary education, and blood glucose monitoring education (88, 67, 81% respectively, were "confident" or "very

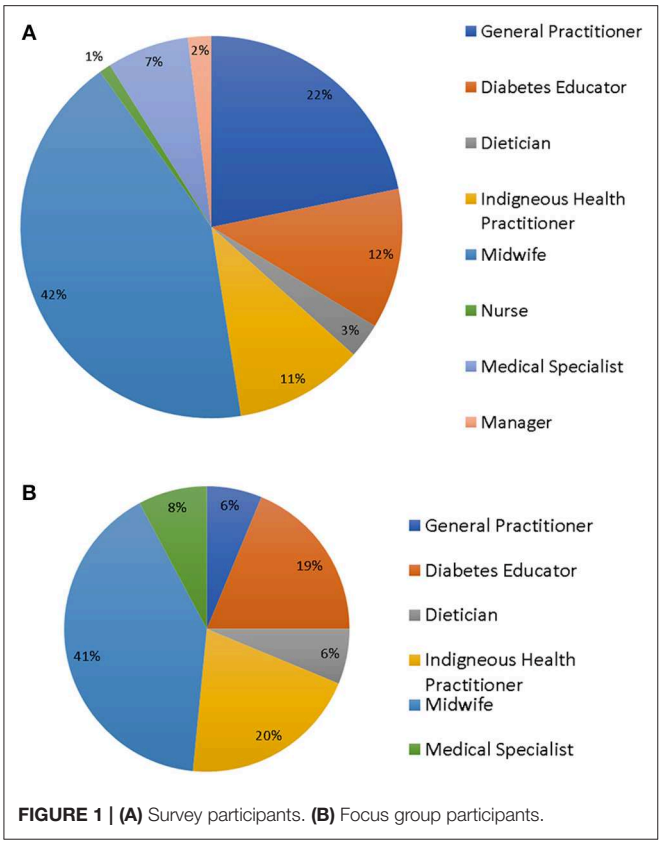

FIGURE 1 | (A) Survey participants. **(B)** Focus group participants.

TABLE 1 | Health Professional Survey: respondent demographics.

Characteristic	Frequency (%)
MAIN WORK SETTING	
Remote	29 (29)
Regional	34 (34)
Urban	24 (24)
Other*	12 (12)
CLIENT BASE	
Aboriginal women	21 (21)
Torres Strait Islander women	7 (7)
Non-indigenous women	4 (4)
All of the above	68 (68)
TIME IN CURRENT POSITION	
<1 year	10 (10)
1–5 years	44 (44)
5–10 years	18 (18)
>10 years	28 (28)
WHAT PERCENTAGE OF WOMEN WOULD YOU HAVE ALSO SEEN FOR PRE-PREGNANCY COUNSELING?	
0–20%	67 (83)
20–40%	11 (14)
>40%	3 (3)
WHAT PERCENTAGE OF WOMEN WOULD YOU ALSO SEE POST-PARTUM?	
0–20%	34 (42)
2–40%	9 (11)
>40%	38 (47)

Total respondents varied per questions and was as follows: Main work setting, n = 99; Client base, n = 100; Percentages of women seen pre-pregnancy and post-partum, n = 81.

confident" in these areas) but only 50% were confident in providing information regarding administration and storage of insulin (**Figure 3**). There was no difference in confidence according to time in job or location of practice (regional vs. remote). However, midwives, dietitians, and diabetes educators reported significantly greater confidence when compared to general practitioners and nurses (74 vs. 43%, $p = 0.002$).

HP reported that they used a variety of resources for their own and patient education, with 63% reporting regular reference to Queensland Health Clinical Guidelines. Their preferences for ongoing education included talks from specialists (32%), online learning modules (30%), and as part of a conference or symposium (22%). Seventy- seven percent of respondents suggested women with DIP would benefit from more education for local health workers.

Referrals, Communication, and Care Coordination

Referral methods described were most commonly facsimile (50%), followed by email (34%), phone calls (27%), directly via electronic record (12%), and mail (10%). Most (91%) did not report making referrals to medical specialists, yet 93% did make referrals to allied health specialists. Satisfaction with the referral process and communication from medical and allied health specialists was mostly positive (**Table 2**). However, only 1% reported being satisfied with written information received from client hospital admissions and the timeliness of information. Respondents reported that medical specialists should be involved in managing women with DIP about the same (56%), more (26%), and much more (11%).

