Metabolic risk factors in mothers and metabolic profile of neonates

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Abstract

High body-mass-index (BMI), hyperglycaemia, and hypercholesterolemia are the leading risk factors for mortality and morbidity worldwide. As obesity has become an epidemic in all age groups globally, more and more women enter pregnancy with obesity. Pregnant women with high BMI often present with excessive gestational weight gain, hyperglycaemia, and dyslipidaemia during pregnancy, therefore expose the foetus to unfavourable intrauterine environment. Although maternal obesity and gestational hyperglycaemia have been associated with a series of adverse pregnancy outcomes in previous literature, lifestyle interventions during pregnancy do not confer significant benefit for composite maternal and neonatal health outcomes. Meanwhile, gestational dyslipidaemia has been recognised as an ignored metabolic risk factor for adverse pregnancy outcomes. Therefore, the aim of this project is 1) to explore the association between gestational dyslipidaemia and neonatal adverse metabolic conditions; 2) to establish the most influential maternal metabolic risk factors for maternal and neonatal adverse metabolic conditions; 3) to investigate the metabolic profile in babies with different birthweight percentiles.

In this project, a comprehensive systematic review followed by a prospective cohort study and an exploratory study were conducted to address the above objectives. In summary, maternal lipid levels are secondary to maternal metabolic dysfunction with no clear causal links to adverse neonatal metabolic conditions, although it has strong associations with adverse birthweight outcomes. High maternal pre-pregnancy BMI is the most influential upstream metabolic risk factor for both maternal and neonatal metabolic health outcomes, therefore weight management should be addressed from the preconception period. The differential

metabolic and inflammatory profile in small-for-gestational-age and large-for-gestational-age babies might be crucial for developing subsequent obesity, diabetes, and cardiovascular diseases. Tailored intervention strategies in babies with different birthweight percentiles are needed to prevent metabolic dysfunctions in adult life.

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List of Abbreviations

A	ABN	Additive Bayesian networks
	ACOG	American College of Obstetricians and Gynaecologists
	ADA	American Diabetes Association
	AGA	Appropriate-for-Gestational-Age
B	BeAT	Beige Adipose Tissue
	BMI	Body Mass Index
	BIGCS	Born in Guangzhou Cohort study
C	CENTRAL	CINAHL Plus and Cochrane library
	CI	Confidence Interval
	CRP	C-Reactive Protein
	CVs	Coefficients of Variation
D	DAGs	Directed Acyclic Graphs
	DBP	Diastolic Blood Pressure
	DHA	DocosaHexaenoic Acid
	DNL	De Novo Lipogenesis
	DVD	Ductus Venous Doppler
E	EDTA	EthyleneDiamineTetraacetic Acid
	ELISA	Enzyme-Linked Immune Sorbent Assay

	EPA	EicosaPentaenoic Acid
	ESC	European Society of Cardiology
F	FFAs	Free Fatty Acids
G	GAM	Generalized Additive Model
	GCT	Glucose Challenge Test
	GDM	Gestational Diabetes Mellitus
	GLM	Generalized Linear Model
	GLMM	Generalized Linear Mixed Model
	GLUT	GLUcose Transporter
	GWCMC	Guangzhou Women and Children's Medical Centre
	GWG	Gestational Weight Gain
H	HAPO	The Hyperglycaemia and Adverse Pregnancy Outcome study
	HDL-C	High-Density Lipoprotein Cholesterol
	HIV	Human Immunodeficiency Virus
	HR	Hazard Ratio
I	IADPSG	International Association of Diabetes and Pregnancy Study Groups
	IDF	International Diabetes Federation
	IL-6	Interleukin 6
	IOM	Institute of Medicine
	IQR	Inter Quartile Range

	IR	Insulin Resistance
	IUGR	IntraUterine Growth Restriction
J	JAMA	The Journal of the American Medical Association
L	LBW	Low BirthWeight
	LDL-C	Low-Density Lipoprotein Cholesterol
	LGA	Large-for-Gestational-Age
M	MD_{adj}	Adjusted Mean Difference
	MetS	Metabolic Syndrome
	MLR	Multivariable Linear Regression
	MOOSE	Meta- analysis Of Observational Studies in Epidemiology
N	NA	Not Applicable
	ND	No Documented
	NICE	National Institute of Health and Clinical Excellence
	NOS scale	Newcastle-Ottawa Scale
	NS-	Negative Screenees of OGTT test
O	OGTT	Oral Glucose Tolerance Test
	OR	Odds Ratio

P	PRISMA	Preferred Reporting Items for Systematic reviews and Meta-analyses
	PS+	Positive Screenees of OGTT test but reach GDM diagnostic threshold value
R	RC	Regression Coefficients
	RCT	Randomized Controlled Trial
	RCOG	Royal College of Obstetricians and Gynaecologists
	Ref	Reference group
	RR	Risk Ratio
S	SAT	Subcutaneous Adipose Tissue
	SBP	Systolic Blood Pressure
	SD	Standard Deviation
	SGA	Small-for-Gestational-Age
	STROBE	STrengthening the Reporting of OBservational studies in Epidemiology
T	T1	First Trimester
	T2	Second Trimester
	T3	Third Trimester
	TC	Total Cholesterol
	TG	TriGlycerides
	TNF-α	Tumour Necrosis Factor - alpha

U	UAD	Umbilical Artery Doppler
	US	United States
	UK	United Kingdom
V	VLDL-C	Very-Low-Density Lipoprotein Cholesterol
W	WAT	White Adipose Tissue
	WHO	World Health Organisation
	β_{std}	standardized adjusted regression coefficient
	β_{adj}	adjusted regression coefficient

Chapter 1 Introduction

1.1 Definition and health burden

Overweight and Obesity

Definition and diagnosis criteria

'Overweight' and 'obesity' are terms used to refer to conditions of abnormal or excessive body fat accumulation possibly leading to short or long-term adverse health outcomes (1). Body Mass Index (BMI) is the most commonly used estimate of evaluating of body fat mass in individuals, and is calculated through dividing a person's weight in kilograms by the square of their height in meters (2). The World Health Organisation (WHO) defines adults with a BMI greater than or equal to 25 kg/m^2 as overweight, and those who have a BMI greater than or equal to 30 as obese (1). Obesity is further classified into three categories based on BMI values: Class Ⅰ, 30.0 - 34.9 kg/m^2; Class Ⅱ, 35.0 - 39.9 kg/m^2; Class Ⅲ, ≥40 kg/m^2 (3).

The general WHO definition of BMI classifications was derived by morbidity and mortality data of white European populations, therefore hard to be generalised to other populations. The BMI classifications of overweight and obesity vary among different ethnic groups. WHO Asia-Pacific defined that adults who with a BMI value below 18.5 kg/m^2 are underweight, who with a BMI value between 18.5 and 22.9 kg/m^2 are normal weight, and who with a BMI value between 23 and 25 kg/m^2 are overweight, and who with a BMI value over or equal to 25 kg/m^2 are obese (4). The Indian Consensus Group proposed that the BMI criteria for the Indian population is 23 - 24.9 kg/m^2 for overweight and ≥ 25 kg/m^2 for obesity (5). Clinical guidelines from the Department of Disease Control Ministry of Health in China classified BMIs of 24 - 27.9 kg/m^2 and ≥ 28kg/m^2 as overweight and obesity, respectively (6).

It has been argued that BMI is not a perfect measurement for body fat mass, because it does not assess fat mass and its distribution directly (7). However, evidence has shown that BMI is strongly associated with body fat mass measured by Multi-Compartment Models (gold-

standard methods), and has excellent performance when predicting disease risks (8, 9). BMI is also the easiest way for clinicians, scientists, and individuals to judge the level of body fat and their risk of associated health conditions. Other available measurements of fat mass include waist circumference, skinfold thickness, Bioelectrical Impedance Analysis, and Dual-Energy X-ray Absorptiometry (10).

Prevalence of risk factors

Overweight and obesity have become major public health concerns globally. It is widely considered to be a leading risk factor for total mortality and morbidity (11). The WHO estimates that about 26% (more than 1,250 million) and 13% (650 million) of adults (≥18 years) worldwide in 2016 were overweight or obese, respectively (1). The prevalence of overweight in male and female are almost equal (12). The age-standardised prevalence of overweight and obesity have increased substantially over the recent decades in both developed (e.g. United State: 41% in 1975 vs. 67.9% in 2016) and developing countries (e.g. China: 9.9% in 1975 vs. 32.3% in 2016) (13).

It has been estimated that approximately 24.5 million and 14.6 million pregnant women were overweight or obese worldwide in 2014, respectively (14). India and China had the largest number of pregnant women who were either overweight or obese (4.30 million in India and 4.29 million in China), which altogether accounted for 22.1% of the global overweight burden in 2014 (14). Meanwhile, around 18% of children and adolescents aged 5-19 years were either overweight or obese in 2016 (15). The largest increase in trend occurred in China, where the crude estimate of overweight and obesity prevalence among children and adolescents doubled in the last 10 years from 13.8% to 28.5% (15).

Dyslipidaemia

Definition and diagnosis criteria

Dyslipidaemia refers to the altered amount of lipids or lipoproteins in the blood. It is normally characterized by increased total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), very-low-density lipoprotein cholesterol (VLDL-C), and triglycerides, as well as decreased high-density lipoprotein cholesterol (HDL-C) (16). Dyslipidaemia therefore also known as hyperlipidaemia, hypercholesterolemia, hyperlipidaemia, or hypertriglyceridemia. There is no established cut-off point between normal and abnormal lipid thresholds (17). BMJ best practice indicates that patients whose serum TC, LDL-C, apolipoprotein B, or lipoprotein (a) concentrations were over the 90^{th} percentile, or HDL-C or apolipoprotein A-I concentrations were less than the 10^{th} percentile, for the general population could be diagnosed as dyslipidaemia (18). The interpretation of lipid profiles in adults can be found in **Table 1**. In addition, the NHS indicated that TC levels should be 5 mmol/L or less and LDL-C levels should be 3 mmol/L or less for healthy adults (19).

Table 1 Interpretation of lipid profiles in adults (20, 21)

Lipids profile	mg/dL	mmol/L	Interpretation
TC	<200	<5.2	Desirable
	200-239	5.2-6.2	Borderline
	>240	>6.2	High
HDL-C	<40	<1.0	Undesirable; risk increased
	41-59	1.0-1.5	Okay, but not optimal
	>60	>1.55	Good; risk lowered
LDL-C	<100	<2.6	Most desirable
	100-129	2.6-3.3	Good
	130-159	3.4-4.1	Borderline high
	160-189	4.1-4.9	High and undesirable
	>190	>4.9	Very high
TG	<150	<1.69	Normal
	150-199	1.70-2.25	Borderline high
	200-499	2.26-5.63	High
	≥500	≥5.64	Very high

Abbreviation: TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

Prevalence of risk factors

In 2008, the WHO estimated that the global prevalence of raised TC (≥ 5.0 mmol/L) was 39% in adults. The estimate of raised TC prevalence was highest in the WHO Region of Europe (54%) and was lowest in the WHO African Region (22.6%) (22). As a major risk factor for cardiovascular diseases, dyslipidaemia is estimated to cause 7.9% of total mortality (4.4 million deaths) and 2.8% of total disability adjusted life years (40.4 million) (23). It has become a major health burden in both the developed and developing countries due to its contribution to rising morbidity, mortality, and medical costs (24).

In 2015-2016, data from the Centres for Disease Control and Prevention showed approximately 95 million United States (US) adults (aged over 20 years) have TC levels greater than 200 mg/dL, and 71 million US adults have high LDL-C levels (≥100 mg/dL) (25, 26). In 2015-2017, the age-standardized prevalence of dyslipidaemia in rural areas of Henan Province (China) was 32.21% (Male: 42.85% & Female: 26.16%). Only around 22.45% of patients with dyslipidaemia received treatment, with half of their lipid levels being well controlled (27).

Except diet and physical interventions, available pharmacologic therapy for dyslipidaemia management containing statins (target on LDL-C), fibrates (only for severe hypertriglyceridemia), omega-3 fish oil (for severe hypertriglyceridemia), niacin (an adjunct for reducing triglycerides), bile acid sequestrants (for reducing LDL-C and modestly increasing HDL-C, but may increase triglycerides), cholesterol absorption inhibitors (for reducing LDL-C), and proprotein convertase subtilisin/kexin type 9 inhibitors (in combination with statin for lowering LDL-C in individuals with familial hypercholesterolemia) (21).

Gestational weight gain

Definition and diagnosis criteria

Once women became pregnant, their weight changed in line with their pregnancy progression. Total gestational weight gain (GWG) can be defined as the altered weight in pregnant women between conception and the last prenatal visit before labour. In 2009, the Institute of Medicine (IOM) recommended that the optimal GWG of singleton pregnancy should range between 12.5-18 kg, 11.5-16 kg, 7-11.5 kg, and 5-9 kg for women underweight, normal weight, overweight, and obese prior to pregnancy, respectively (28). Women whose GWG were over the upper limit of the optimal range should be considered to have excessive GWG, and those whose GWG were below the lower limit of the optimal range should be considered to have inadequate GWG (28).

In practice, women normally realize they are pregnant at or after four weeks of gestation. Therefore, pre-pregnancy weight information used for GWG calculation is often self-reported. Although women may potentially underestimate their weight, evidence suggests that most women still remain in the same BMI categories using self-reported pre-pregnancy weight (29). Since obesity has become an epidemic in both developed and developing countries, more and more women are now severely obese when they become pregnant. The IOM recommendations did not differentiate the optimal GWG ranges among women with different obesity classes , due to a lack of both short- and long-term evidence of GWG on severe obese women (30).

The generalizability of the IOM GWG recommendations to Chinese women is a controversial issue, because the IOM recommendation was developed based on Caucasian and black populations. Tan et al. applied IOM criteria in 4,567 pregnant women living in Chengdu and concluded that the IOM recommendation is fit for Chinese women with underweight and normal weight, but its criteria for overweight and obese women warrant further modification

(31). Similarly, Huang et al. suggested that the IOM recommendation is generally fit for Chinese women, but the BMI cut-off point remains to be legislated by Chinese guideline (32).

Prevalence of risk factors

A report from the US Centres for Disease Control and Prevention found that the overall prevalence of inadequate GWG was 20.4% and the prevalence of excessive GWG was 47.5% in 2012-2013 (33). Meanwhile, about one third of women gained excessive weight during pregnancy in Sydney (34). Compared to women with normal weight, women who were underweight before pregnancy had a 50% lower risk of excessive GWG, whereas women who were overweight or obese had an increased risk of excessive GWG (177% for overweight women and 166% for obese women) (35). Meanwhile, women who were underweight or obesity Ⅱ or Ⅲbefore pregnancy, had an increased risk of inadequate GWG (underweight: 40%; obesity Ⅱ: 25%; obesity Ⅲ: 86%) (35).

Gestational Diabetes Mellitus

Definition and diagnosis criteria

Gestational diabetes mellitus (GDM) refers to glucose tolerance that develops or is first recognised during pregnancy, and usually disappears after delivery (36, 37). The screening and diagnostic approaches and thresholds vary across countries.

Women meeting one of the following criteria were recognized to have an increased risk of GDM: 1) obesity; 2) previously delivered a baby over 4.5kg; 3) had GDM before; 4) has a parent or sibling with diabetes; 5) family origins are Asian, Chinese, African-Caribbean, or Middle Eastern (38). The National Institute of Health and Clinical Excellence (NICE) in the United Kingdom (UK) recommends screening those women with one or more risk factors for

GDM (38). Other than NICE, guidelines and recommendations argue that using historical factors to identify high GDM risk population will miss about half of women with GDM, thus the impetus for GDM screening and diagnostic tests to be conducted in all pregnant women (39-44).

There are two major strategies used for GDM screening and diagnosis: 1) One-step approach: 75-g oral glucose tolerance test (OGTT); 2) Two-step approach: 50-glucose challenge test (GCT) followed by 100-g OGTT (in positively screened women) (45). The diagnosis thresholds for these two strategies can be found in ***Table* 2**. The majority of organizations recognize the one-step approach designed by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) in 2010 (38, 40, 42-44), the American College of Obstetricians and Gynaecologists (ACOG) use the two-step approach (39), while the American Diabetes Association (ADA) accepts both two methods (41).

Table 2 Screening and diagnosis thresholds of GDM (39, 41)

Cut-off point at different time points (mmol/L)	One-step	Two-step		
	75-g OGTT	50-g GCT	100-g OGTT	
			Carpenter-Coustan	NDDG
Fasting	5.1	-	5.3	5.8
1 hour	10.0	7.2 to 7.8	10.0	10.6
2 hour	8.5	-	8.6	9.2
3 hour	-	-	7.8	8.0

One-step strategy: 75-g OGTT should be performed at 24-28 weeks of gestation in overnight fasting status. The diagnosis of GDM is made when any of the cut-off points of glucose values at three time points are met or exceeded.
Two-step strategy: 50-g GLT should be performed at 24-28 weeks of gestation in non-fasting status. If the one-hour plasma glucose measurement is reached or is over 7.2 mmol/L, 7.5 mmol/L, or 7.8 mmol/L, proceed to 100-g OGTT. There are two diagnostic thresholds for three-hour OGTT listed in this table. Without clear comparative evidence, one set of diagnostic criteria cannot be recommended over the other. The diagnosis of GDM is made when at least two of the cut-off points of glucose values at four time points are met or exceeded.
Abbreviation: OGTT, oral glucose tolerance test; GCT, glucose challenge test; NDDG, National Diabetes Data Group

Prevalence of risk factors

The estimate of GDM prevalence vary widely both within and between countries. A report based on WHO data shows that the median prevalence of GDM ranges from 5.8 % to 12.9% around the world between 2005 and 2015 (46). The estimate of GDM prevalence in Singapore was the highest (25.1%, based on the IADPSG criteria), while it in Ireland was the lowest (1.8%, using NICE criteria) (46). In 2015, approximately 4.4% of 956,861 pregnancies in the UK were diagnosed as GDM (47). A recent systematic review reported that the prevalence of GDM in China varies from 5.12% (Xinjiang) to 22.8% (Tianjin) (48). In the United States (US), around 7.6 % pregnant women were diagnosed as GDM, 19.7% of them developed to type 2 diabetes postpartum (49). Another meta-analysis summarised that about half of pregnant women with previous GDM are at risk of GDM recurrence (50).

Gestational Hypertension

Definition and diagnosis criteria

Gestational hypertension is defined as hypertension presenting after 20 weeks of gestation without significant proteinuria (over 300 mg protein in a validated 24-hour urine collection result, or creatinine ratio ≥ 30 mg/mmol) or previous hypertension (51, 52). The diagnosis threshold of gestational hypertension is a diastolic blood pressure (DBP) greater than or equal to 90 mmHg or a systolic blood pressure (SBP) greater than or equal to 140 mmHg (51, 52). Both NICE and European Society of Cardiology (ESC) further classified gestational hypertension as three categories: 1) Mild hypertension: DBP 90-99 mmHg or SBP 140-149 mmHg; 2) Moderate: DBP 100-109 mmHg or SBP 150-159 mmHg; 3)Severe DBP ≥ 110 mmHg or SBP ≥ 160 mmHg (52, 53).