Telephone or video case conferencing was reported as useful for client care by 83% of those who had used it, however, only 42% reported actually using telehealth. That telehealth should be used more often was reported by 21%, and about the same by (54%). Most used an electronic medical record system (80%) but 55% also still used hand held paper records. Use of the nation-wide "My eHealth" record was not widespread (13%).

Seventy-nine percent thought a DIP Clinical Register in FNQ would be useful. The main benefits were thought to be improved care coordination (62%) with the ability to review care delivered by other providers (53%), offering follow-up screening recall lists (62%), and improved inter-pregnancy care (45%). Other benefits included using the DIP Clinical Register as a quality assurance tool for DIP services (41%) and using information from the register to assist planning of future services (42%).

Focus Groups

The primary findings from the focus groups highlighted the complex and fragmented models of care for DIP across regions and organizations in Far North Queensland. The health system is impacted by a range of factors including multiple information

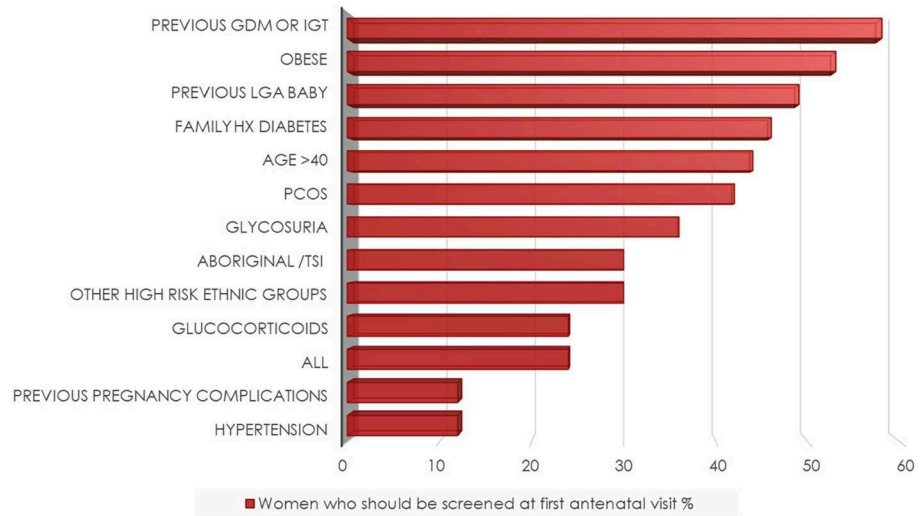

FIGURE 2 | Risk factors* determining screening at the first antenatal visit. *Respondents could choose multiple answers, see question 10 in **Supplement 1**.

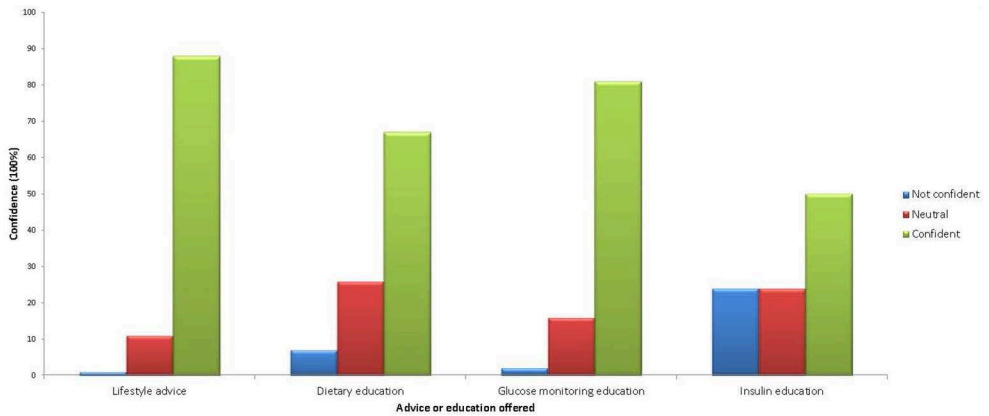

FIGURE 3 | Health professionals' confidence in providing advice or education.

systems, disjointed communication, challenges with logistics and access to care in a service which spans a very large demographic.