Prevalence of risk factors

The prevalence of gestational hypertension varies in different populations. In the US, 30.6 women in every 1,000 deliveries experienced gestational hypertension in 2004 (54). Data from NHS Maternity Statistics summarised that about 6% of England women were reported as having hypertension during pregnancy in the period 1998-99 to 2000-01 (55). In 2011, reports of the prevalence of gestational hypertension in China range from 1.23% to 7.44% (56). The estimated prevalence of gestational hypertension was 7.5% in Brazil (1991-95) and was 5.9% in Nigeria (2016) (57, 58). Evidence also suggests that women with twin pregnancies may be more likely to experience gestational hypertension than women with singleton pregnancies (59).

Low and high birthweight

Definition and diagnosis criteria

The term of 'birthweight' refers to the body weight of newborns at birth. Babies with a birthweight less than 2,500 grams are described as 'low-birth-weight (LBW)' (60), and those with a birthweight more than 4,000 grams are defined as having 'macrosomia' (61). As birthweight changes along with the gestation week of delivery and neonatal sex, the International foetal and newborn growth consortium developed the INTERGROWTH-21st Newborn Size at Birth Chart to help identify the relative position of individual newborns among the whole population (62). Babies with a birthweight below the 10th percentile for their gestational age are considered as 'small-for-gestational-age (SGA)', while those with a birthweight greater than 90th percentile for their gestational age are considered as 'large-for-gestational-age (LGA)'(63).

The cut-off points for defining SGA and LGA were suggested by the WHO expert committee in 1996 and has been criticised for being arbitrarily chosen. Using data from 17,979,120

singleton live births, Xu et al. suggested the optimal cut-off points for defining SGA and LGA for the risk of neonatal death and low 5-min Apgar score might be the 15th and 97th percentiles of birthweight, respectively (64).

As a crucial indicator of prenatal developmental conditions for newborns, birthweight has been associated with both short- and long-term health outcomes, including stillbirth, infant mortality, obesity, type 2 diabetes, and cardiovascular diseases (65-67). Birthweight is one of the most intuitive and accessible indicators that can be precisely recorded in clinics, therefore it has been widely used by both clinicians and researchers. It is worth noting that birthweight is an imperfect proxy for neonatal development and body fat mass, so that it needs to be considered with caution (68).

Prevalence of risk factors

The WHO estimated that there are more than 20 million LBW newborns accounts for about 15% - 20% of all births worldwide in 2012 (69). In fact, around half of perinatal and one-third of neonatal overall mortalities can be attributed to LBW (70). The vast majority of LBW births (95.6%) occurred in low- and middle- income countries, especially in South Asia (data exclude China), where 28% of infants were born with LBW (71, 72).

Data from WHO's Global Survey on Maternal and Perinatal Health shows that the prevalence of macrosomia varies from 0.5% (India) to 14.9% (Algeria) among 24 developing countries in Africa, Latin American, and Asia (73). In the US, about 7% of infants were affected by macrosomia and 1% of infants had birthweight over 4,500g in 2015 (74). Similarly, in 2012, approximately 11.78% newborns in the UK have a birthweight greater than or equal to 4,000 grams (75). A 2011 national cross-sectional survey in China summarised that the overall estimate of macrosomia prevalence was 7.3%, ranging from 4.1% to 13.4% by province (76).

1.2 Current guidelines and recommendations

Weight management before and during pregnancy

Weight management before pregnancy

Advice on pre-pregnancy weight management is limited and ambiguous. One NICE public health guideline on weight management recommends that health professionals should encourage, educate, and help women with obesity to lose weight (ideally to reduce their BMI within 24.9 – 18.5 kg/m^2) before becoming pregnant. Meanwhile, it also suggests that the NHS commissioner/managers and public health directors should ensure that health professionals appreciate the importance of weight management before pregnancy (77).

Similarly, another guideline from the Royal College of Obstetricians and Gynaecologists (RCOG) in the UK suggests that primary care services should advise all women of childbearing age during their contraceptive consultations on their weight and lifestyle to ensure the best chance of optimising their weight before pregnancy, especially those with BMI of 30 kg/m^2 or more. Women who have received bariatric surgery should wait for at least 12-18 months before conceiving (78).

In addition, the IOM suggests that women should be informed of the importance of conceiving with a BMI within the normal range by federal, state, and local agencies (28). None of these guidelines focus on weight management in adults provide any specific suggestion for women of childbearing age to prevent adverse pregnancy outcomes (79-82).

Weight management during pregnancy

Recommendations on weight management during pregnancy are controversial. The IOM suggests that the weight gain of women during pregnancy should be routinely monitored by the Department of Health and Human Services (28). Both IOM and ACOG advise that health

professionals should provide guidance on weight gain, diet, and physical activity throughout the pregnancy to ensure that gestational weight gain is within the appropriate range recommended by IOM (28, 83).

On the contrary, the NICE does not recommend regular weighing of women during pregnancy to ensure 'appropriate' gestational weight gain, as there is still lack of consensus on optimal weight gain during pregnancy (77, 78). It also suggests that all pregnant women should be offered practical and tailored advice on diet (both dieting and 'eating for two' are not recommended) and moderate-intensity physical activity (77). Women with a BMI of 30 kg/m^2 or more should be weighed again in the third trimester, and should be provided both with necessary information about the potential risk of obesity during pregnancy and with dietetic suggestions by professionals (78). Additionally, it is necessary to provide nutritional surveillance and specialised nutritional advice to women who have had previous bariatric surgery (78).

Gestational diabetes mellitus

Preconception planning and care

The ADA suggests that health professionals should discuss the importance of family planning, effective contraception, and glycaemic control with women and their partners at the time of preconception counselling (84). Although all pregnant women should be screened for GDM at the end of the second trimester, an early pregnancy screening for undiagnosed diabetics in high-risk populations is also recommended by the ACOG (***Table* 3**) (39).

Table 3 Strategy for Screening Undiagnosed Diabetes or Early GDM (39)

Women who are overweight or obese with at least one of the following risk factors need to be tested:

- Physical inactivity
- First-degree relative with diabetes
- High risk race or ethnicity (e.g. African American, Latino, Native American, Asian American, Pacific Islander)
- Have previously delivered a macrosomia
- Previous GDM, impaired glucose tolerance/fasting glucose, or $HbA_{1c} \geq 5.7\%$
- History of hypertension
- HDL-C < 0.90 mmol/L, triglycerides > 2.82 mmol/L
- With polycystic ovarian syndrome (PCOS)
- Insulin resistance relevant clinical conditions (e.g. acanthosis nigricans)
- History of cardiovascular disease

Glycaemic control during pregnancy

The NICE suggests that health professionals should explain the implications of GDM and the importance of glycaemic control during pregnancy to women who were diagnosed as having GDM (38). Women with GDM need to be taught how to self-monitor their fasting and postprandial blood glucose levels (38, 84).

Individualised and practical counselling of nutrition and exercise needs to be offered to women with GDM by a registered dietitian or other relevant health professionals (38, 39, 84). With lifestyle modification alone, the majority of women with GDM could meet the glycaemic targets successfully (***Table* 4**) (84).

If women with GDM fails to maintain adequate glycaemic control with lifestyle therapy alone, pharmacologic treatment needs to then be recommended (38, 39, 84). Both ADA and ACOG suggest that insulin is the preferred choice for GDM, since it cannot cross the placenta (39, 84), while the preferred method suggested by NICE is metformin (38). Other than insulin and metformin, glibenclamide could be considered as alternative treatment for women with GDM as well (38, 39, 84). The ACOG further points out that health care providers should discuss the potential safety issues of oral agents with women with GDM (39).

Table 4 Glycaemic targets of women with GDM (38, 84)

Glycaemic targets at different time points	ADA	NICE
Fasting	5.3 mmol/L	5.3 mmol/L
1-hour postprandial	7.8 mmol/L	7.8 mmol/L
2-hour postprandial	6.7 mmol/L	6.4 mmol/L

The glycaemic level of women with GDM need to achieve the glycaemic target at fasting status and either the glycaemic targets at 1-hour postprandial or 2-hour postprandial.
Abbreviation: GDM, gestational diabetes mellitus; ADA, American Diabetes Association; NICE, National Institute for Health and Care Excellence.

Intrapartum care

The NICE advises health professionals to discuss the timing and mode of delivery with women with GDM at the third trimester (38). In particular, for women with GDM who have an ultrasound-diagnosed macrosomic foetus, both NICE and ACOG indicate that the risks and benefits of each delivery mode need to be clearly explained by health professionals (38, 39).

The NICE suggests that women with GDM should be advised to delivery no later than 40+6 weeks, especially those with maternal or foetal complications (38). For women with GDM who have not yet given birth by that time, health professionals should offer an elective birth, including induction of labour and caesarean section (38). During labour, the capillary plasma glucose of women with GDM should be measured hourly and needs to be maintained between 4 and 7 mmol/L (38).

Meanwhile, the ACOG recommends that the timing of giving birth for women with GDM using non-pharmacologic therapy should be in the range of 39 to 40 + 6 weeks of gestation (39). For women with GDM who are taking pharmacologic therapy, the optimal timing of delivery is 39 weeks of gestation (39).

Postpartum care

Both ADA and ACOG suggest screening women who are diagnosed as GDM 4-12 weeks postpartum to identify women with diabetes, impaired glucose tolerance, or impaired fasting glucose levels (39, 84), while the screening time suggested by NICE is 6 - 13 weeks postpartum (38). The ADA recommends using the 75-g OGTT with nonpregnancy criteria for postpartum screening (84). Conversely, the NICE prefers the fasting plasma glucose test, and suggests that the 75-g OGTT test should not be offered routinely (38). If a women has not performed a fasting

plasma glucose test by 13 weeks postpartum, a HbA1c test could be an alternative option if the fasting plasma glucose test is not possible after 13 weeks postpartum (38).

For women who are diagnosed as GDM with a moderate risk of developing type 2 diabetes (fasting plasma glucose level < 6.0 mmol/L or HbA1c < 5.7%), the NICE recommends offering lifestyle advice and an annual HbA1c test. For women who are diagnosed as GDM with a high risk of developing type 2 diabetes (fasting plasma glucose level: 6.0 - 6.9 mmol/L or HbA1c: 5.7 – 6.4%), advice, guidance, and interventions need to be offered to prevent the onset of type 2 diabetes. Women with fasting plasma glucose ≥ 7.0 mmol/L or HbA1c level ≥ 6.4% at postnatal test could be directly diagnosed as type 2 diabetes (38).

Both ADA and ACOG suggest that women who are diagnosed as GDM with a negative result of their postnatal test should be screened for type 2 diabetes every 1-3 years (39, 84). In future pregnancies, the NICE recommends that women with previous GDM should be offered an early pregnancy OGTT test or asked to self-monitor glucose levels (38).

Gestational hypertension

Management of hypertension in pregnancy

For pregnant women with both diabetes and chronic hypertension, the ADA recommends that the blood pressure should be remained in the range of DBP 80 - 105 to SBP 120 - 160 mmHg (84). However, the ESC suggests that there is not enough evidence in support of setting up a blood pressure target in pregnancy (53).

The NICE suggests that additional assessment and follow-up needs to be carried out in women who are diagnosed as gestational hypertension with the following risk factors: nulliparity, age ≥ 40 years, pregnancy interval > 10 years, family or previous history of pre-eclampsia, multiple

pregnancy, BMI ≥ 35 kg/m^2, previous gestational hypertension, or pre-existing vascular/kidney diseases (52). The management process of gestational hypertension is shown in ***Table* 5**.

The ESC suggests pregnant women with a blood pressure of 170/110 mmHg or more are considered as emergency cases in need of hospitalization (53). The ACOG also indicates that antihypertensive treatment should be administered for those with acute-onset persistent severe hypertension within 30 - 60 minutes (51). Contrary with the NICE guidelines, the ESC suggests that all three medicines (labetalol, oral methyldopa, and nifedipine) could be used as first-line antihypertensive treatment (53). Use of i.v.hydralazine, i.v. urapidil, and sodium nitroprusside (last resort) could be carefully considered in women with severe hypertension, while angiotensin-converting-enzyme inhibitors, angiotensin receptor blockers, and direct renin inhibitors should be strictly contraindicated (53). In addition, magnesium sulphate, suggested by the ACOG, could be used to prevent seizure in women with gestational hypertension (51).

The effect of non-pharmacological treatment on gestational hypertension is limited. The ESC advises that women who are diagnosed as gestational hypertension with obesity might need to be recommended regular exercise carefully to prevent excessive gestational weight gain (≥ 6.8 kg) (53). Bed rest is not suggested for women with gestational hypertension (52).

Table 5 Gestational hypertension management (52)

Degree of hypertension	Mild hypertension (140/90 to 149/99 mmHg)	Moderate hypertension (150/100 to 159/109 mmHg)	Severe hypertension (≥160/110 mmHg)
Hospitalization	No	No	Yes
Treatment	No	First-line: Labetalol Second-line: Methyldopa & nifedipine	First-line: Labetalol Second-line: Methyldopa & nifedipine
Treatment target	No	<150/100 to 150/80 mmHg	<150/100 to 150/80 mmHg
Blood pressure measurement	≤ Once a week	≥ Once a week	Inpatients care: ≥ Four times a day Outpatients care: twice a week
Proteinuria test	Creatinine ratio measured at each visit	Creatinine ratio measured at each visit	Inpatients care: Creatinine ratio measured daily Outpatients care: urine test twice a week
Blood tests	Routine antenatal care	Test if there is proteinuria, including kidney function, electrolytes, full blood count, transaminases, bilirubin	Inpatients care: Test at presentation and then monitor weekly: kidney function, electrolytes, full blood count, transaminases, bilirubin Outpatients care: once a week

Intrapartum care

The ACOG recommends expectant management up to 37 + 0/7 weeks of gestation for women with gestational hypertension without severe features (51). After that, delivery is the best option rather than expectant management (51, 53). The NICE argues that the timing of birth should be discussed between the senior obstetrician and women, for those women with a blood pressure of 160/110 mmHg or less after 37 weeks (52). Delivery is recommended after maternal stabilization or with labour/pre-labour rupture of membranes, if a women with gestational hypertension has severe features at or after 34 0/7 weeks (51). In addition, women with gestational hypertension taking steroids should give birth as soon as possible (51, 52).

Postpartum care

Blood pressure should be monitored continuously in women who had gestational hypertension after giving birth (52). If blood pressure remains high (≥ 130/80 mmHg), women need to take antihypertensive medicines (except methyldopa) after delivery (52, 53). Medical review and specialist assessment needs to be offered to women who had gestational hypertension at the postnatal review (6-8 weeks after delivery) (52). Women who had gestational hypertension should receive a care plan and be transferred to community care (52).

Low and high birthweight

Low birth weight (LBW)

The WHO suggests that very low birth weight (VLBW) and LBW infants should preferably be fed human milk exclusively until 6 months of age. Standard infant formula should be given from the time of discharge to six months postpartum to infants who could not be fed human milk. VLBW infants who fail to gain weight with adequate standard infant formula should be

fed preterm infant formula. VLBW infants fed with human milk should be given Vitamin D, Calcium, phosphorus, and iron daily for an appropriate time within 6 months of age (85).

Small-for-gestational age (SGA)

To prevent SGA birth, the RCOG suggests that the primary prevention efforts should be made to promote smoking cessation. In particular, antiplatelet agents need to be offered to women with a high risk of preeclampsia before or at 16 weeks of gestation, since this may be effective in reducing the risk of SGA. Umbilical Artery Doppler (UAD) is the primary surveillance tool to reduce the risk of perinatal morbidity and mortality, while Ductus Venous Doppler (DVD) has the predictive value at delivery for acidaemia and adverse outcomes in preterm SGA with abnormal UAD. For a SGA foetus with normal UAD and DVD, the timing and mode of delivery should be consulted with a senior obstetrician, and the time should be no later than 37 weeks of gestation (86).

The Queensland Clinical Guideline recommends promoting and supporting breastfeeding for full-term SGA infants. Feeding should be in response to neonatal needs. If infants fail to suck breast milk, a feeding volume of 60 mL/kg should be offered in the first day of birth, and should then be increased by 30 mL/kg every day subsequently (87).

Large-for-gestational age (LGA) or Macrosomia

The NICE recommends the induction of labour should not be considered in women with LGA/macrosomic foetus if there is no other indication (88). No other recommendation for LGA or macrosomic infants was found.

1.3 Existing epidemiological evidence

Maternal obesity

Maternal pre-pregnancy obesity has been associated with adverse health conditions in both mothers and their offspring.

Maternal obesity and maternal health

The impact of maternal high pre-pregnancy BMI starts during early pregnancy. A large case-control study shown that the risk of early miscarriage (odds ratio [OR] = 1.20, 95% confidence interval [CI] 1.01 to 1.46) and recurrent early miscarriages (OR = 3.5, 95% CI 1.03 to 12.01) in women with obesity were significantly higher than the risk in lean women (89). Compared with women with a normal pre-pregnancy BMI, the risk of stillbirth increased by 47% (95% CI 8% to 94%) and 107% (95% CI 59% to 174%) among women with overweight or obesity, respectively (90).

Women who were overweight/obese prior to pregnancy have an increased risk of developing GDM, gestational hypertension, and pre-eclampsia, compared with women with a normal BMI (91). The odds ratios of GDM increased by the degree of obesity, which are 1.97 (95% CI 1.77 to 2.19) for overweight women (BMI 25 - 29.9 kg/m^2), 3.01 (95% CI 2.34 to 3.87) for moderately obese women (BMI 30 - 34.9 kg/m^2), and 5.55 (95%CI 4.27 to 7.21) for morbidly obese women (BMI $\geq$ 35kg/m^2), respectively (92). Other than metabolic complications, women with pre-pregnancy obesity are more likely to experience depressive symptoms in pregnancy (OR = 1.43, 95% CI 1.27 to 1.61) and postpartum (OR = 1.30, 95%CI 1.20-1.42) (93).

During the period of labour, infants of women with either pre-pregnancy overweight or obesity have increased risks of shoulder dystocia, caesarean section, and perinatal death, compared with lean women (91, 94). Maternal pre-pregnancy overweight is associated with both pre-term birth

(RR = 1.06, 95% CI 1.01 to 1.11) (95) and post-term birth (OR = 1.24, 95% CI 1.15 to 1.34) (96).

In the postpartum period, compared with women with normal weight, obese women are less likely to initiate breastfeeding and more likely to have a delayed lactogenesis onset and inadequate milk supply (97).

Maternal obesity and offspring health

It is well known that maternal pre-pregnancy overweight/obesity has substantial impacts on the development of offspring.

Evidence indicated that neonates born to women with overweight/obesity have an increased risk of neural tube defects (overweight: OR = 1.20, 95% CI 1.04 to 1.38; obese: OR = 1.87, 95% CI 1.62 to 2.15), compared with neonates born to lean women (98, 99). Meanwhile, the offspring of obese mothers were more likely to be affected by congenital anomalies, including spina bifida, hydrocephaly, anorectal astresia, limb reduction anomalies, cardiovascular anomalies, cleft palate, cleft lip and palate, and septal anomalies (99).

It has shown that the risk of compromised neurodevelopmental outcomes (e.g. autism spectrum disorder, attention deficit-hyperactivity disorder, developmental delay, and emotional/behavioural problems) were significantly higher in children whose mother were overweight (OR = 1.17, 95% CI 1.11 to 1.24) or obese (OR = 1.51, 95% CI 1.35 to 1.69) prior to pregnancy than the risk in children with lean mothers (100). Another meta-analysis also indicated that the risk of cerebral palsy increased by 51% (95% CI 24% to 84%) among children born to overweight/obese mothers (101).

Consistent findings indicated that maternal high pre-pregnancy BMI is significantly associated with the increased risk of LGA (OR = 2.42, 95% CI 2.16 to 2.72), macrosomia (OR = 2.17, 95%

CI 1.92 to 2.45) (102), childhood obesity (103), and cardiovascular and metabolic diseases in adulthood (104, 105). Another systematic review concluded that maternal high pre-pregnancy BMI is associated with altered offspring DNA methylation levels, which may contribute to the elevated life-long risk of metabolic syndromes in offspring (106). In addition, infants born after maternal bariatric surgery have significantly reduced fat mass and an elevated fasting insulin level, compared with their siblings born before surgery (106).

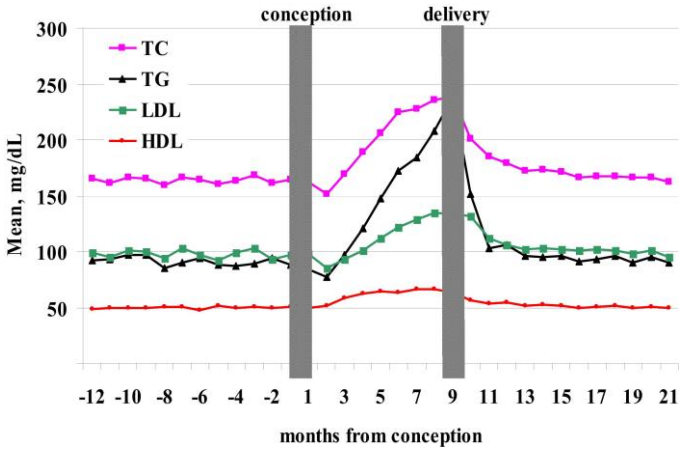
Maternal dyslipidaemia

Maternal circulating lipid levels, including TC, HDL-C, LDL-C, and triglycerides, slightly decreased in the first 6 weeks after conception, and then dramatically increased in line with the progression of pregnancy. After childbirth, maternal lipid levels gradually dropped to the level before pregnancy (***Figure 1***) (107).

Maternal dyslipidaemia and maternal health

Maternal lipid levels during pregnancy have been associated with the incidence of GDM, pre-term delivery, as well as pre-eclampsia. Compared with non-GDM women, women who are diagnosed as GDM have a significant elevated triglycerides level throughout pregnancy and a significant reduced HDL-C level in the second and third trimesters (108). The latest systematic review concluded that women with either low or high lipid levels (TC, HDL-C, LDL-C, and triglycerides) during pregnancy might have an increased risk of preterm delivery (109). Moreover, a meta-regression analysis demonstrated that the elevated maternal TC and triglycerides levels throughout pregnancy and the decreased maternal HDL-C levels in the third trimester are associated with the incidence of pre-eclampsia (110).

Figure 1 The average concentrations of maternal lipid levels one year before, during, and one year after gestation (107)



Maternal dyslipidaemia and neonatal health

Apart from maternal lipid levels during pregnancy, maternal pre-pregnancy weight, gestational weight gain, and glucose levels during pregnancy have been associated with a series of adverse neonatal health outcomes in previous literature (99, 102-104, 111-115). Although maternal lipid levels during pregnancy are associated with the increased risk of pre-term delivery and pre-eclampsia (109, 116), evidence regarding the association between maternal lipid levels during pregnancy and neonatal health outcomes remains inconsistent and controversial (117-119), which needs to be appropriately addressed. No previous systematic review has produced a synthesis for the association between maternal lipid levels and neonatal health outcomes. Therefore, I conducted a systematic review to synthesis evidence on the association between maternal lipid levels during pregnancy and neonatal health outcomes, including birthweight, metabolic factors, and inflammatory parameters (Chapter 3).

Gestational weight gain

Gestational weight gain and maternal health

A narrative review published in 2015 indicated that maternal excessive weight gain in the first and second trimester is an independent risk factor of impaired glucose tolerance during pregnancy, GDM, type 2 diabetes, gestational hypertension, and pre-eclampsia (120). Meanwhile, among women with obesity before pregnancy, inadequate GWG was also associated with decreased risk of gestational hypertension (OR = 0.70, 95% CI 0.53 to 0.93) and pre-eclampsia (OR = 0.90, 95% CI 0.82 to 0.99) (121). However, another systematic review (2017) including six studies on the association between GWG and GDM concluded that there is no sufficient evidence for this relationship (111).

Compared with women with optimal GWG, women with inadequate GWG have an increased risk of preterm delivery (OR = 1.70, 95% CI 1.32 to 2.20), while women with excessive GWG have a higher risk of caesarean section (OR = 1.30, 95% CI 1.25 to 1.35) and a lower risk of preterm delivery (OR = 0.77, 95% CI 0.69 to 0.86) (111). Evidence also indicates that women with obesity who gaining excessive GWG were at risk of preterm delivery as well (OR = 1.54, 95% CI 1.09 to 2.16) (122). There is also a clear association between excessive GWG and maternal postpartum weight retention (123).

Gestational weight gain and neonatal health

It has been established that women with inadequate GWG are at a higher risk of having SGA babies (OR = 1.53, 95% CI 1.44 to 1.64), while women with excessive GWG are at a higher risk of having LGA babies (OR = 1.85, 95% CI 1.76 to 1.95) and macrosomia (OR = 1.95, 95% CI 1.79 to 2.11) (111). Maternal excessive GWG was also recognised as one of those leading risk factors for early childhood obesity (103).

Maternal excessive GWG may be associated with neonatal fat mass and long-term metabolic health, but the quality of evidence was poor (120, 124). In addition, a genetic systematic review demonstrated that maternal GWG has no association with differentially methylated cord blood, which may imply that there is limited influence on neonatal metabolic health (106).

Interventions for optimising gestational weight gain

Concerted efforts have been made to optimise GWG in women for preventing adverse pregnancy outcomes but end up with conflicting results. A recent meta-analysis indicated that healthy diet and/or physical activity during pregnancy are effective in control of gestational weight gain (125).

A 2017 systematic review indicated that combining diet and exercise interventions during pregnancy and reduced GWG were associated with decreased risk of GDM and caesarean section, but have no clear association with gestational hypertension, mortality, LGA, perineal trauma, neonatal hypoglycaemia, and childhood adiposity (126). In the same year, an individual-patient-data meta-analysis concluded that diet and physical activity interventions during pregnancy only succeeded in reducing GWG and lowering the risk of caesarean section but has no effect on composite adverse outcomes in mothers and infants (127).

In 2015, another systematic review concluded that exercise during pregnancy could help to reduce the risk of GDM (Risk ratio [RR] = 0.69) (128). In particular, for women with obesity, exercise during pregnancy could only help to reduce the risk of pre-term delivery (RR = 0.62, 95% CI 0.41 to 0.95), but has no effect on the risk of GDM, caesarean delivery, low birthweight, macrosomia, and stillbirth (129).

Gestational diabetes mellitus

Gestational diabetes mellitus and maternal health

It is well established that GDM has a major impact on the maternal short- and long-term health. Evidence indicates that the incidence of pre-eclampsia and gestational hypertension in women with GDM is higher than that it in women without GDM (112). During childbirth, women with GDM have an increased risk of pre-term birth, caesarean section, perineal trauma, shoulder dystocia, as well as induction of labour (38, 130, 131).

Approximately 30% to 84% of women with a history of GDM tend to have a recurrence GDM in their next pregnancy (132). Almost half of those women who were diagnosed as GDM would develop to type 2 diabetes within ten years (133). Studies have also shown that women who were diagnosed as impaired glucose tolerance during pregnancy or GDM have a significantly

increased risk of developing cardiovascular disease within ten years after childbirth (134-136). Additionally, women with a history of GDM have a higher incidence of postpartum depression (RR = 1.67, 95% CI 1.22 to 2.28) and non-alcoholic fatty liver disease than women who have never experienced GDM (137, 138).

Gestational diabetes mellitus and neonatal health

Neonates born to mothers with GDM have an increased risk of macrosomia (OR = 1.71, 95% CI 1.52 to 1.94), LGA, neonatal hypoglycaemia, and a higher fat mass at birth (112-114, 139). Compared to neonates of non-GDM women, an increased incidence of anorectal malformations and congenital heart defects were observed in neonates born to women with GDM (115, 140). Maternal GDM seems to be associated with offspring's higher BMI Z-Score and 2-hour plasma glucose level in their childhood. It remains unclear whether the association is independent of maternal BMI (141). Children born to mothers with GDM have a 62% higher risk of autism spectrum disorders, compared to that in children of non-GDM mothers (142). GDM seems to have a negative impact on neonatal cognitive development, but needs to be evidenced (143). In addition, GDM was found to significantly increase neonatal methylation levels, therefore might have life-long impacts on neonatal health (106).

Interventions for glycaemic control in GDM women

Treatments for GDM could significantly lower the risk of shoulder dystocia (OR = 0.40, 95% CI 0.21 to 0.75) and LGA (OR = 0.48, 95% CI 0.38 to 0.62) (112). Both acute and chronic prenatal exercise could effectively reduce the level of circulating blood glucose levels in women with GDM (144). Meanwhile, low glycaemic index diet was demonstrated to reduce the risk of macrosomia among women with GDM (145). If pharmacotherapy is needed for women with

GDM, evidence suggests that insulin and metformin are preferable choices, but not glibenclamide (146). GDM is also considered a disease with heterogeneous physiological characteristics, therefore individualized therapies to maximize the health outcomes of mother and neonates warrants further investigations (147).

Gestational hypertension

Gestational hypertension and maternal health

Hypertensive disorders in pregnancy are closely associated with the incidence of pre-eclampsia (148). Recurrence of hypertensive disorders in pregnancy is associated with the increased risk of pre-term delivery (149).

Although maternal blood pressure typically fall back to a normal level, vascular dysfunctions could even persist in women with a history of gestational hypertension postpartum (150). Evidence indicates that women with a history of gestational hypertension have an increased risk of a series of cardiovascular diseases after childbirth. It containing chronic hypertension (RR = 3.70, 95% CI 2.70 to 5.10), coronary heart disease (RR = 2.50, 95% CI 1.43 to 4.37), stroke (RR = 1.81, 95% CI 1.29 to 2.55), atrial arrhythmias (Hazard ratio [HR] = 1.48, 95% CI 1.10 to 1.98), heart failure (RR = 4.19, 95% CI 2.09 to 8.38), as well as cardiovascular death (RR = 2.21, 95% CI 1.83 to 2.66) (151-153). However, there is no statistically significant differences in the level of lipids and glucose homeostasis parameters observed in women with or without a history of gestational hypertension (151).

Gestational hypertension and neonatal health

Gestational hypertension is associated with an increased risk of hypospadias (OR = 1.68, 95% CI 1.46 to 1.93) and bronchopulmonary dysplasia (OR = 1.59, 95% CI 1.11 to 2.26) in offspring (154, 155).

Neonates born to gestational hypertension mothers probably have higher risks of cardiovascular events (156), low cognitive ability (157), autism spectrum disorder, attention-deficit, hyperactivity disorder (158), schizophrenia, mental and behavioural disorders (159), and retinopathy of prematurity (160).

Interventions for blood pressure control during pregnancy

Antihypertensive drugs (such as oral nifedipine, labetalol, and methyldopa) are safe choices for treatment of gestational hypertension during pregnancy, but have no effect on reducing risk of neonatal cerebral palsy (161-163). Evidence indicates that calcium supplementation in women with the risk of gestational hypertension could potentially prevent preterm delivery (164).

Maternal pre-pregnancy weight, gestational weight gain, and glucose concentration during pregnancy are associated with birthweight and neonatal metabolic health in previous observational studies (102, 111, 113). Meanwhile, maternal lipid levels during pregnancy have been recognised as an ignored metabolic risk factor (118). Most of previous studies only focused on one specific metabolic trait or on the number of metabolic disorders (165, 166). However, these metabolic risk factors highly interact with each other, therefore it is necessary to consider the underlying metabolic network when exploring the association between maternal metabolic risk factors and neonatal metabolic conditions. To address this we conducted an additive Bayesian network analysis to quantify the inter-dependency between maternal

metabolic risk factors and their association with birthweight and cord blood insulin level (Chapter 4).

Low and high birthweight

Birthweight and short- and long-term health outcomes

There is a debate between the use of absolute birthweight and percentile charts. In 1999, a systematic review found that the overall mortality and morbidity is raised among neonates with birthweight less than or equal to the lowest 3rd percentile for their gestational age (65). Another systematic review (2014) found a significantly increased risk of mortality in very low birthweight (VLBW, < 1,500 g) neonates, but not in neonates with low birthweight percentile for gestational age (167). A recent Bayesian meta-analysis also indicates that SGA babies have a significantly higher risk of intrauterine foetal demise (OR = 7.8, 95% CI 4.2 to12.3), neonatal death (OR = 3.5, 95% CI 1.1 to 8.0), perinatal death (OR = 5.8, 95% CI 3.8 to 7.8) and neonatal intensive care unit admission (OR = 3.6, 95% CI 2.0 to 5.5), compared to non-SGA babies. There is no elevated risk of perinatal death, hypoglycaemia, and intensive care unit admission was observed in LGA babies (168). Evidence indicates that there is a mild association between childhood mobility and SGA (OR = 1.49, 95% CI 1.02 to 2.19), but not with LBW (169). Evidence indicates that birthweight might be associated with cognitive ability in childhood (170). Compared to AGA, SGA neonates have relatively lower scores on neurodevelopmental outcomes (171). Similarly, VLBW neonates were found to be twice as likely to have anxiety problems in later life, compared to term non-VLBW neonates (172). In addition, impairment of motor function throughout childhood was also observed in those children with VLBW (173). Macrosomic babies were found to have an increased risk of obesity from childhood to early adulthood (66), while LBW babies were found to have an increased risk of cardiovascular

dysfunction (e.g. endothelial dysfunction, reduced arterial diameters, and altered vascular structure) in their later life (174). Compared to babies with appropriate birthweight (2,500 – 4,000 g), both LBW (OR = 1.32, 95% CI 1.06 to 1.64) and macrosomic (OR = 1.27, 95% CI 1.01 to 1.59) babies were found to associated with an increased risk of developing type 2 diabetes (67).

Babies born small-for-gestational-age (SGA) and large-for-gestational-age (LGA) are at similar risks of developing obesity, diabetes, and cardiovascular diseases (65-67). The metabolic profiles in SGA and LGA babies are largely unknown, therefore cannot reliably inform clinical practice and recommendations to avoid subsequent metabolic dysfunctions. The ectopic fat deposition is considered a common feature linking SGA/LGA babies to the risk of subsequent metabolic dysfunctions (175). However, it is hard to explain why SGA babies with moderate catch-up-growth still show impaired insulin sensitivity in their childhood (176). To investigate whether SGA and LGA babies have similar metabolic profile at birth that lead to subsequent adverse health outcomes, in Chapter 5 I have evaluated the association of birthweight with cord blood metabolic parameters (glucose, lipids, and insulin).

Feeding infants with low or high birthweight

Breastfeeding is the preferable way to feed all infants. Evidence indicated that human milk feeding in LBW babies could help to reduce the risk of late onset sepsis, retinopathy of prematurity and severe necrotising enterocolitis, but has no effect on neurodevelopment and bronchopulmonary dysplasia (177).

Additionally, vitamin A supplementation in VLBW infants seems to have minor effects on reducing the risk mortality at one month of age and chronic lung disease, but has no effect on neurodevelopment (178).

1.4 Potential mechanism

Obesity, insulin, glucose, and lipid metabolism

Adipose tissue and insulin

There are three kinds of adipose tissue in the human body: white, beige, and brown adipose tissue (179). Obesity is characterized by adipose tissue expansion, especially white adipose tissue (WAT) (180). WAT is a key endocrine organ that regulates metabolic homoeostasis. Both 'too much' and 'too little' adipose tissue have been associated with the risk of developing type 2 diabetes mellitus, non-alcoholic fatty liver disease, cardiovascular disease, polycystic ovary syndrome, as well as several cancers (180). Other than the amount of adipose tissue, the distribution of adipose tissue also does matter (181). For people with normal weight, the majority of adipose tissue is distributed in the subcutaneous tissue. Ectopic fat accumulation in visceral and other non-adipose tissues is considered to be more harmful than the subcutaneous adipose tissue (SAT), and plays an important role in development of metabolic dysfunction (182).

Insulin is a peptide hormone secreted by β cells embedded in the pancreatic islets (183). It regulates an integrated anabolic metabolism by binding to the insulin receptor on the cell membrane of target cells (184). Insulin receptors are expressed in many somatic cells, including, but not limited to, WAT, skeletal muscle, liver, neurons, and glial cells (185, 186). Postprandial insulin secretion rises steeply, which is stimulated by the ingestion of glucose, amino acids, and fatty acids (183). The elevated insulin directly stimulates glucose uptake in skeletal muscle and WAT, promotes glycogen synthesis in skeletal muscle and liver, activates lipogenesis in liver and WAT, suppresses lipolysis in WAT, and blocks gluconeogenesis and glycogenolysis in the liver (187). Thereafter, the blood glucose level will fall gradually back to normal levels.

Insulin resistance (IR) occurs when somatic cells do not respond adequately to a normal insulin plasma levels (183). As a result of a compensatory response, the pancreas must secrete more insulin into the bloodstream to achieve its previous anabolic effects (188). It is considered a pathological condition because the long-term IR is likely to induce β cells decompensation, and often eventually progress to type 2 diabetes mellitus (189). In general, chronic overnutrition is considered the most important reason for IR.

As the major lipid-storage organ, the WAT is very sensitive to insulin (183). Stimulation of glucose/fatty acid transport and inhibition of lipolysis are the two major physiological function of insulin in WAT (190-192). As a consequence of insulin action and excessive nutrients supply, the size of individual adipocytes increases. As adipocyte grow, it gradually becomes resistant to insulin to avoid further expansion and lipid overload (191). Importantly, the WAT, especially the SAT, has limited capacity for hyperplasia (recruit preadipocyte) and hypertrophy (enlargement) in a given individual (180). The expansion capacity of SAT is likely determined jointly by genetic, epigenetic, and environmental factors (180).

When lipids accumulation in adipocyte approaches its critical boundary, multiple cytotoxic stresses can trigger inflammatory signalling cascades, that results in necrosis of adipocytes (193, 194). The contents of dead adipocytes, mainly lipids, are then released into the extracellular space (195). Chemo-attractants secreted by stressed adipocytes can drive macrophage infiltration, thereby inducing chronic inflammatory response locally (185, 191). Chronic inflammation has been shown to induce IR in WAT by 'two-hit' model, including macrophage activation and the secretion of proinflammatory cytokines (such as TNF-α, IL-6, IL-1β and IL-8) (185, 196). The elevated circulating proinflammatory cytokines levels will also induce chronic low-grade inflammatory response in non-adipose tissues, such as in coronary arteries, pancreases, and liver (185, 197).