Communication and Information Systems

Participants described challenges in communication between health professionals and organizations, with inconsistent access to electronic medical records and a heavy reliance on emails, facsimile and hand-held records. An Indigenous Health Worker described how:

> *There's no information sharing. You can't just hop on to our computer system, you rely onsomeone [. . .] put[ting] you into the correspondence or contact[ing] you.*

Emailing handheld records to the antenatal clinic was one strategy suggested by a midwife for overcoming disjointed communication because *"email gets checked by whoever is on, every day."* Yet, problems were described with email and facsimile with one medical officer highlighting variability in information transfer being dependent on *"if there's a really diligent midwife."* A diabetes educator suggested *"communication processes"* could be improved *"just by having generic emails, so it's not person-dependent."*

Concerns around confidentiality were raised, including whether information was being delivered to the intended recipient. A midwife commented *"I don't know where it's going"* and often *"we don't [receive the information]."* Furthermore, reference was made to the *"legalities around sending secure information by email"* and that *"we actually have a policy [. . .] we are not allowed to email"* [diabetes educator/midwife 1].

Hand-held records are relied upon in some settings. However, Midwife 1 described how *"most women choose not to carry [their hand-held record]"* which creates *"another big problem for us."* As reflected on by another midwife, this is *"really hard"* as everything has *"to be duplicated and kept the same [. . .] trying to make sure they're all correct in each location."* Midwife 1 summarized

TABLE 2 | Health professionals' satisfaction with care coordination.

	Dissatisfied or very dissatisfied	Neutral	Satisfied or very satisfied	Not applicable
SATISFACTION WITH REFERRALS AND COMMUNICATION				
The process of referring to medical specialists	9 (11)	20 (24)	50 (61)	3 (4)
The communication received back from medical specialists	27 (33)	17 (21)	33 (40)	5 (6)
The process of referring to allied health specialists	7 (9)	20 (24)	54 (65)	2 (2)
The communication received back from allied health specialists	12 (14)	17 (20)	51 (61)	3 (4)
SATISFACTION WITH SPECIALIST OR HOSPITAL APPOINTMENTS AND ADMISSIONS				
Written information received from client hospital admissions	29 (37)	50 (63)	1 (1)	0
Timeliness of information received from client hospital appointments or admissions	32 (40)	47 (59)	1 (1)	0
Process of arranging appointments in the nearest hospital or specialist clinic	14 (17)	64 (79)	3 (4)	0

Total respondents varied per question and was as follows: Satisfaction with referrals and communication to: medical specialists (n = 82) and allied health specialists (n = 83); Satisfaction with specialist or hospital appointments and admissions: Written information (n = 80), Timeliness (n = 80), Process of arranging appointments (n = 81).

the state of current information systems and said that they are *"hoping to eventually get rid of the written disaster and go with an electronic record."*

Care-Coordination

The inconsistencies and fragmentation of communication and IT systems were reported on as negatively impacting care coordination. Women in remote and many regional areas are often transferred to Cairns to access specialist care and to birth. However, issues with care coordination and lack of systematic processes for referrals and discharge summaries arise from communication breakdown. Midwife 2 described how in their organization "*there's no discharge summary, I don't know whether she had a normal birth, caesarean, and I'm doing a post-natal visit on her and she's been back here for a month."* Similarly, health professionals in Cairns described a lack of information transfer from referring health professionals in other regions. For example, a diabetes educator said that for *"anyone coming down from the Cape, I don't know when they're coming down."* Despite this, one Medical Officer reported how *"in the last half a year"* improvements have been made to patient summaries, including *"any changes to medications"* which contrasts to previously when *"the information we g[o]t back wouldn't be very good."*

The recent introduction of the nurse navigator role at Cairns Hospital for women with GDM aims to improve care coordination, particularly for vulnerable women. As articulated by a midwife, this role will *"help [disengaged women] to access services,"* advocate for their needs and *"coordinate their care [. . .] getting them extra supports"* as required.

Impact of Workforce on Care-Coordination

The high turnover of health staff was frequently identified by participants as a workforce challenge impacting on the delivery of care. A diabetes educator/midwife in Cairns said that care coordination *"does fall over every couple of years [. . .] it's change of staffing, all that sort of stuff."* Again, care-coordination is fraught with issues around person dependence and how when *"health professionals go on leave and [have] not [. . .] been replaced, you might be putting that referral through but it could be months before the client is seen"* [diabetes educator].