Lipotoxicity and insulin resistance

Increased lipolysis caused by insulin intolerance and net lipids egress from dead adipocytes contribute to the elevated free fatty acids (FFAs) and triglycerides in the blood plasma (185). Long-term lipids overload is considered to induce lipids accumulation in non-adipose tissue, which is known as ectopic lipid storage (180, 198). Excessive lipids accumulation in liver and skeletal muscle plays an important role in the development of IR and other metabolic dysfunctions (185). This whole process is referred as lipotoxicity (198).

Chronic nutrients oversupply drives de novo lipogenesis (DNL) in liver through substrate-push (e.g. FFAs, glucose, and amino acids) mechanism that is independent of insulin signalling (199-202). Lipids overflow from adipose tissue, DNL, together with dietary lipids, lead to elevated levels of intrahepatic lipids (203). Moreover, the chronic low-grade inflammation induced by proinflammatory cytokines could further contribute to the fat deposition in non-adipose tissues (197).

The intracellular FFAs metabolism mainly occurs in mitochondria through β-oxidative pathway (197). Some bioactive lipid intermediates (e.g. diacylglycerol, ceramides, and acyl-carnitines), rather than inertly stored triglyceride, have been demonstrated to activate inflammatory signalling pathway, inducing the autocrine/paracrine secretion of proinflammatory cytokines, thereby enhancing inflammation locally and systemically (204). The accumulation of toxic lipid intermediates could also increase oxidative and endoplasmic reticulum stress, thereafter inducing mitochondrial dysfunctions (205). It is known that both low-grade inflammation and mitochondrial dysfunctions contribute to pathophysiology of IR and type 2 diabetes (185). Therefore, lipid intermediates are increasingly recognized as key components in the pathway linking the accumulation of lipids and IR (185).

Other than liver and skeletal muscle, evidence indicate that the ectopic fat deposition in pancreas might induce β-cell dysfunction and insulin resistance (206). Additionally, insulin resistance in neurons and glial cells might result in inability to suppress appetite, leading to diet-induced obesity (207). It is also indicated that gut-brain-liver axis, hypothalamic-pituitary-adrenal axis, and brown adipose tissue may also participate in the glucose homeostasis, but these haven't been thoroughly investigated in detail (185).

Maternal metabolism and placental nutrient transport

Maternal metabolism in pregnancy

To meet the nutrient requirements of foetal growth and developments, maternal metabolism changes with the progression of pregnancy (208). Maternal normal metabolism during pregnancy is characterized by 'accelerated starvation' and 'facilitated anabolism' (209). Accelerated starvation refers to an intensified response to overnight fasting in pregnant women compared to the response in non-pregnant women, including significantly decrease in glucose and amino acids, as well as elevated FFAs and ketones in plasma (208, 209). The process of maternal metabolism adaptation to ensure adequate nutrients supply to foetus is referred to as 'facilitated anabolism' (209).

It is well known that maternal fasting plasma glucose and amino acids concentrations decrease throughout pregnancy (210, 211). Pregnant women are considered to be in the lipogenesis state during the first half of pregnancy, and then switch into a catabolic state in the late pregnancy (212). ***Figure 1*** showed that maternal circulating TC, LDL-C, and TG levels drop slightly in the first trimester and then enhance dramatically later on, while HDL-C concentration slightly increases with the progress of pregnancy (107). As the insulin sensitivity decreases gradually

during pregnancy, the maternal fasting insulin concentration is about three times higher than it was before pregnancy (208, 212).

Maternal fuels and its placental transfer

As the principle nutrient substrate, glucose could be transported from maternal side to neonatal side freely by facilitated diffusion through the glucose transporter (GLUT) family members, especially the GLUT1 (213). Since gluconeogenesis in foetus is minimal, the entire foetal glucose is almost transported from the maternal side (214, 215).

The vast majority of maternal circulating FFAs (98%) are stored as triglycerides in triglyceride-rich lipoproteins in plasma (216). Triglycerides themselves cannot cross the placenta directly. They have to break down into FFAs and glycerol by lipases in placenta, and then transported to foetal side by specific transport proteins and diffusion (215). The lipid metabolites delivered from maternal side become the majority substrate for neonatal *de novo* lipogenesis in liver and muscle (216). Evidence also indicates that human placenta could actively take up maternal lipoprotein cholesterol and dietary chylomicrons through specific lipoprotein receptors-mediated transport or endocytosis (217, 218).

The placenta can take up maternal circulating amino acid actively through amino acid transporters (219). Moreover, the amino acid concentration in foetus is normally higher than in mothers (220). Also maternal circulating insulin cannot cross the placenta, but it may significantly influence the efficiency of nutrient transport in the placenta (221).

Intrauterine nutritional programming

Foetal origins hypothesis

In 1990s, David Barker, a British epidemiologist, first proposed a hypothesis that intrauterine growth retardation may be the original causality for the development of cardiovascular diseases (222-225). This hypothesis is certainly limited in scope, but it has a profound impact on people's understanding on the origins of chronic metabolic diseases.

The foetal origin hypothesis, also known as 'foetal programming', has been widely recognised and supported by evidence from epidemiological and epigenetics studies (226). It proposes that the intrauterine environment, especially the nutritional environment, has a life-long impact on the developmental health and chronic metabolic conditions of the offspring (227).

Nutritional programming

The foetal programming starts from preconception period. Evidence shows the physiology of the gametes changed significantly in people with obese, compared with non-obese people (228). The quality and content of oocyte is significantly influenced by maternal diet and obesity through lipotoxicity and oxidative stress mechanism (229-231). Although evidence regarding the programming effects of paternal obesity is limited, some studies indicated that paternal BMI is significantly associated with DNA damage and methylation, altered miRNA content, spermatogenesis, and function in sperms (232-236). The preconceptional exposure to negative nutritional environments seems have persistent impact on the epigenetic and translation profile of the developing embryo, thereby leading to altered phenotypes in offspring (228).

All organs of foetus are formed during pregnancy. Therefore, gestation period is recognised as a sensitive time window for foetal development. The structural or functional changes of tissues or biological system in response to different environmental input is referred to as 'adaptive

developmental plasticity' (237, 238). The early developmental regulation events in foetus are particularly sensitive to the intrauterine nutritional environment. Stress induced by the deficient or overwhelming nutrient exposure in utero can systematically shape foetal development, leading to permanent changes of metabolic function in offspring (237).

Compared to lean women, women with higher BMI tend to progress to lipid catabolic state earlier and often present with a more severe dyslipidaemia during pregnancy (239). In addition, evidence indicates that women with GDM have a significant higher triglycerides and lower HDL-C than non-GDM women throughout three trimesters (108). About 90% fat deposition in foetus occurs in the third trimester, coincident with the maximal lipids placental transport period (240).

Evidence indicates that a steep concentration gradient across the placenta due to high levels of maternal circulating triglycerides can accelerate lipid placental transport rates, leading to fat deposition in foetus. A two-thirds alteration of placental gene expression in women with type 1 diabetes or GDM is relevant to lipid pathways, while only 9% of which is relevant to glucose pathways (241). Meanwhile, the activity of lipid transport protein and lipoprotein lipases in the placenta could potentially be upregulated by maternal hyperinsulinemia and hyperglycaemia, resulting in elevated lipids placental uptake and transport (242). Additionally, just like other tissues, it seems that maternal obesity could induce low-grade inflammatory response in the placenta, resulting in possible functional changes (243). However, the underlying mechanism have not yet been well established.

Excessive lipid exposures in uterus may potentially influence the development of foetal metabolic organs (e.g. liver, adipose tissue, muscle, brain and pancreas) through several pathways. First, the increased intrauterine FFAs exposures and its intracellular fatty acid intermediates could induce systematic chronic low-grade inflammation, leading to insulin

resistance in the foetus (244). Secondly, increased lipids input may induce ectopic fat deposition in non-adipose tissues (239). Thirdly, the ectopic lipid accumulation in foetal liver and muscle could result in oxidative stress and mitochondrial dysfunction (245, 246). Fourthly, the excessive FFAs exposure in utero may induce β-cell apoptosis in pancreas through endoplasmic reticulum stress pathway (247). Finally, the increased maternal circulating lipids could potentially change the feeding behaviour of their offspring through regulating appetite relevant neuronal genes and hypothalamic-pituitary-adrenal axis (248, 249).

Glucose transported from maternal side could stimulate *de novo* insulin synthesis in the foetus. As insulin plays a central role in foetal growth and development, the additive effects of maternal hyperglycaemia may have a profound impact on metabolic health of offspring. GDM pregnancies have been linked with placental structural dysfunction, and increased inflammatory response as well as oxidative stress in placenta, thereby leading to chronic foetal hypoxia (250-252). It is also indicated that neonates born to GDM mothers have reduced β-cell mass, because the development of pancreas in foetus is particularly sensitive to intrauterine hyperglycaemia (253). Compared to neonates with non-GDM mothers, neonates with GDM mothers were observed to have altered DNA methylation levels in their placenta and cord blood (254). It demonstrated that intrauterine hyperglycaemia exposure may have a persistent influence on neonatal metabolic health.

1.5 Objectives

The aim of this thesis was to determine the metabolic risk factors in mothers and their babies, and to investigate how these metabolic risk factors influence one another. Technical details will be presented in Chapter 2. Specifically, this project aimed to evaluate:

1. The association between maternal lipid levels during pregnancy and adverse birthweight outcomes (Chapter 3)
2. The interdependency between maternal metabolic risk factors and birthweight as well as cord blood insulin level (Chapter 4)
3. The association between birthweight and cord blood metabolic parameters (Chapter 5)
4. The association of birthweight and cord blood triglycerides with Interleukin 6, C-Reactive Protein, and Tumour Necrosis Factor-alpha levels in cord blood (Chapter 6)

Chapter 2 General Methods

In this chapter, I will present the systematic review protocol (2.1), then describe the BIGCS cohort study along with an overview of study designs (2.2), and finally, highlight the key statistical methods used in the analysis (2.3).

2.1 How does maternal dyslipidaemia influence neonatal birthweight, metabolic and inflammatory parameters: A systematic review protocol

Abstract

Background

Metabolic risk factors, including BMI, glucose, and dyslipidaemia, and its subsequent health outcomes have become one of the most important public health concerns globally. *In vivo* evidence indicates that maternal metabolic disorders, especially dyslipidaemia, might hold a key role in the process of foetal programming, which might increase the risk of metabolic dysfunctions or even further induce early onset of cardiovascular disease in offspring. The effects of intrauterine over-nutrition over the life-course might be a vicious cycle across generations. However, most of these metabolic risk factors are controllable and preventable. In this review, existing primary epidemiological evidence will be collected and synthesized to understand the association of maternal lipid levels in pregnancy with neonatal birthweight and metabolism.

Selection Methods:

Electronic searches will be performed in the Embase (1974 to current), MEDLINE (1946 to current), PubMed (1950 to current), Scopus (1960 to current), PsycINFO (1967 to current), CINAHL Plus (EBSCO,1937 to current) and Cochrane Library (1974 to current) databases.

Secondary searches will be conducted in Grey literature, Open grey and google scholar for potential grey literatures and relevant reviews. No language limitation will be applied in this review.

Selection criteria

Study design: Longitudinal observational studies

Population: Studies focused on general pregnant women, pregnant women with gestational diabetes mellitus or obesity are eligible for this review.

Exposures: Maternal lipid levels during pregnancy (Total cholesterol, high-density-lipoprotein cholesterol, low-density-lipoprotein cholesterol, very low-density-lipoprotein cholesterol, triglycerides, and free fatty acids).

Outcomes: The primary outcome of this review is birthweight. The secondary outcomes include neonatal metabolic as well as inflammatory parameters.

Comparators: Not applicable.

Data extraction, quality assessment and analysis

A designed data extraction form and the adapted Newcastle-Ottawa Scale (NOS scale) will be used in this review. The data extraction and quality assessment process will be undertaken by the first author and the second author independently. If any disagreement exists, a third individual (expert) will be invited to discuss it before reaching a final decision. Different format of data will be converted into same format if applicable. Meta-analysis method (random-effects model) will be used to synthesis the extracted data. I^2 statistics will be used to measure the degree of heterogeneity between studies for each outcome. Funnel plot will be used to detect the existence of publication bias visually.

Systematic review registration:

PROSPERO 2016: CRD42016048568

Introduction

Approximately 7% newborns in United States (2015) as well as in China (2011) are diagnosed as having macrosomia (255, 256). It has been well established that neonatal birthweight is related to the risk of developing subsequent obesity, type 2 diabetes and cardiovascular diseases in later life (66, 67, 257). Maternal metabolism during pregnancy has been demonstrated as a key component that might program foetal health systematically from the beginning of life (258, 259). Apart from maternal glucose level during pregnancy, recent literature has shown that maternal lipid levels during pregnancy might also play an important role in the process of foetal programming (239, 260).

Dyslipidaemia caused by obesity might further induce pre-diabetes status among non-pregnant population

As a lipids storage organ, the buffering function of adipose tissue decrease when lipid accumulation exceeds its storage capacity, leading to elevated serum free fatty acids (FFAs) and triglycerides (TG) (261-263). This results in the net lipid flux to non-adipose organs, which is called ectopic fat stores (262). As obesity progresses, chemoattractants secreted by unhealthy and larger adipocytes could drive macrophage infiltration in adipose tissue (264, 265). When ectopic lipid accumulation occurs in liver, muscle and pancreas, pro-inflammatory cytokines produced by macrophages can directly engender insulin resistance (IR) in those organs and may result in diabetes through NFκβ, c-Jun N-terminal kinases and oxidation stress pathways (262, 264, 266, 267).

How maternal dyslipidaemia might work on neonatal health?

During the first half of pregnancy, pregnant women are mainly in lipogenesis state, then they switch to catabolic state (268-270). With the progression of pregnancy, women's ability to inhibit lipolysis decrease as a result of relative insulin resistance, resulting in steep concentration gradient across the placenta. This results in lipids transport into foetus through placenta (239, 271). Compared to pregnant women with normal weight, obese women will not only progress to catabolic state earlier, but have severe hyperlipidaemia, which presents as lower high-density lipoprotein cholesterol (HDL-C) level and higher very low-density lipoprotein cholesterol (VLDL-C)/ free fatty acids (FFAs) levels (239),

Human placenta could actively/passively take up maternal lipoproteins and dietary chylomicrons through specific lipoprotein receptors-mediated transport or endocytosis (217, 272). The changes in maternal lipid profile could alter both the quantity and quality of transplacental lipids (217). In addition, Radaelli et al. reported that 67% of changes in placental gene expression in GDM women relates to lipids transport pathways, but only 9% to glucose transport pathways (241). In the placenta of obese mothers, it has been observed there is enhanced expression of proinflammatory cytokines and significant accumulation of heterogeneous macrophage population (243). This indicates that persistent low-grade inflammation in placenta might be one of the mechanisms of excessive lipids accumulation in pregnancy (239).

In vivo evidence has shown that excess maternal intrauterine lipids exposure could affect the development of foetus organs systematically, such as skeletal muscle, adipose tissue, liver, pancreas, and brain, through several metabolic pathways (239). It containing oxidative stress pathways, inflammatory pathway, Peroxisome Proliferator-Activated Receptor γ pathway, hypothalamic leptin pathway, and metabolic epigenetic programming pathway, which can alter

initial foetus metabolism and even feeding behaviours permanently (239). Foetus metabolic abnormalities mediated by maternal obesity and high-fat diet often manifest as increased body weight, fat mass, blood glucose, cholesterol, and blood pressure levels, as well as decreased insulin sensitivity, and ectopic lipid storage (239).

Why it is important to do this review?

Maternal dyslipidaemia during pregnancy and its consequent health outcomes have produced heavy burden on economy and health globally. However, these are controllable and preventable. One review proposed that the effects of intrauterine overnutrition over the life-course might be a vicious cycle across generations, likely increasing the risk and/or accelerating the onset of metabolic disorders like obesity and diabetes in the offspring (243). As an upstream risk factor, if the maternal dyslipidaemia is demonstrated as the key node of the whole metabolic pathway, early interventions on maternal lipids profile during pregnancy might be a viable option to reduce the risk of GDM and other related adverse health outcomes.

There are several systematic reviews surrounded this topic, but none of them discussed the effect of maternal dyslipidaemia on neonatal health outcomes. Evidence around the impact of maternal dyslipidaemia on neonatal birthweight and metabolic condition is still inconclusive. Therefore, it is necessary to conduct a review to synthesis existing evidence systematically to have a better understanding. This review aims to investigate the relationship between maternal dyslipidaemia and neonatal birthweight, metabolic condition, as well as inflammatory factors.

Objectives

Primary objective

To investigate the association between maternal lipid levels in pregnancy and birthweight.

Secondary objectives

1) To investigate the association between maternal lipid levels in pregnancy and neonatal metabolic parameters.

2) To identify studies that might have tested the potential mechanisms linking maternal lipid levels in pregnancy and neonatal birthweight as well as metabolic parameters.

Methods

Search strategy

Electronic searches will be performed in the Embase (1974 to current), MEDLINE (1946 to current), PubMed (1950 to current), Scopus (1960 to current), PsycINFO (1967 to current), CINAHL Plus (EBSCO,1937 to current) and Cochrane Library (1974 to current) databases (273). The best study designs for causality studies are cohort study and randomized controlled trial (RCT), therefore, we limit our searches by using cohort and RCT study filters to identify the most robust evidence for this study.

Secondary searches will be conducted in Grey literature, Open grey, and Google Scholar for potential grey literatures and relevant reviews. Abstracts, conference, and symposia proceeding from relevant organisations will be identified as well. References lists will be checked carefully to identify any available primary studies or reviews. Therefore, it is reasonable to believe that every eligible study in these three databases will be picked up through our primary and secondary searches.

Screening for the search results will be undertaken by the first author (JW) and the second screener independently to confirm the accuracy of selection. If there is any disagreement of study selection after discussion, the third person (expert in this field) will be asked to resolve any issues. Non-English studies that eligible for this review will be translated into English by

an independent translation company to ensure there is no language restrictions in our searches. Results of search will be entered into EndNote X7 to facilitate record keeping, duplicate removal, study selection and document writing.

Inclusion and exclusion criteria and selection strategy

- Types of studies

Longitudinal observational studies and secondary analysis of randomised controlled trials will be included in this review.

- Types of participants

Studies focused on general pregnant women, pregnant women with GDM (treated with either diet or insulin) or obesity are eligible for this review. The diagnostic criteria for GDM and obesity are different in different populations. Therefore, we accept the concept of GDM/obesity defined by the study author. Any studies focussing on population of pregnant women with diseases/conditions that could influence maternal metabolic status before pregnancy (hepatitis, polycystic ovary syndrome, familial hyperlipidaemia, human immunodeficiency virus [HIV] infection, type 1 & type 2 diabetes, hypertension, thrombophilia history of thromboembolism, rheumatologic disorders, cardiac dysfunction, or history of taking relevant lipid-lowering medicines) will be excluded.

- Types of exposures

Maternal lipid levels during pregnancy (TC, HDL-C, LDL-C, VLDL-C, TG and FFAs).