Effective care coordination was often described in relation to health professionals' time in their roles. For example, a dietitian described how some "c*ommunities in the Cape have [a] really strong health worker workforce and have had senior health workers in those positions for decades."* They explained how the implication of this is that "*there's really strong relationships with community"* which, as articulated by an Indigenous Health worker, is important to *"build[ing] that relationship and that trust with the girls [making] follow-ups [. . .] easy.*"

Logistics and Access

Given the remote context of FNQ, many women are required to travel to access care which can create challenges. An Obstetrician described that:

> *"The main reason why [women] don't come is because they're reluctant to leave their children behind [. . .] or they want to be able to bring them down [but] they need someone with them who can care for their children when they're birthing [. . .] Or some women are [. . .] scared and they don't want to be in Cairns alone."*

As reported by an Indigenous Health Worker, women *"can have an escort [. . .] in the last four weeks [. . .] of their first pregnancy"* which may overcome some barriers, however, for subsequent pregnancies *"financially it is horrendous"* and *"the cost of flights astronomical."* Furthermore, as explained by an outreach midwife, the lack of formal care coordination often results in women *"get[ting] a bit lost in the system."* In town, transport services offered were generally described as being sufficient, with travel officers often facilitating this process, although not all women have access to these services. Another barrier to care was clinic waiting times at the hospital and primary care clinics which can be *"a huge deterrent"* for women. One midwife described how:

"*I've had so many [clients] that have walked out, they've been waiting and waiting.*"

Additional barriers to accessing care include appropriateness of accommodation, access to food, financial security. As summarized by a midwife navigator:

"If you overcome a lot of those barriers and also got women to come down and relocate, thatdoesn't necessarily guarantee they're still going to engage with services."

Support workers were described as being critical to enhancing all women's access to care.

Knowledge of Guidelines

Women with DIP are a high priority for health professionals in FNQ. As described by an Outreach Diabetes Educator, *"someone with an abnormal BMI [. . .] or GDM or type 2, [. . .] become a higher priority [than other women]."*

Many health professionals reported adherence to local clinical guidelines in the antenatal period. For example, one midwife commented on how *"most [women] get a HbA1C on their first bloods."* However, screening practices in the post-partum period are more varied and challenged by disjointed care-coordination, which:

". . . can be tricky because [women] might be coming back for their annual health checks, but if it's not documented in the chart that she had diabetes then it's not done [. . .] we should be able to [enter information in to the electronic medical record] that she needs that annual follow-up forever, but at the moment we don't have the capacity to do that."

Midwife

DISCUSSION

This mixed methods study revealed the complexities and challenges faced by multidisciplinary services working across large geographic locations, which are relevant to many areas of Australia and other countries with similar high-risk populations. It was apparent that caring for women with DIP was a high priority for the health professionals who engaged in the workshops and survey. Issues were similar to those previously identified in the Northern Territory (13) although there were differences in priorities. The key findings were that disjointed communication disrupts the current system, largely because of information technology difficulties. This in turn affects care-coordination along with other factors such as high staff turnover and remoteness. Knowledge of guidelines and screening recommendations as reported by participants was reasonable but selected areas could be improved.

A common theme regarding communication was the variance in electronic and paper records, with multiple services using different platforms that do not interact with each other. This leads to information being lost or communicated to the wrong health provider particularly when women live rurally and seek care from multiple practices. Practitioners therefore often rely on personal communication which is unsustainable when there is high staff turnover. One suggested solution was that of generic email addresses for midwives at a certain location rather than one person as the sole recipient of communications.

Better integration of systems is a common and ongoing challenge, similar to previous reports from the NT, a comparable geographical setting (16). Queensland Health have recognized the need for better integration at a State-wide level and have introduced the Integrated Electronic Medical Record at Cairns Hospital, a "scalable, reliable, and flexible information-sharing capability that allows integration with new and existing systems, across care settings" (21). However, Aboriginal Controlled Community Health Organizations, private practice, and Queensland Health clinics outside of Cairns Hospital are still not able to fully access the hospital electronic record, although there is a staged plan to increase access. Generating a diabetes related discharge summary from the DIP Clinical Register is an innovation which will be trialed by the DIPPINQ to reduce described gaps in communication.

Information technology could also be used more effectively for recall systems. It was evident that many staff involved in pregnancy care were not involved in either pre-pregnancy or postnatal care beyond 6 weeks, or long-term follow-up screening, where an "inter-pregnancy" window to give opportunistic lifestyle and pre-conception counseling exists (22, 23). A need for more structured follow-up systems has been suggested by others (24). The potential for the DIP Clinical Register to generate follow-up lists to inform primary health centers across the region is currently being explored.