- Types of outcome measures

Subjects: all living neonates (less than three years old)

The primary outcome of this review is birthweight, which should be measured in the first week after delivery.

Secondary outcomes

1. Neonatal birthweight parameters, including low birth weight (LBW), small for gestational age (SGA), large for gestational age (LGA) and macrosomia (as defined by the study author)

2. Neonatal metabolic status

a) Anthropometric indicators (weight gain, BMI, skinfold thickness)

b) Circulating metabolic parameters (glucose, TC, HDL-C, LDL-C, VLDL-C, triglycerides, FFAs, and insulin) measured in cord blood or blood samples taken from neonates.

3. Inflammatory factors level, involving Monocyte Chemoattractant Protein-1, interleukin 6, tumour necrosis factor-alpha and 11-beta-Hydroxysteroid Dehydrogenase Type 1, and C-reactive protein, measured in cord blood or blood samples taken from neonates.

4. Leptin level measured in cord blood or blood samples taken from neonates.

- Types of comparators

Currently, there is no guideline mentioned about what is the 'normal' range of maternal lipid levels during pregnancy clearly. Given the knowledge of maternal lipid levels changes with progression of pregnancy, no comparator limitation will be applied in this review.

Data extraction and quality assessment strategy

The designed form for data extraction will be applied in this review. To evaluate the quality of included cohort studies under our topic, an adapted 'star system' quality assessment tool, the NOS scale will be used in this review (274). Both the data extraction and quality assessment process will be conducted by the first author and the second screener independently. Where disagreements exist, the third individual (expert) will be invited to review and discuss the issue before reaching a final decision.

Methods of analysis/synthesis

- Measures of effect size

For continuous outcomes (insulin, glucose, lipids, inflammatory factor and leptin levels, and early postpartum weight gain), results will be summarised and represented with tables in its original format. For categorical outcome variables with clear gold-standard cut-off point (e.g. macrosomia, LGA, AGA and SGA), odds ratios will be used for pooling.

- Dealing with missing data

A comprehensive strategy has been developed for dealing with missing data. For all included studies, the percentage of missing data and its reasons will be recorded in data extraction form. Authors will be contacted for unreported results. Sensitivity analysis will also be conducted to evaluate the impact of including or excluding potentially unreliable results due to missing data.

- Assessment of heterogeneity

I^2 statistics will be used to measure the degree of heterogeneity between studies for each outcome (275). Once heterogeneity is identified (I^2-value $\geq$ 50%), reasons behind it will be discussed based on the baseline characteristics of study design (276).

- Assessment of reporting biases

Funnel plot, a scatterplot of common effect against measure of study size, will be used to detect the existence of publication bias visually (277, 278). Theoretically, a symmetric funnel shape indicates low risk of publication bias (277). Conversely, asymmetric funnel indicates the existence of publication bias and substantial heterogeneity. Therefore, a result with an asymmetric funnel should be explained carefully (279, 280). Otherwise, a funnel plot will be used only for over 10 studies in this review (281).

- Data synthesis

Random-effects model will be performed to compare dichotomised outcomes (odds ratio). Meta-analytic software (Review Manager 5.3) will be used for calculating and combining data (282). Variability among effect sizes will be evaluated by the Q statistics and the I2 index. For dichotomous data, the result of random-effects analysis will be presented as the average effect size with 95% confidence intervals, and the estimate value of I^2.

- Subgroup analysis and investigation of heterogeneity

We plan to conduct subgroup analysis among obese GDM group, obese non-GDM group, lean GDM group and lean non-GDM group. Once substantial heterogeneity is identified, subgroup analysis will be performed based on its study characteristics. Additionally, the effect of maternal dyslipidaemia on different ethnic populations will also be analysed through subgroup analysis. To avoid selective reporting biases, all planned analyses will be presented in this protocol. Any additional analyses will be marked as 'post'.

- Sensitivity analysis

Sensitivity analysis will be performed based on methodological quality, ethnic populations, publication status and sample size scale.

2.2 The association between maternal metabolic risk factors and neonatal anthropometric and cord blood metabolic parameters: A research proposal

Abstract

Background and objective

Maternal pre-pregnancy body mass index (BMI), gestational weight gain (GWG), and gestational diabetes mellitus are independent risk factors for macrosomia. Current clinical guidelines provide pregnant women advice on diet and exercise for reducing the risk of adverse metabolic pregnancy outcomes. However, recent evidence shown that life style changes during pregnancy achieved limited success in reducing GWG and did not have any effect on composite maternal and foetal outcomes. The aim of study is to investigate the interdependent association of maternal metabolic risk factors with birthweight and cord blood insulin, and to explore the association between birthweight and cord blood metabolic parameters.

Methods and analysis

Data from the Born in Guangzhou Cohort study (BIGCS), an ongoing prospective longitudinal birth cohort, will be used in this study. Primiparous women with a singleton pregnancy and their offspring who participant in BIGCS study are eligible for this study. Maternal fasting blood samples and cord blood samples will be assayed for metabolic parameters. Other relevant information will be extracted either from the clinical records or questionnaires. Descriptive analysis followed by multivariable regression model, Bayesian network analysis, as well as generalised additive model will be applied to analyse the data.

Ethics and dissemination

The protocols for this study of BIGCS will be reviewed and approved by the Institutional Ethics Committees at the women and children centre in Guangzhou.

Introduction

Metabolic disorders have become a serious health concern in China

Metabolic risk factors, including central obesity, dyslipidaemia, hypertension, and impaired glucose tolerance, that might progress to diabetes mellitus, cardiovascular diseases, and certain cancers in later life (283, 284). Apart from the long-term effect of these metabolic risk factors among the adult population, evidence indicates that metabolic dysfunctions before and during pregnancy in mothers, such as diabetes mellitus, obesity, and dyslipidaemia, might significantly influence neonatal metabolic conditions (285, 286). The effect of intrauterine overnutrition and its subsequent long-term health outcomes might become a vicious cycle across generations for human (287).

Around 27.9% of Chinese female in childbearing age (18 - 44 years old) were overweight or obese, and 29.7% of the female population were found to have hypertriglyceridemia (288-290). On the other hand, studies have shown that increased neonatal birth weight might be related to increased adiposity and early on-set metabolic disorders (66, 291, 292). Data from the China Health and Nutrition Survey showed that the standardized incidence of overweight (new cases between 1991 and 2000, and between 2000 and 2011) of children aged 2-6 years old across two decades increased from 2.3% to 8.3% (293). These data indicate that metabolic disorders of mothers and their offspring have become one of the most important public health concerns in China currently.

The altered maternal metabolism in pregnancy

Once women become pregnant, their energy homeostasis is challenged by the increased demands of pregnancy when suboptimal adaptive responses may be noted. Consequently, the glucose/lipid levels and weight of pregnant women change dramatically during pregnancy,

which might in turn influence the metabolism of their offspring from the early beginning of their life. In 2008, the prevalence of GDM was 6.2% among women living in urban Tianjin (China) (294). A systematic review published in 2015 demonstrated that dyslipidaemia in pregnancy might be an independent risk factor for GDM, although there is no clear definition for gestational dyslipidaemia to date (108).

Maternal metabolic disorders are critical for foetal programming

It has been established that maternal pre-pregnancy body mass index (BMI), gestational weight gain (GWG), and GDM are independent risk factors for macrosomia, but recent studies also indicated only a quarter of the difference of birth weight in multivariate models could be explained by maternal hyperglycaemia (239, 295, 296). Among GDM women with well-controlled glucose level, researchers found that maternal fasting serum TG and free fatty acids (FFAs) level may be independent risk factors for neonatal high birth weight and fat mass (166, 297). Therefore, we hypothesised that maternal metabolic conditions, including obesity, glucose, and lipid levels, might play important role in the process of foetal programming jointly (295, 298).

It is necessary to investigate the association between maternal metabolic risk factors and neonatal health

Epidemiological studies have demonstrated that maternal metabolic risk factors (high pre-pregnancy BMI, excessive GWG, and GDM) could results in high birth weight of their offspring (299-302). However, none of these studies investigated the impact of maternal metabolic disorder on neonatal metabolism. Importantly, an individual patient data meta-analysis concluded that diet and lifestyle interventions in pregnancy only achieved modest

success in reducing gestational weight gain and had no effect on composite maternal and foetal outcomes (303). Meanwhile, gestational dyslipidaemia is gaining attention from researchers and clinicians, and has been considered as an ignored risk factor for adverse pregnancy outcomes. Evidence show that excessive intrauterine lipids exposure can affect the development of foetus organs systematically through oxidative stress and also by inflammatory and metabolic epigenetic programming pathways (239). If we could figure out how maternal metabolic parameters influence neonatal birthweight and metabolism, then early optimal interventions on the most critical risk factors might be a viable option to avoid subsequent adverse health outcomes. Therefore, the aim of this study is to disentangle the association and interdependency of maternal metabolic risk factors during pregnancy with neonatal anthropometric parameters and metabolic markers in cord blood through a large well characterised cohort study.

Objectives

Primary objective

To investigate the association of maternal metabolic risk factors (pre-pregnancy BMI, mid-pregnancy glucose, total cholesterol, HDL-C, LDL-C, and triglycerides) with birthweight. As insulin plays a central role in the foetal growth and development, the association between maternal risk factors and cord blood insulin will be further explored as well.

Secondary objectives

1. To investigate the interdependency of maternal metabolic risk factors and their association with birthweight as well as cord blood insulin;
2. To investigate the association between birthweight and cord blood metabolic parameters.

Study design

Setting: The BIGCS study

- What is the BIGCS study

The Born in Guangzhou Cohort Study (BIGCS) is an ongoing prospective longitudinal birth cohort study, which was established to investigate the short- and long-term health consequences in the younger generation, whose parents have experienced one of the world's most rapid societal and epidemiological transition in their lifetime. It aims to recruit 20-30,000 pregnant women in Guangzhou within 5-10 years from 2011 and follows them and their offspring. The main investigations of BIGCS include maternal lifestyle, nutrition, psychosocial stress, medical, and environmental conditions before, during, and after pregnancy as well as neonatal health conditions in early and late childhood.

- Design of the BIGCS study

Participants: All pregnant women attending the maternity units in Guangzhou Women and Children's Medical Centre (GWCMC) are eligible for the study. Pregnant women who do not live in Guangzhou or plan to leave Guangzhou within 3 years are excluded from this study. Women who attend pregnant routine antenatal examinations or come for the first pregnancy visit (usually during weeks 6 - 16) were invited to participate in the BIGCS.

Follow-up: The schedule for collection of data and biological samples is attached below in ***Table 6***.

Funding: The establishment of the BIGCS has been supported by grants from Guangzhou Municipal Government and Guangzhou Women and Children's Medical Centre.

- Implementation of the BIGCS

Since then to date it has recruited 30,000 number of patients.

Table 6 Schedule for collection of data and biological samples

Time point	Visit	Questionnaire (Q)	Biological samples collected
Antenatal			
Before week 11	Early pregnancy clinic visit		
Weeks 11-16	First trimester clinic visit/ Down Syndrome screening	Q1	Parental blood
Weeks 20-24	Second trimester clinic visit	Q2 (food frequency questionnaire)	Maternal blood
Weeks 30-33	Third trimester clinic visit	Q3	Maternal blood, urine
At birth	Delivery at hospital	Q4	Maternal blood, cord blood, placenta and umbilical cord tissues
Postnatal			
3 months	Telephone interview / home visit	Q5	
6 months	Telephone interview / home visit	Q6	
12 months	Children's health care visit	Q7	Blood, urine
18 months	Telephone interview / home visit	Q8	
36 months	Children's health care visit	Q9	Blood
72 months	Children's health care visit	Q10	

Participants

All healthy adult (aged over 18 years old) primiparous women with a singleton pregnancy who have fasting blood samples collected during the second trimester in BIGCS study are eligible for this study. Women with conditions that influence their metabolic response during pregnancy were excluded from the study. Conditions excluded were liver diseases, kidney diseases, pancreas diseases, polycystic ovary syndrome, immune system diseases (human immunodeficiency virus infections or erythematosus), inherited metabolic diseases (familial hyperlipidaemia or 21-hydroxylase deficiency), type 1/ type 2 diabetes, thyroid dysfunction, hypertension, thrombophilia, history of thromboembolism, rheumatologic disorders cardiac dysfunction, history of organ transplant before pregnancy, records of Intrauterine infections, miscarriage and stillbirth during this pregnancy/labour, have neonatal with inherited metabolic diseases and congenital abnormalities, have missing samples of cord blood, or dropped out before labour.

Sample size

We powered the study for the potentially least associated maternal metabolic risk factor (TG) for birthweight. Knopp et al. reported a correlation between maternal TG and birthweight of r = 0.09 ($p < 0.05$) in non-GDM women (304). We conservatively assumed an effect size of 0.08. Stata 14.0 was used to calculate the sample size. After using 'Fisher's z tests comparing one correlation to a reference value' tool, a sample of 1,225 will give 80% power to detect a correlation of 0.08 at 5% significance level (two-sided). We conservatively assumed 20% attrition rate due to missing data and loss to follow up, thus giving a sample size of 1,531.

Samples collection and storage

Maternal fasting blood samples are collected (for OGTT test and storage) in the second trimester clinic visit. The umbilical cord vein blood samples are collected after labour. All samples are taken into an Ethylenediaminetetraacetic acid (EDTA) tube for serum and plasma separately, which are stored in the biobank at -80°C until analysis.

Exposures

Once pregnant women are enrolled into the BIGCS study, baseline information (such as maternal age, height, pre-pregnancy weight, ethnicity, education level, marital status, social-economic level, working status, family and housing environment, smoking status, alcohol use and medicine history) would be collected via the Q1 questionnaire (self-completed).

Participants were routinely given a 75g, 2h OGTT for GDM screening in a prenatal care visit (20 - 28 weeks). The diagnosis of GDM is made when any of the following blood glucose values is met or exceeded based on the criteria developed by the International Association of Diabetes and Pregnancy Study Groups: fasting, 5.1 mmol/l; 1h, 10.0 mmol/l; 2h, 8.5 mmol/l (40).

Maternal pre-pregnancy BMI and paternal BMI are calculated using the weight in kilograms and the height in metres (305). Based on the recommendations of the China Obesity Task Force of the Chinese Ministry of Health, pre-pregnancy BMI is classified as two groups: lean group (< 23.9 kg/m^2) and overweight group (≥ 24 kg/m^2) (306). Gestational weight gain is defined as the increase in maternal weight during pregnancy, which is calculated by maternal terminal weight minus pre-pregnancy weight.

Maternal fasting blood samples will be analysed for lipids profile, which including total cholesterol (TC), HDL-C, low-density lipoprotein cholesterol (LDL-C) and TG. Automated

clinical chemistry analyser, which is based on enzymatic colorimetric assays, will be used to perform samples analysis. Evidence have shown that there is no significant changes in the stability of lipids profile test measurements based on samples stored at -80°C for a period up to 24 months (correlation between fresh plasma and samples stored for 24 months are 0.990 for TC and HDL-C, and 1.000 for TG). Multiple freezing and thawing of plasma samples have no influence on the measured studied lipids profiles as well (307, 308).

Outcomes

- Primary outcome

The primary outcome of this study is birthweight and cord blood insulin concentrations. The value of birthweight will be extracted from clinical records. On the basis of Intergrowth 21st Newborn Size Standard and Tools, newborns will be classified into large for gestational age (LGA, birthweight $\geq 90^{th}$ percentile for gestational age), appropriate for gestational age (AGA, birthweight $>10^{th}$ or $<90^{th}$ percentile for gestational age) and small for gestational age (SGA, birthweight $\leq 10^{th}$ percentile for gestational age)(309). Macrosomia is defined as birthweight over 4000g (310). In China, the overall prevalence of macrosomia in 2011 was 7.3%. The prevalence of macrosomia in southern China is 5.6%, which is much lower than in northern China (8.5%) (256). Cord blood insulin will be measured by monoclonal antibody-based sandwich enzyme-linked immune sorbent assay (ELISA) (311).

- Secondary outcomes

The secondary outcomes of this study are glucose and lipids concentrations in venous cord blood. The concentrations of plasma glucose in venous cord blood samples will be assayed using automatic biochemistry analyser. The analytical stability of glucose level will not be influenced by numerous freeze-thaw and up to 3 months -20℃storage (312). Venous cord blood

samples will be analysed for TC, HDL-C, LDL-C and TG, using automated clinical chemistry analyser. There is scarce literature reporting lipid profile distribution in cord blood. In one study conducted in Iran (n = 442), the reported distribution of lipids profile in cord blood was TC 76.9mg/dL (SD = 28.9 mg/dL), HDL-C 30.1mg/dL (SD = 28.9 mg/dL), LDL-C 34.1mg/dL (Standard deviation [SD] = 11.7 mg/dL) and TG 67.5 mg/dL (SD = 20.1 mg/dL) (313).

Covariates

Information on maternal terminal weight, parturition methods (caesarean section, assisted delivery or eutocia), preterm birth (defined as birth of newborns less than 37 weeks gestational age), pre-eclampsia and eclampsia, intrauterine growth restriction and neonatal gender will be extracted from clinical records of subjects in BIGCS study.

Statistical analysis

Data analysis will be performed using Stata 14.0. The following analyses will be included:

1. Descriptive analysis
2. Multivariable regression model (to qualify the linear associations between independent variables and dependent variables)
3. Generalized additive model (to explore the potential non-linear associations between independent variables and dependent variables)
4. The Bayesian Net-work analysis (to identify potential pathways of action if there were to be a positive relationship between maternal metabolic risk factors and neonatal outcomes linked to or predisposing to metabolic dysfunctions)

Technical details of those statistical methods will be described in Chapter 2.3.

Ethical permission

The protocol for this study in BIGCS was reviewed and approved by the Institutional Ethics Committees at the women and children centre at Guangzhou. Written, informed consent was obtained from all participants. All participants were voluntarily joining BIGCS and can withdraw their consent at any time. The identity of participants was covered by a unique number. This number was used to links all data acquired from the participant, her partner (biological father of the offspring), and her offspring (including multiple births). All records and biological samples used for this study have been collected by researchers in BIGCS.

Discussion

The BIG study is a unique large prospective birth cohort resource for disentangling the effect of maternal metabolism on neonatal health outcomes during pregnancy (272). To our knowledge, it is the first study trying to elaborate a comprehensive disease casual network of maternal and neonatal metabolisms in the same population.

The BIG study is conducted in Guangzhou, a Southern Chinese city, where there are less obese women (18.4%, BMI $\geq$ 25, 2001) than Northern city in China (36.9%, BMI $\geq$ 25, 2001) and Western countries (33.3%, BMI $\geq$ 30, 2001) (314, 315). The dietary habits, lifestyle and climate environments have distinct differences compared with other populations (315). As a tertiary hospital-based cohort study, the population of this study mainly come from urban areas, with features of modern lifestyle. We are unsure how these features will influence our results.

2.3 Introduction to statistical methods

Multivariable regression model

Multivariable regression model refers to a statistical method that can be used to determine the relationship between one dependent variable and a number of independent variables (316). There are three multivariable regression models that are commonly used in the public health literature: multivariable linear regression, multivariable logistic regression, and multivariable Cox proportional hazards regression (317). A multivariable linear regression model could be defined by this equation: $Y= \alpha + X_1\beta_1 + X_2\beta_2 + \ldots + X_k\beta_k + \varepsilon$ (316).

The model structures of logistic regression and Cox proportional hazards regression are similar to the linear regression model. The multivariable logistic model could be used to assess the association between a dependent binary outcome and more than one independent variables (318). The multivariable Cox proportional hazards regression model could be used to investigate the association between several independent variables and the time at which a specific event occurs (319).

Additive Bayesian Network analysis

A Bayesian network is a probabilistic graphical model that represents a set of variables and their conditional dependencies via directed acyclic graphs (DAGs) (320). It is a well-established unsupervised machine learning methodology that is typically referred to as structure discovery model for dealing with multidimensional data (321). Unlike other widely used multivariate approaches, such as principal component analysis, propensity score matching analysis and multivariate regression model, graphical modelling does not involve any dimension reduction. Most graphical models, including path analysis and structural equation modelling, rely on a pre-specified structure, whereas Bayesian network is entirely data driven.

Unlike the contingency table parameterization in standard Bayesian network models, Additive Bayesian networks (ABN) allow us to obtain interpretable DAGs where each node in graph comprises a generalized linear model (GLM) or a generalized linear mixed model (GLMM, if binary variable involved) (322, 323). There are two mutually dependent parts in ABN model: a network structure (i.e. the DAG) and a set of parameters. Each node (corresponding to the variables in the dataset) in the DAG is the equivalent of a potential dependent variable in a Bayesian GLM or GLMM regression model. While other DAG nodes where relevant as identified by the unsupervised learning act as covariates, having a role of corresponding parameters. Therefore, an ABN model is ideally suited to analysing highly complex epidemiological data comprising many inter-dependent variables.

Generalized additive model

The generalized additive model (GAM) is a combination of GLM and additive model (324). Unlike the general linear regression model, the dependent variable of GLM does not have to be normally distributed or be continuous (325). A multivariable linear regression model could be considered a special case of GLM. It could be described as: $Y= g (\alpha + X_1\beta_1 +X_2\beta_2 + \ldots + X_k\beta_k + \varepsilon)$, where g (…) is an identity function. A link function (gi), the inverse function of g (…), is used to connect the association between independent variables and dependent variable in GLM: $gi (muY) = \alpha + X_1\beta_1 +X_2\beta_2 + \ldots + X_k\beta_k + \varepsilon$, where muY represents the expected value of Y. The general regression model also has the nature of an additive model. Instead of using the linear equation ($X_k\beta_k$), the non-parametric function $f_k (X_k)$ of each independent variable is used to achieve the best prediction of the dependent variable value. The additive model could be described as a formula like this: $Y = \alpha + f_1(X_1) +f_2(X_2) + \ldots + f_k(X_k)$.

The form of GAM is gi (muY) = $\sum k(f_k(X_k))$, which assumes the link functions are additive and its components are smooth (324). GAM allows us to explore non-linear, linear, and non-monotonic relationships between dependent variable and independent variables.

Chapter 3 Gestational Dyslipidaemia and Adverse Birthweight Outcomes: a systematic review and meta-analysis

Abstract

Background

Low and high birthweight is known to increase the risk of acute and longer-term adverse outcomes, such as stillbirth, infant mortality, obesity, type 2 diabetes and cardiovascular diseases. Gestational dyslipidaemia is associated with a numbers of adverse birth outcomes, but evidence regarding birthweight is still inconsistent to reliably inform clinical practice and treatment recommendations.

Objective

The aim of this study was to explore the relationship between maternal gestational dyslipidaemia and neonatal health outcomes, namely, birthweight, metabolic factors and inflammatory parameters.

Methods

We searched systematically Embase, MEDLINE, PubMed, CINAHL Plus and Cochrane Library up to 1 August 2016 (with an updated search in MEDLINE at the end of July 2017) for longitudinal studies that assessed the association of maternal lipid levels during pregnancy with neonatal birthweight, or metabolic and inflammatory parameters up to 3 years old.

Results

Data from 46 publications including 31,402 pregnancies suggest that maternal high triglycerides and low high-density-lipoprotein cholesterol levels throughout pregnancy are associated with increased birthweight, higher risk of large for gestational age and macrosomia

and lower risk of small-for-gestational age. The findings were consistent across the studied populations, but stronger associations were observed in women who were overweight or obese prior to pregnancy.

Conclusions

This meta-analysis suggested that the potential under-recognized adverse effects of intrauterine exposure to maternal dyslipidaemia may warrant further investigation into the relationship between maternal dyslipidaemia and birthweight in large prospective cohorts or in randomized trials.

Manuscript (Published)

Introduction

Low and high birthweight has been linked to the risk of stillbirth and infant mortality (65). In a longer life course, both low birthweight or small for gestational age (SGA), and large for gestational age (LGA) or macrosomia are known to increase the future risk of obesity, type 2 diabetes and cardiovascular disease (66, 67). The estimated prevalence of macrosomia in developed countries varies from 5% to 20%, and a parallel increase in macrosomic births was observed in both developed and developing countries over the last two to three decades (73). These life course associations have often been attributed to the impact of an adverse intrauterine environment, particularly fuels (glucose, lipids and amino acids) transported from the maternal end (326). Previous reviews have shown that maternal obesity and gestational diabetes mellitus (GDM) are two identified risk factors of low and high birthweight (95, 327, 328). However, as one of common metabolic disorders, the adverse effects of gestational dyslipidaemia on neonates' birthweight/birthweight centiles are not widely recognized in clinical practice.

Dyslipidaemia has been considered a risk factor for a number of adverse health outcomes, in particular cardiovascular disease and type 2 diabetes (329, 330). Previous reviews have shown that dyslipidaemia during pregnancy are associated with increased risk of GDM, pre-eclampsia and pre- term delivery (108, 331, 332), but epidemiological evidence on birthweight is conflicting (117-119). Furthermore, previous evidence indicates that excessive maternal intrauterine lipid exposures may program the development of foetal organs from early life, resulting in metabolic dysfunction (239, 333). If maternal dyslipidaemia is a significant contributor to birthweight and implicated in neonatal metabolic dysfunction, then interventions before and during pregnancy to mitigate dyslipidaemia might improve offspring's adverse birth and metabolic health outcomes.

We performed a comprehensive systematic review and meta- analysis to explore the association and quantify the magnitude of effect between maternal dyslipidaemia and neonatal outcomes, namely, birthweight, metabolic factors and inflammatory parameters.

Methods

Search strategy and selection criteria

The protocol for this review was registered on PROSPERO (CRD42016048568), and the review is reported in accordance with the Preferred Reporting Items for Systematic reviews and Meta- analyses (PRISMA) (334) and Meta- analysis Of Observational Studies in Epidemiology (MOOSE) (335) guidelines. We searched systematically Embase, MEDLINE, PubMed, Scopus, CINAHL Plus and Cochrane library (CENTRAL) up to 1 August 2016, without language or year restrictions. An updated search was made in MEDLINE before manuscript submission until the end of July 2017. The search of bibliographic databases combined index and free- text terms relating to lipids (e.g. 'lipids', 'lipoproteins', 'fatty acids', 'triglycerides' and 'cholesterol') with those relating to pregnancy (e.g. 'pregnan*', 'gestation*', 'gravidity' and 'mothers') and birthweight (e.g. 'birth weight', 'small for gestational age', 'large for gestational age' and 'macrosomia'). The full strategies are provided in ***Supplementary material S1***. Cohort and randomized controlled trial (RCT) filters were used to target longitudinal observational studies and the secondary analysis of RCT studies (336). Additional searches were conducted in Grey Literature Report and Open Grey. Reference lists of included studies were screened and checked for relevance.

Search results, after removal of duplicates, were screened for relevance using title and abstract information. Full texts of relevant articles were assessed for eligibility against the selection

criteria. Screening and selection were undertaken by two reviewers independently in consultation with a third reviewer when required.

This review included studies of healthy pregnant women and pregnant women with GDM or obesity, which investigated the association between maternal lipid levels during pregnancy (total cholesterol [TC], high- density lipoprotein cholesterol [HDL- C], low- density lipoprotein cholesterol [LDL- C], very- low- density lipoprotein cholesterol [VLDL- C], triglycerides [TG] and total free fatty acids [FFAs]) and neonatal anthropometric, metabolic and inflammatory parameters.

Studies of pregnant women with conditions that could influence maternal metabolic status before pregnancy (hepatitis, polycystic ovary syndrome, familial hyperlipidaemia, acquired immunodeficiency syndrome, type 1 and type 2 diabetes, hypertension, thrombophilia, history of thromboembolism, rheumatologic disorders, cardiac dysfunction or history of taking relevant lipid- lowering medications) were excluded.

The primary outcome was birthweight measured within the first week after delivery. Neonatal anthropometric parameters, including low birthweight, SGA, LGA and macrosomia, were considered as different indexes of birthweight. Secondary outcomes included the following: anthropometric parameters in children younger than 3 years (e.g. weight gain after delivery, body mass index [BMI] and skinfold thickness), biological indicators (glucose, TC, HDL- C, LDL- C, VLDL- C, TG, FFAs and insulin levels; and insulin resistance) and neonatal inflammatory factors (monocyte chemoattractant protein- 1, interleukin 6, tumour necrosis factor alpha and 11- beta- hydroxysteroid dehydrogenase type 1 and C- reactive protein, as well as leptin levels) measured in cord blood or blood samples taken from neonates (< 3 years old). Owing to the diverse definition of GDM, obesity, SGA, LGA and macrosomia in different populations, we accepted the definition specified by authors.

Data extraction and quality assessment

A STrengthening the Reporting of OBservational studies in Epidemiology (STROBE)- based pre- designed form (337) was used for data extraction, including the following information: study characteristics (study name, design, language and location), participants (setting, eligibility/exclude criteria and sample size), maternal characteristics (age, parity, pre-pregnancy BMI and gestational length), follow- up (enrolment time, length of follow- up, data collection methods and loss to follow- up rate), exposures (definition, fasting status, measured gestational weeks and measurement methods) and outcomes (definition and measurement time point) (***Supplementary material S2***).

The Newcastle–Ottawa Scale was used to characterize and stratify the methodological quality of included studies (***Supplementary material S3***) (274). Studies' quality was classified as 'low' (≤ 5), 'medium' (6 and 7) or 'high' (8 and 9). In addition, domains relating to sample selection, comparability between groups and method of outcome assessment were considered separately. Data extraction and quality assessment were conducted by two reviewers independently in consultation with a third reviewer when required. Missing information was requested from authors by email.

Data synthesis

Included studies were categorized by trimester on the basis of the mean/median gestational age for the lipid measurement (first trimester [T1], 1 – 13; second trimester [T2], 14 – 27; and third trimester [T3], ≥ 28 gestational weeks). For studies reporting lipid levels multiple times within one trimester, data from the trimester with the largest sample size were adopted. Studies with different types of population (example GDM or obesity) were divided into two or three subsets

to enable us to assess and report separately. Lipid measurements reported in milligrams per decilitre were converted to millimoles per litre using standard unit conversion factors (338).

Results of birthweight were reported in various ways, e.g. regression coefficients (RC) and correlation coefficients. Findings were summarized in tables and visually represented as horizontal histogram, displaying the direction as well as statistical significance of results comprehensively (post- analysis).

Summary estimates were pooled using random- effects meta- analysis, according to assessment of outcomes (birthweight, LGA, SGA and macrosomia), timing of lipids measurement (T1/T2/T3) and statistic reported in the primary study (RC, odds ratio [OR] or mean difference). Unadjusted and adjusted estimates reported in the articles were entered into random- effects models separately. Confounding factors that were adjusted (maternal age, pre- pregnancy BMI, gestational weight gain, gestational glucose level, pre- term birth, gestational lipid levels, gestational age and neonatal gender) for each result were recorded for further sensitivity analyses. The I^2 statistic was used to quantify the degree of heterogeneity beyond that expected by chance in each analysis (275). The potential for publication bias could not be assessed via funnel plots as the requirement for 10 or more studies per meta- analysis was not met (281). Owing to the heterogeneity in baseline characteristics of included studies, we were not able to compare non- GDM women with GDM women. Sensitivity analysis was performed by choice of covariates controlled for in the model. All analyses were conducted using Review Manager version 5.3 (Nordic Cochrane Centre, Copenhagen, Denmark) and R 3.3.2 (The R Foundation for Statistical Computing).

Results

Study selection

Of the 13,705 unique records identified by the searches, 46 publications (117-119, 165, 166, 297, 301, 339-377) reporting from 42 studies were included in the review (***Figure 2***). These studies included 31,402 pregnancies. Of the 46 included publications, 16 contributed to the quantitative analysis owing to the diversity of reporting formats (RC, correlation coefficients, mean differences, trend analyses or without exact effect estimates) and lack of data required for calculations. No additional eligible studies were found in the updated search till July 2017.

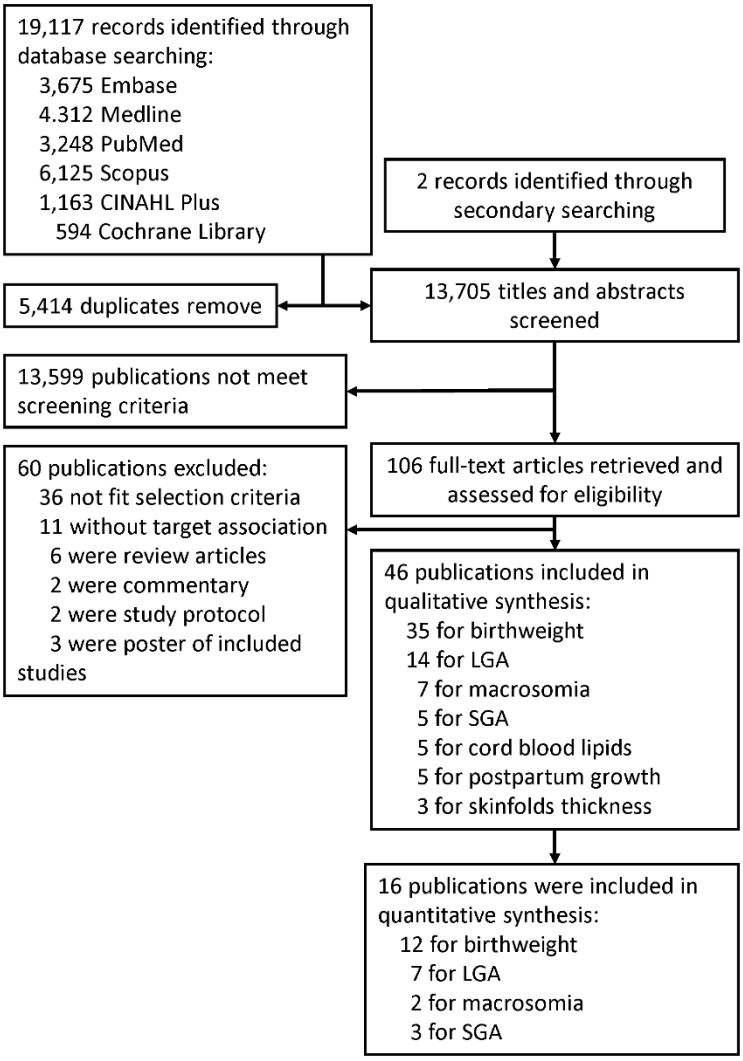


Figure 2 Flow-chart

Characteristics of included studies

Table 7 describes the baseline characteristics of the 46 included publications. Most articles were published in English language and as full text articles with only one 44 study written in German and one 43 published as an abstract. The studies were published between 1985 and 2016. The number of pregnancies ranged from 38 to 5,535. On the basis of the World Bank Income Classification of countries (378), 25 out of 42 studies were from high- income economies (117, 119, 166, 297, 340-347, 350-353, 355, 358-360, 367, 368, 370, 373, 374), 16 from upper- middle- income economies (118, 165, 301, 339, 348, 349, 354, 356, 357, 363-366, 369, 376, 377) and 1 from middle- income economies (372). Forty studies were prospective cohorts (118, 119, 165, 297, 301, 339-341, 343-346, 348-365, 367-372, 374-377), three were retrospective cohorts (347, 366, 373) and three were secondary analyses of cohorts in RCTs (117, 166, 342).

Table 7 Baseline characteristics of included studies

Study ID	Study design	Locations	Population (N)	TC	HDL	LDL	TG	VLDL	FFAs	Tri.	Outcomes
Ye et al.2015	Prospective observational study	China	non-GDM (n=1,243)	√	√	√	√			3	Birthweight LGA, SGA
Wang et al.2015	Prospective cohort study	China	General (n=636)	√	√	√	√			2	Birthweight
Crume et al.2015	Prospective cohort study	American	General (n=804)	√	√		√		√	2,3	Birthweight
Hwang et al.2015	Prospective cohort study	Korea	non-GDM (n=1,011)				√			2,3	Birthweight
Kulkarni et al.2013	Prospective cohort study	India	non-GDM (n=631)	√	√		√			2,3	Birthweight
Vrijkotte et al.2012	Prospective cohort study	Netherlands	non-GDM (n=4,008)	√			√			1	LGA, SGA
Retnakaran et al.2012	Prospective cohort study	Canada	non-GDM (n=472)	√	√	√	√			3	Birthweight LGA
Hou et al.2014	Prospective observational study	China	non-GDM (n=2,790)	√	√	√	√			3	LGA
Kramer et al.2014	Prospective cohort study	Canada	General (n=340)	√	√		√			3	Infant weight gain at 3 months
Son et al.2010	Retrospective longitudinal observational study	Korea	GDM (n=104)	√	√	√	√			3	Birthweight LGA
Ahmad et al. 2006	Controlled prospective study	Malaysia	non-GDM (n=246)	√			√			3	Birthweight LGA
Di et al. 2005	Prospective observational study	Italy	OGTT+ (n=83)	√	√	√	√			2	Birthweight LGA
Couch et al.1998(1) Couch et al.1998(2)	Prospective observational study	American	GDM (n=20) Non-GDM (n=20)	√	√	√	√	√	√	3	Birthweight Cord vein lipids profile
Ortega et al. 1996	Prospective cohort study	Spain	General (n=292)	√	√	√	√	√	√	3	Birthweight Cord arteriovenous lipids profile
Alberti-Fidanza et al. 1995	Prospective observational study	Italy	General (n=70)	√	√		√			1-3	Mixed venous-arterial cord blood lipids profile