Care-coordination is a function of not only electronic communication systems, but also referral pathways and access to care. Our study participants reported that referral pathways were sometimes unclear and that information received from the hospital, in particular, was lacking or untimely. The DIPPINQ models of care component aims to improve transparency of these pathways, by working with local providers to clarify processes in each district. Cairns Hospital has recently created a nurse navigator role, to guide women with GDM through the complexities of multiple appointments, procedures, and travel. This model has been used successfully in other areas and involves a woman-centered intervention using trained personnel to mitigate barriers for women as they access health services, with a particular focus on ameliorating social disadvantage (25, 26). This nurse will work closely with the DIPPINQ team to improve care-coordination. Barriers to healthcare access described by participants included remoteness and cultural factors. It has been reported that using culturally appropriate resources and improving Indigenous workforce involvement are key areas on which to focus (24). One of the main suggestions for improvement was continued education and up-skilling for local health practitioners, including Indigenous Health Workers.

Telehealth is also used routinely in Cairns to assist with the "hub and spoke" model of providing specialist care (18), striving to counter the problems associated with vast distances. Many practitioners were in favor of this approach

and thought it could be used more frequently. Telehealth has the potential to improve patient access to health care, reduce travel and inconvenience for patients, families, carers, and health professionals and provide health professionals with access to peer support and education (27). One limitation, however, is that obstetric care including examinations and tertiary level ultrasound scans are not always possible via telehealth. One opportunity identified in both this study and by Edwards et al. (13) was that of increased utilization of telehealth for case conferencing with multiple disciplines to improve care for complex cases.

The majority of health professionals were comfortable with universal screening at 24–28 weeks gestation as per the WHO, ADIPS, and IADPSG (28) guidelines. Who should be screened in early pregnancy still raised uncertainty among the study participants, despite the current routine guidelines being in place for 2 years in the region. This raises the possibility that women at high risk are not being screened appropriately and are subsequently missing out on potential treatment. Participants reported confidence in their knowledge of how to manage DIP, with an exception being education of patients in use of insulin. This may contribute to therapeutic inertia and lack of escalation of treatment from dietary to medical therapy, and has been described in both pregnant (29) and non-pregnant patients with T2DM (30). Future education sessions conducted by DIPPINQ will concentrate on the early screening component of the guidelines and appropriate use of medication in DIP. Empowering midwives in particular to be confident regarding insulin education is a strategy which will be explored. Regular audits and review of data from the DIP Clinical Register by DIPPINQ will assist in assessing whether these strategies are effective.

There were a number of limitations to this study. Study findings may have limited generalizability as invitations to participate were through professional networks and may not have included all relevant health professionals. There was some potential bias in that those who responded were more likely to have an interest in DIP management and the dominant perspective was from midwives, the most represented group. Despite this, roles of other participants were quite varied and located across the geographic regions. Participation was voluntary and the response rate was not obtained. Additionally, some questions may not have been relevant to all health professionals. These issues led to missing data for some of the variables, and this limited our ability to interpret some specific results. Stakeholders had to be available to attend the workshops in person which was difficult for those in rural and remote locations, however, multiple subsequent workshops and telehealth options were offered to be flexible and maximize attendance as much as possible.

The strengths of this study were wide representation of practitioners across urban, rural, and remote settings and participation from many different health professions including Indigenous health workers, doctors, nurses, and allied health professionals. Quantitative data from the survey and qualitative results from the focus groups were comparable and themes were consistent across the two methods. Overall the information gathered was extremely useful to inform priorities for the DIPPINQ models of care work.

CONCLUSION

Mapping practitioners' experience providing care for women with diabetes in pregnancy reveals that logistics and management of these women in Far North Queensland can be challenging. There are opportunities for improvement in all the following key themes identified as current concerns: communication systems, information technology, care-coordination, access, and education for health professionals. A complex health systems intervention to address each of these themes is currently being undertaken by the DIPPINQ, with prospective evaluation planned.

ETHICS STATEMENT

Ethics approval for this study was obtained from the Far North Queensland Human Research Ethics Committee (HREC/16/QCH/15).

AUTHOR CONTRIBUTIONS

AM: study design, data collection and interpretation, primary author of manuscript. RK: study design, data collection and interpretation, second primary author. SC, CC, JBo, AB, JM, MW, HM, JO, and AS: study design and manuscript review. CW and JBa: data collection and manuscript review. FB: data interpretation and manuscript review. LM-B: study design, data interpretation, manuscript review, and corresponding author.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2019.00192/full#supplementary-material

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