Study ID	Study design	Locations	Population (N)	TC	HDL	LDL	TG	VLDL	FFAs	Tri.	Outcomes
Schaefer-Graf et al. 2008	Secondary analysis of RCT study	German	GDM (n=150)	√			√		√	3	Birthweight, cord blood lipids LGA
Swierzewska et al. 2015	Prospective observational study	Poland	General (n=136)	√	√	√	√			3	Birthweight
Sommer et al. 2015	Prospective cohort study	Norway	General (n=699)	√	√	√	√			3	Birthweight, sum of skinfolds
Slagjana et al. 2014	Prospective cohort study	Yugoslavia	non-GDM (n=200)	√	√	√	√			3	Birthweight LGA, SGA
Laleh et al. 2013	Prospective cohort study	Iran	GDM (n=112)	√	√	√	√			3	LGA, macrosomia
Whyte et al. 2013	Prospective cohort study	Ireland	General (n=189)	√	√	√	√			2	Birthweight
Zhou et al. 2012	Prospective cohort study	China	General (n=1,000)	√	√	√	√			2	Macrosomia
Vrijkotte et al. 2011	Prospective cohort study	Netherlands	General (n=2,052)	√			√			1	Birthweight Postpartum growth
Vinod et al.2011(1) Vinod et al.2011(2)	Prospective cohort study	American	Overweight (n=71) Normal weight (n=72)	√	√	√	√			1-3	Birthweight
Zawiejska et al. 2008	Prospective observational study	Poland	GDM (n=357)		√		√			2	Birthweight Macrosomia
Clausen et al. 2005	Prospective cohort study	Norway	General (n=2,050)	√	√	√	√			2	Macrosomia
Mathews et al. 2003	Prospective cohort study	UK	General (n=798)	√						2,3	Birthweight
Olmos et al.2014(1) Olmos et al.2014(2) Olmos et al.2014(3)	Prospective observational study	Chile	GDM + lean (n=128) GDM + overweight (n=105) GDM + obese (n=46)	√	√		√			2,3	Birthweight

Study ID	Study design	Locations	Population (N)	TC	HDL	LDL	TG	VLDL	FFAs	Tri.	Outcomes
Emet et al.2013	Prospective observational study	Turkey	General (n=801)	√	√	√	√			3	Birthweight, infant weight at 3 months
Liu et al.2016	Retrospective cohort study	China	General (n=1,546)	√	√	√	√			2	Birthweight
Brunner et al. 2013	Secondary analyses of RCT study	German	General (n=208)				√			3	Birthweight, postpartum growth, skinfolds thickness
Knopp et al.1992	Prospective observational study	American	NS- (n=521) PS+ (n=264) GDM (n=96)				√			3	Birthweight
Knopp et al.1985	Prospective observational study	American	General (n=283)		√	√		√	√	3	Birthweight
Schaefer-Graf et al. 2011	Prospective observational study	German	non-GDM (n=190)	√			√		√	3	Birthweight, Cord blood metabolic parameters
Nolan et al.1995	Prospective observational study	Australia	General (n=388)				√			1	Birthweight
Lin et al.2013	Prospective observational study	China	General (ND)				√			ND	Macrosomia
Friis et al.2012	Prospective observational study	Norway	General (n=207)	√	√		√		√	3	Birthweight
Lei et al.2016	Prospective cohort study	China	General (n=5,535)		√		√			2	LGA, SGA
Kitajima et al. 2001	Prospective observational study	Japan	OGTT + (n=146)	√			√		√	3	Birthweight LGA
Mossayebi et al. 2014	Prospective cohort study	Iran	General (n=154)	√	√	√	√			3	Birthweight LGA, macrosomia
Geraghty et al. 2016	Secondary analyses of RCT study	UK	non-GDM (n=331)	√	√	√	√			2,3	Birthweight Postpartum growth, sum of skinfolds
Jin et al. 2016	Prospective cohort study	China	non-GDM (n=934)	√	√	√	√			1-3	LGA, SGA, macrosomia

Study ID	Study design	Locations	Population (N)	TC	HDL	LDL	TG	VLDL	FFAs	Tri.	Outcomes
Brockerhoff 1986	Prospective observational study	German	ND (n=112)		√	√		√		2	Cord blood lipids profile
Harmon et al. 2011	Prospective observational study	American	non-GDM (n=38)				√		√	1	Birthweight
Robin et al. 2007	Retrospective cohort study	American	General (n=957)	√						2	Birthweight
Charles et al. 2016	Perspective observational study	Mediterranean countries	General (n=1062)	√	√	√	√			3	Birthweight

Oral Glucose tolerance test (OGTT), Negative screenees of OGTT test (NS-), Positive screenees of OGTT test but reach GDM diagnostic threshold value (PS+), No documented (ND).

Quality of included studies

Forty- five publications, excluding the abstract (365), were assessed for methodological quality. Ten, 21 and 14 studies were assessed as methodologically high (118, 343, 344, 346, 350, 352, 355, 362, 375, 377), moderate (117, 119, 301, 339, 341, 342, 345, 347, 351, 354, 357-361, 363, 364, 366, 367, 374, 376) and low quality (165, 166, 297, 340, 348, 349, 353, 356, 368-373), respectively (***Supplementary material S4***). Three (7%) of 45 included studies had low risk for study selection while 40 (93%) had medium risk. For comparability bias, 15 (33%) had low risk, 13 (29%) had medium risk and 17 (38%) had high risk. Sixteen (36%) studies were regarded to have a low risk of outcome assessment bias, with the rest (29 studies) having medium risk.

Maternal lipid levels during pregnancy and birthweight

Figure 3 shows the relationship between maternal lipid levels during pregnancy and birthweight (***Supplementary material S5***). There were strong associations noted for HDL- C and TG throughout pregnancy with birthweight. For HDL- C, both studies 55 reporting in T1, 6 (117, 118, 346, 365, 374, 376) out of 11 (117, 118, 165, 297, 301, 346, 358, 366, 374, 376) studies reporting in T2 and 11 (118, 119, 343, 346, 348, 351, 360, 370, 374, 377) out of 18 (117-119, 301, 343, 345, 346, 348, 349, 351, 360, 369, 370, 372-374, 377) studies reporting in T3 showed an inverse association with birthweight, while 1 (118) in T2 and 1 (117) in T3 reported a positive association. For TG, 4 (367, 374, 375) out of 5 (353, 367, 374, 375) studies reporting in T1, 10 (118, 165, 297, 346, 355, 358, 366, 374, 376) out of 12 (117, 118, 165, 297, 301, 346, 355, 358, 366, 374, 376) studies reporting in T2 and 20 (117, 118, 301, 339, 343, 345, 346, 348, 349, 351, 355, 359, 361, 372-374, 377) out of 27 (117-119, 166, 301, 339, 342, 343, 345, 346, 348, 349, 351, 355, 359, 361, 369-374, 377) studies reporting in T3 found a positive

association with birthweight, while 3 (119, 342, 370) studies in T3 reported an inverse association. Of the seven studies reporting the association between maternal FFAs level in T3 and birthweight (166, 345, 346, 351, 359, 360, 371), four reported a positive association (346, 359, 360, 371), while none reported inverse association. For TC, 7 (117, 118, 346, 347, 350, 366, 374) out of 12 (117, 118, 297, 301, 346, 347, 350, 358, 366, 374, 376) studies in T2 and 8 (117, 118, 339, 348, 350, 359, 374, 377) out of 22 (117-119, 166, 301, 339, 343, 345, 346, 348-351, 359, 368-374, 377) studies in T3 reported a positive association, while 1 (374) in T2 and 3 (343, 370, 374) in T3 found an inverse association. There was no evident association between maternal LDL- C level and birthweight (117, 119, 297, 301, 343, 345, 348, 349, 358, 360, 366, 369, 370, 372-374, 376, 377) or between maternal VLDL- C level and birthweight (345, 360).

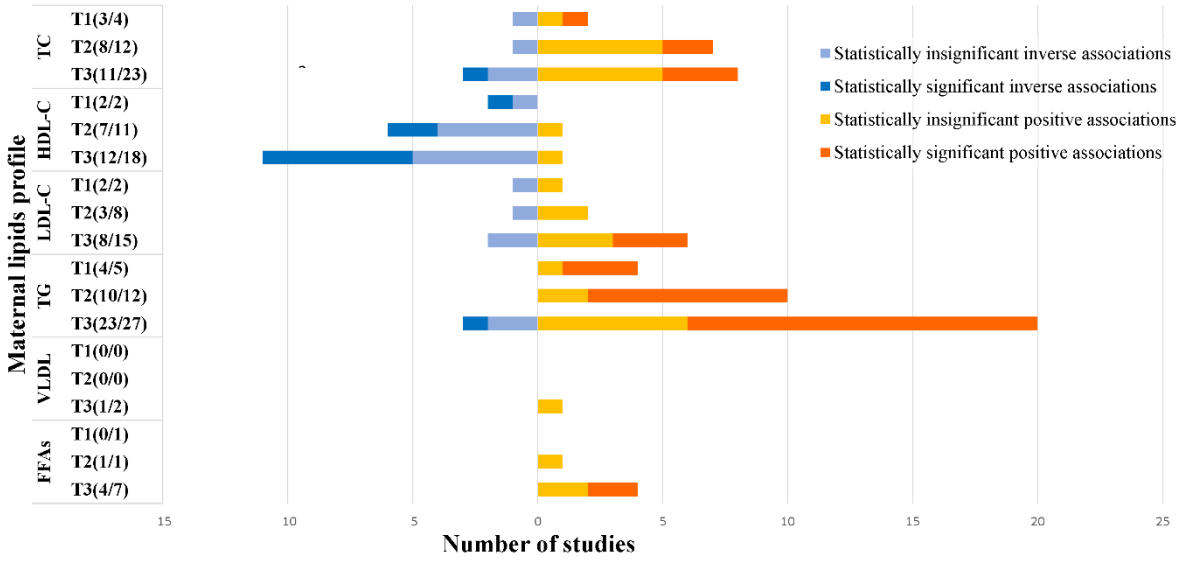


Figure 3 Results summary of the association of maternal lipid levels with birthweight throughout pregnancy.

The numbers in parenthesis are the number of studies shown in this figure/the overall number of studies reporting the target associations. Studies reporting statistically insignificant results without its direction or those that did not report their results are not shown in the figure. FFAs, total free fatty acids; HDL- C, high- density lipoprotein cholesterol; LDL- C, low- density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; VLDL, very-low- density lipoprotein.

Figure 4 shows the pooled estimates for the effect of maternal lipids throughout pregnancy on birthweight using all available data (***Supplementary material S5***). In general, the results of meta-analyses are consistent with the overall results summary (***Figure 3***). Maternal HDL-C was inversely associated with birthweight, particularly in T3 (adjusted RC, −70.17 g $mmol^{-1}$ L^{-1}, $p < 0.001$). Increased maternal TG levels were significantly associated with birthweight for T1 (adjusted RC, 86.72 g $mmol^{-1}$ L^{-1}, $p < 0.001$) and T3 (adjusted RC, 89.58 g $mmol^{-1}$ L^{-1}, $p = 0.01$). Positive associations between TC and birthweight were observed in T1 (adjusted RC, 22.67 g of birthweight per mmol L^{-1} maternal lipid, $p = 0.02$), T2 (adjusted RC, 24.74 g $mmol^{-1}$ L^{-1}, $p = 0.01$) and T3 (adjusted RC, 9.14 g $mmol^{-1}$ L^{-1}, $p = 0.13$).

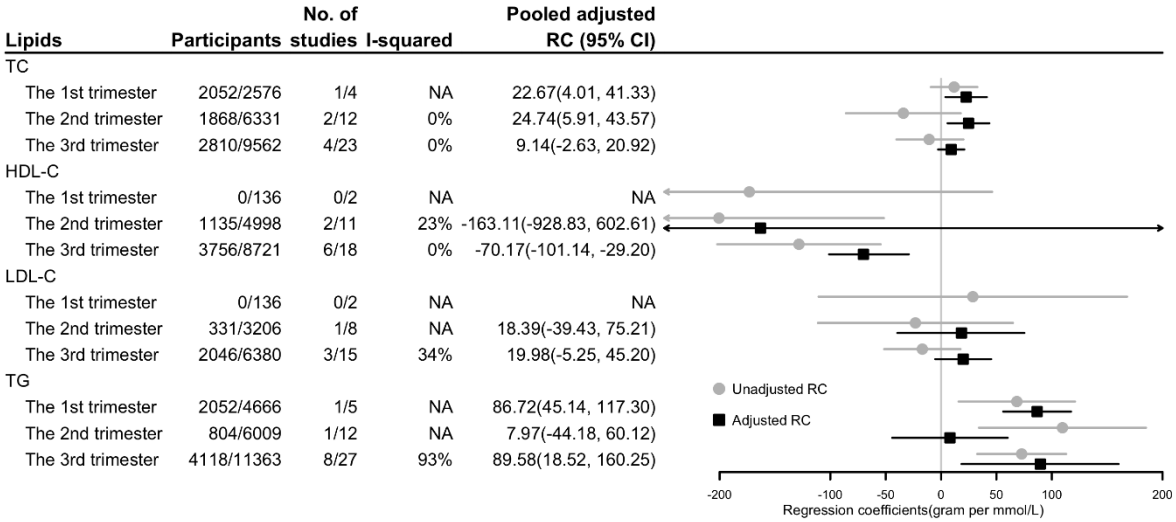


Figure 4 Summary of findings of meta- analysis for the associations between maternal lipids and birthweight throughout pregnancy.

The number of participants (studies) included into quantitative analysis/overall number of participants (studies) that reported the outcome of interest. HDL- C, high- density lipoprotein cholesterol; LDL- C, low- density lipoprotein cholesterol; NA, not applicable; RC, regression coefficients; TC, total cholesterol; TG, triglycerides.

Stronger associations were observed among pregnant women with pre- pregnancy overweight or obesity in the two relevant studies (Appendix S5) (301, 374). The degree of heterogeneity within all meta- analyses in T3 was detected with I^2 values ranging from 0% to 93%. The heterogeneity decreased markedly when studies controlled for pre- pregnancy BMI, gestational weight gain, glucose level and gestational age (***Supplementary material S5***).

Maternal lipid levels during pregnancy and LGA, SGA and macrosomia

Figure 5 shows the pooled adjusted OR for LGA as well as SGA, according to each type of maternal lipids in T3 (***Supplementary material S6 and S7***). Pooled estimates for rising maternal HDL- C level revealed potentially decreased odds of LGA (OR, 0.77; 95% CI, 0.59 to 1.01; $p = 0.06$) and significantly increased odds of SGA (OR, 1.96; 95% CI, 1.04 to 3.71; $p = 0.04$). In contrast, increased maternal TG levels were associated with increased odds of LGA (OR, 1.08; 95% CI, 1.01 to 1.15; $p = 0.02$) and decreased odds of SGA (OR, 0.66; 95% CI, 0.49 to 0.90; $p = 0.007$). In addition, 10 (166, 339, 348, 354, 357, 359, 363, 372, 373, 377) out of 11 (119, 166, 339, 348, 354, 357, 359, 363, 372, 373, 377) studies reporting the association between maternal TG and LGA in T3 reported positive statistically significant associations. Of six studies investigating the relationship between maternal HDL- C and macrosomia (165, 344, 348, 356, 357, 363), four studies reported decreased risk of macrosomia (three statistically significant) (165, 344, 356, 357), especially for T2 with higher HDL- C (***Supplementary material S8***). For the relationship of TG with macrosomia, five (344, 356, 361, 363, 365) out of six (344, 356, 357, 361, 363, 365) studies reported statistically significant positive OR values across three trimesters. No association was observed between maternal TC as well as LDL- C levels and LGA, SGA and macrosomia.

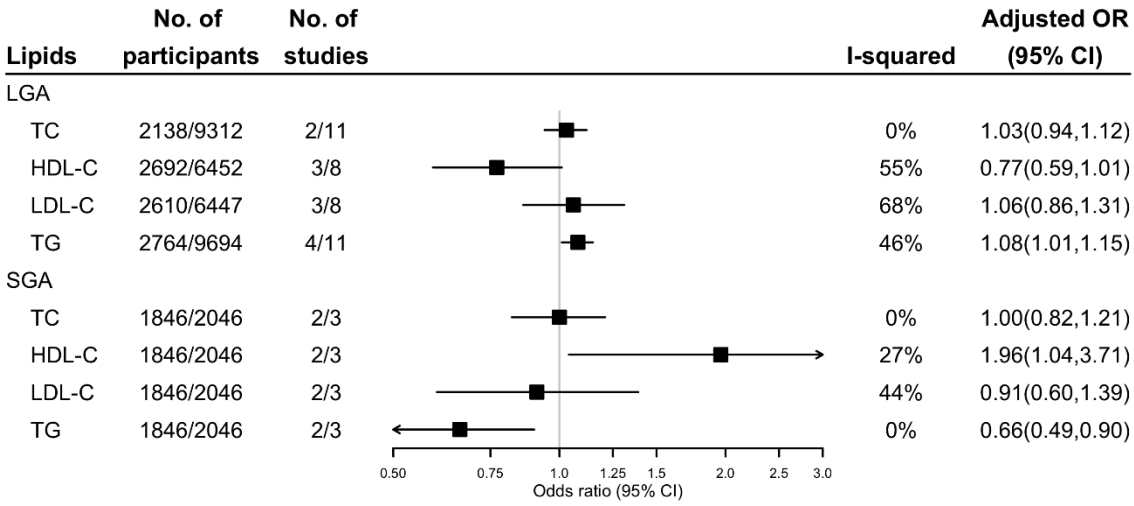


Figure 5 Summary of findings of meta- analysis for the associations between maternal lipids and LGA/SGA in the third trimester.

The number of participants (studies) included into quantitative analysis/ overall number of participants (studies) that reported the outcome of interest. HDL- C, high- density lipoprotein cholesterol; LDL- C, low- density lipoprotein cholesterol; LGA, large for gestational age; SGA, small for gestational age; TC, total cholesterol; TG, triglycerides.

Maternal lipid levels during pregnancy and other outcomes of interest

For secondary outcomes, positive correlations were found by all six publications investigating the association between different maternal lipids and different cord blood lipids, but results are inconsistent with each other (166, 340, 341, 345, 368, 371). No association was observed between maternal lipids and infant post- natal weight, weight gain or sum of skinfold thickness up to two years old (117, 342, 362, 375). No study investigated the relationship of maternal lipid levels during pregnancy with neonatal glucose, insulin, inflammatory factors and leptin levels in our searches.

Discussion

Summary of the findings

This is the first systematic review pooling data from 40 longitudinal observational studies and two RCT secondary analysis studies providing quantitative estimates of the magnitude of association between maternal lipid levels at various stages of pregnancy and neonatal health outcomes. Throughout pregnancy, low maternal HDL- C and high TG levels are associated with increased birthweight. Low HDL- C and high TG increased the risk of LGA/macrosomia and lowered the risk of SGA babies. Maternal TC level throughout pregnancy and FFAs level in the third trimester are positively associated with a small increase in birthweight. Associations are stronger among populations with pre- pregnancy obesity. The findings provide evidence for the critical role of dyslipidaemia in gestational metabolism and neonatal health and will contribute to future research and management of gestational dyslipidaemia.

Potential mechanisms

The results are mostly consistent with previous published evidence. Maternal lipid metabolism is mainly in lipogenesis state in the earlier half of pregnancy, but then switches into catabolic state (269, 270). When the lipid accumulation exceeds the storage capacity of adipose tissue, the buffering function of the adipocytes is decreased, leading to elevated serum FFAs and TG (261, 263, 379). Compared with pregnant women with smaller pre- pregnancy BMI, women who are overweight or obese not only will progress to catabolic state earlier but also have less capacity to inhibit lipolysis (239). Women with obesity prior to pregnancy usually present with more central adipose accumulation and severe dyslipidaemia (380, 381), resulting in steep concentration gradient across the placenta (271).

Both in vivo and epidemiological evidence suggest that excessive maternal intrauterine lipid exposure could affect the development of foetal organs systematically, which can then alter initial foetal metabolism and feeding behaviours permanently (239, 382). Previous animal studies observed that foetal metabolic abnormalities mediated by maternal obesity and high-fat diet often manifest as increased body weight, fat mass, blood glucose, cholesterol and blood pressure levels and decreased insulin sensitivity and ectopic lipid storage in newborns (239). The latest multi- ancestry genome- wide association study meta- analysis also demonstrated that cholesterol biosynthesis is one of the most important metabolic pathways involved in birthweight (333).

Strengths and weaknesses

The major strengths of this study are the comprehensive searches, adherence to robust review methodology and thorough analyses. Special care was taken in the handling of missing data, which was addressed by personal contact with the authors in an attempt to minimize reporting

bias. The inclusion of longitudinal studies ensured the temporal association between exposures and outcomes, which also permitted a trimester- specific analysis. The major limitation of the study was the substantial heterogeneity, possibly owing to the diversity of settings, study populations, lipid measurement methods and diverse gestational age of the studied populations. However, this heterogeneity was addressed by subgroup analysis.

It would be intriguing to explore the effects of maternal dyslipidaemia independent of maternal hyperglycaemia. Unfortunately, this was not feasible owing to the nature of data reported in individual study. GDM women are known to have higher TG levels and lower HDL- C levels than do non- GDM women (108). However, elevated maternal TG levels and lower HDL- C levels are associated with the risk of LGA and macrosomia in both GDM women (166, 363, 373) and non- GDM women (339, 354, 357, 372, 375, 377). For women with type 1 diabetes/GDM, maternal hyperglycaemia is not the sole contributor to increased birthweight because foetuses may develop LGA despite them having optimal glycaemic control 80. Several other studies found that lipid levels during pregnancy, similar to glucose levels, are also strong metabolic determinants for foetal growth (118, 166, 343, 344, 351, 353, 359, 361, 362, 364, 366, 376). Our sensitivity analyses result has also shown that there is little effect on the relationship between gestational HDL- C/TG levels and birthweight when removing those studies controlled for glucose (Appendices S7.13 and S7.23). Collectively, this evidence suggests that maternal dyslipidaemia may be an independent, unrecognized risk factor of LGA/macrosomia.

Unfortunately, paucity of the required primary data prevented the pre- specified subgroup analyses on the basis of different definitions used for GDM and obesity across studies. Thus, this should be acknowledged as a source of clinical heterogeneity when interpreting the findings of the present study. Another limitation of this study is that we are unable to control for the

effect of GDM treatment on lipid levels. However, it has been noticed that initiation of therapy (diet control, insulin or metformin) may modestly influence TG levels (383), yet to a direction that would obscure rather than magnify differences between normal and GDM pregnancies. Similarly, our sensitivity analyses show a moderate decrease on TG effect estimate when removing studies that excluded pre- term births (Appendix S7.25).

It should be acknowledged that our primary outcome, birthweight, is a quite inexact measure of foetal growth, although it has been widely measured and utilized in clinical and research areas. We tried to extend our target outcomes from birthweight parameters to other neonatal growth parameters, biological indicators and inflammatory factors; however, we did not find sufficient studies.

Implications

Our results provide compelling evidence on the role of maternal circulating HDL- C and TG levels on birth outcomes and suggest that the under- recognized adverse effects of intrauterine exposure to maternal dyslipidaemia may need further investigation in large prospective cohorts or in randomized trials. Although the importance of screening for preconceptional dyslipidaemia has been noted in recent guidelines to alert for risk assessment for GDM (384, 385), its independent adverse effects remain largely underestimated in routine clinical practice, and recommendations regarding the management of dyslipidaemia preconceptionally or during pregnancy are still lacking. Our findings do question the current clinical practice and support the monitoring of gestational dyslipidaemia before or during pregnancy. Moreover, our findings may be a call for action regarding the implementation of strategies to address maternal dyslipidaemia (such as carefully planned dietary interventions, increasing physical activity and/or omega- 3 fatty acid supplementation). Meanwhile, gestational dyslipidaemia, as an

important feature of obesity and GDM, might be a potential treatment target for clinical interventions. These steps need to be evaluated by global health policy makers through RCTs, evidence synthesis and consensus (272, 386, 387).

Conclusion

Our findings demonstrate that maternal low HDL- C and high TG levels are positively associated with neonatal birthweight. No effect was documented for total or LDL- C. Findings are of clinical importance in considering the management of gestational dyslipidaemia, e.g. using lifestyle interventions and omega- 3 fatty acid supplementation to improve maternal and neonatal outcomes.

Chapter 4 Inter-dependency between maternal metabolic risk factors and their association with birthweight and cord blood insulin: a Bayesian Network Analysis

Abstract

Objective

Maternal obesity, gestational diabetes mellitus, and excessive gestational weight gain (GWG) are associated with adverse pregnancy outcomes. However, lifestyle interventions during pregnancy do not confer significant benefits for composite maternal and neonatal health outcomes. Meanwhile, gestational dyslipidaemia has been recognised as an ignored metabolic risk factor. This study aims to quantify the inter-dependency between those maternal metabolic risk factors and their association with birthweight and cord blood insulin level.

Methods

We used data from 1,522 mother-child pairs from the Born in Guangzhou Cohort Study. Multivariable linear regression (MLR) and Additive Bayesian Network (ABN, a data-driven causality inference model) were used to investigate the association of maternal metabolic risk factors and their interdependency in predicting birthweight and cord blood insulin concentrations. Metabolic risk factors studied were maternal pre-pregnancy body mass index (BMI), fasting glucose, lipid profile (total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], and triglycerides), and early GWG.

Results

High maternal pre-pregnancy BMI was associated with neonatal birthweight (standardized adjusted regression coefficient [β_{std}] = 0.27, 95%CI 0.22 - 0.32) directly; and indirectly with cord blood insulin in ABN. Maternal fasting glucose was positively associated with increased birthweight(adjusted regression coefficient [β_{adj}] = 84, 95%CI 43 – 126 g per mmol/L) and cord

blood insulin (β_{adj} = 2.23, 95%CI 0.89 - 3.57 μU/mL per mmol/L) in MLR, but only with cord blood insulin (β_{std} =0.12, 95%CI 0.07 - 0.17) in ABN. Maternal GWG was associated with birthweight, but not with cord blood insulin in ABN. ABN suggested none of the maternal lipids profile was independently associated with birthweight or cord blood insulin.

Conclusions

High maternal pre-pregnancy BMI is the most influential upstream metabolic risk factor for both maternal and neonatal metabolic health, therefore weight management should be addressed from preconception period. Maternal hyperglycaemia drives neonatal hyperinsulinemia and may lead to adipose tissue accumulation in neonates. Maternal dyslipidaemia appears to be secondary to maternal metabolic dysfunction with no clear links to metabolic adverse outcomes in neonates.

Manuscript (Under review)

Introduction

High fasting plasma glucose, hypercholesterolemia, and high body-mass index (BMI) remain leading risk factors for mortality and morbidity worldwide (11). In pregnancy, poor maternal metabolic health is known to induce adverse outcomes for both mothers and babies. These include: stillbirth, pre-term delivery, low or high birthweight, pre-eclampsia, and maternal postnatal diabetes/cardiovascular disease (388, 389). The prevalence of gestational diabetes mellitus (GDM) varies from 2% to 25% worldwide, and has increased over the last decades in parallel with the increasing obesity prevalence of women in child-bearing age (390, 391).

Evidence has shown that offspring born to mothers with obese, GDM, and/or gestational dyslipidaemia have increased risk of high birthweight (286, 392, 393). However, evidence regarding the association between maternal metabolic disorder and neonatal metabolic programming remains controversial. Previous observational studies reported that maternal obesity and GDM might contribute to an increased risk of short- and long-term metabolic dysfunction (e.g. obesity and diabetes) in offspring with inconsistent results (394-396). This could be explained by the heterogeneity of study settings and inadequate adjustment for confounding factors. Meanwhile, interventions based on diet and physical activity in pregnancy have had limited success in decreasing the risk of adverse pregnancy outcomes such as pre-term birth, macrosomia, and foetal adiposity (303, 397).

The mechanism of how maternal metabolic dysfunction is linked with neonatal health remains uncertain, however, the majority of researchers believe it may be explained by the foetal programming hypothesis of intrauterine over-exposure to macronutrients (glucose, free fatty acids, and amino acids) (196, 398). Previous studies mainly focused on one specific metabolic trait or on the number of metabolic disorders, without assessing the underlying interacted

effects of the natural metabolic network. This is due to the limited analytical ability of classical statistical methods to analyse multidimensional data. Understanding how the metabolic network influences neonatal health is crucial to future interventional studies and potentially to antenatal/pre-natal advice.

In this study, we investigated the association of maternal metabolic traits with birthweight and cord blood insulin in the Born in Guangzhou Cohort Study (BIGCS). To give new insights from data, we further mapped the inter-dependency of metabolic factors on both mothers and their offspring, using Additive Bayesian Network (ABN) analysis, a robust unsupervised machine learning method, which has been widely used in other disciplines (321, 399).

Research Design and Methods

Participants

The design and methods of BIGCS have been described previously (400). In brief, eligible women with Chinese nationality, living in Guangzhou who are < 20 weeks gestation and who intend to deliver at one of the two Guangzhou Women and Children's Medical Centre (GWCMC) campuses were recruited into BIGCS. This study was conducted in a subgroup of BIGCS in whom maternal and cord blood were analysed for metabolic parameters separately. Pregnant women attending BIGCS with a singleton pregnancy who delivered at GWCMC between Jan 2015 and Jun 2016 and had umbilical cord blood retained are eligible for this study. Women were excluded if: 1) maternal blood samples unavailable at 14-27 gestation week; 2) no records of maternal fasting glucose at 20 - 28 gestation week; 3) lacking maternal demographic information; 4) diagnosed with health condition prior to pregnancy, including type 1 or type 2 diabetes, thyroid dysfunction, hypertension, virus hepatitis, and renal diseases. The study was powered for the association between maternal triglycerides (the potential

weakest risk factors among maternal metabolic traits) with birthweight according to literature (***Supplementary material S9***). The eligible mother-child pairs were then selected into this study by computer generated randomization. Ethical permission for the study was granted by the GWCMC Ethics Committee.

Study Procedures

Maternal demographic data, including anthropometric measures, socioeconomic status, family and personal medical history, were collected through a semi-structured questionnaire (Q1) at recruitment. Maternal overnight fasting blood samples were collected during second trimester. At 22 - 28 weeks gestation, women attending their second prenatal visit underwent a standard 2h 75g oral glucose tolerance test (OGTT). Women with OGTT results which met or exceeded at least one threshold of the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria (FPG $\geq$ 5.1 mmol/L, 1h glucose $\geq$ 10.0 mmol/l, and 2h glucose $\geq$ 8.5 mmol/L) were diagnosed as having gestational diabetes mellitus (GDM) (401). For participating children, birth information, including birth characteristics, delivery mode, and perinatal outcomes were obtained from routine medical records. Umbilical cord blood samples were collected by midwives at birth.

Demographic Data

Maternal demographic information (age, height, pre-pregnancy weight, parity, date of last menstrual period, monthly income, education levels, and ethnicity) were collected through Q1 questionnaire. BMI was calculated by dividing weight in kilograms by height in meters squared. Based on the recommendations of the China Obesity Task Force of the Chinese Ministry of Health, maternal pre-pregnancy BMI is classified into two groups: lean group (< 24 kg/m^2) and

overweight group (≥ 24 kg/m2) (6). Maternal second trimester weight was measured to the nearest 0.1 kg using an electronic scale. Maternal early gestational weight gain (GWG) was calculated by subtracting pre-pregnancy weight from maternal second trimester weight, with documentation of the gestational age at measurement. Maternal fasting glucose concentration was obtained from OGTT test zero-time value in hospital records.

Biochemical Test

Sample collection, delivery, pre-treatment, and measurements were blinded. All blood samples were stored and delivered to pre-treatment laboratory centre. Blood samples were then separated to serum and plasma by immediate centrifugation, and were stored in EDTA tube in the bio-bank at -80℃ until analysis. Plasma lipids (TC, HDL-C, LDL-C, and TG) and insulin levels were measured using commercial kits in fully automated clinical analyser (Roche Diagnostics, Mannheim, Germany). Intra- and inter-day coefficients of variation (CVs) were consistently less than 2% for all assays.

Neonatal anthropometry

Gestational age was estimated from ultrasound examination during the first- or second-trimester. Birthweight and other information, including gestational age at delivery, mode of delivery, neonatal sex, and pregnancy complications were obtained from hospital records. Birthweight was measured to the nearest 50g using an electronic scale by midwives immediately after delivery. Birthweight Z-Score and percentile(adjusted for gestational age at delivery and neonatal sex) were calculated using Intergrowth 21st Newborn Size Standard and Tools (309). Large for gestational age (LGA) was defined as a birthweight larger than the 90th

percentile for gestational age by sex, while Small for gestational age (SGA) was defined as a birthweight smaller than the 10th percentile based on the same birthweight reference.

Statistical Analysis

Classic statistical methods

For the baseline table data are summarized as mean ± Standard Deviation (SD), median (Inter Quartile Range, IQR), or counts with percentages. Pearson correlation was used to assess the impact of the long-term -80 °C storage on insulin concentrations in EDTA tube. Adjustments were then made to account for any degradation by correcting the initial value using linear regression methods (***Supplementary material S10***). Similarly, maternal lipid levels were adjusted for gestational age using regression model to account for timing of blood sampling (***Supplementary material S11***) (347).

Initially, linear and logistic regression were used to estimate the association between maternal metabolic parameters and neonatal continuous and binary outcomes, respectively. Further analyses using linear regression model were performed after all exposures were transformed to Z-Scores. This was to enable comparison of the effect size each maternal metabolic parameter had on birthweight Z-Score and cord blood insulin Z-Score. cord blood insulin and maternal triglycerides were log-transformed prior to standardization. Multiple imputation was used to handle missing data (***Supplementary material S12***). Subgroup analyses were conducted in boys and girls respectively. Sensitivity analyses were conducted to compare the estimate differences between GDM and non-GDM participants, fasting blood samples and non-fasting samples, primiparous women and non-primiparous women, lean and overweight group, as well as before and after multiple imputation (***Supplementary material S13***). All statistical tests were two-

tailed and a P-value <0.05 was considered statistically significant. Statistical analyses were performed in Stata version 14.0 (College Station, Texas, USA).

Additive Bayesian Networks (ABN) analysis

To further assess the inter-dependency between maternal metabolic risk factors and their association with birthweight and cord blood insulin, Additive Bayesian Network (ABN) model - an unsupervised machine learning method - was conducted. Bayesian network analysis is a form of structure discovery statistical modelling that derives, from empirical data, a graphical network describing the dependency structure between variables, shown as directed acyclic graphs (DAGs) (321). ABNs comprise of DAGs where each node in the graph comprises a generalized linear model (GLM) or a generalized linear mixed model (GLMM). ABN model is suitable for analysing highly complex epidemiological data comprising many inter-dependent variables (399).

Ten variables were chosen for ABN based on prior knowledge gained from literature and findings of the classical statistical analyses. These ten variables were maternal age, maternal pre-pregnancy BMI, maternal fasting glycaemia in OGTT, early GWG, maternal fasting plasma HDL-C and triglycerides in the second trimester, birthweight Z-Score, cord blood insulin, gestational age at delivery, and neonatal sex. GWG was adjusted for gestational age at weight measurement in mid-pregnancy. Cord blood insulin was adjusted for sample storage duration. All continuous variables were standardized to Z-Scores to eliminate the influence of different measurement units. Mother-child pairs with missing data were excluded (n = 93/1,522, 6%).

Firstly, an optimal DAG with the best goodness of fit (highest log marginal likelihood) was identified. Next, parametric bootstrapping (12,800 samples) was performed to address the potential overfitting. Full technical details are provided in the ***Supplementary material S14***.

ABN analysis was conducted in R 3.4.4 (The R Foundation for Statistical Computing) using 'abn' package (399).

Results

A total of 5,497 women who gave birth between January 2015 and June 2016 were initially included in this study. Blood samples in the second trimester were successfully collected from 4,595 (83.59%) mothers. We excluded women with pre-pregnancy health conditions known to influence metabolism (thyroid dysfunction, n = 106; viral hepatitis, n = 45; renal diseases, n = 20; type 1 or 2 diabetes, n = 5; known hypertension, n = 5), and women without baseline characteristic information (n = 39, 0.7%). Of the 4,039 eligible mother-child pairs, 1,522 were randomly selected as the sub-cohort for additional blood metabolic parameter measurements (***Figure 6*** and ***Supplementary material S9***).

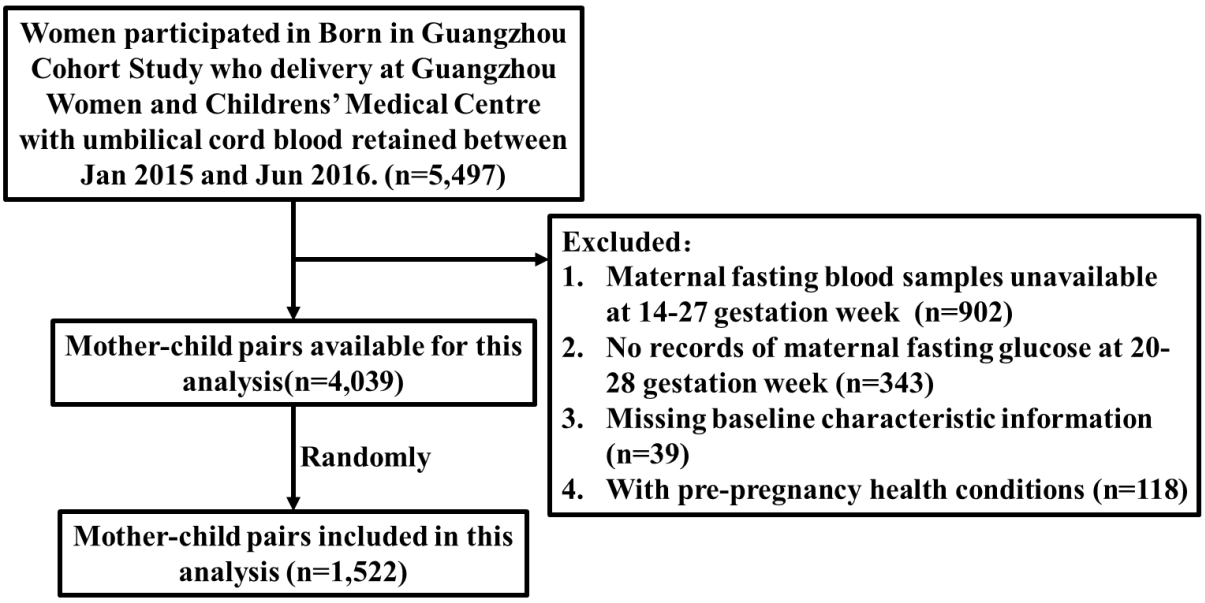


Figure 6 Flow chart

The baseline characteristics of participants are shown in **Table 8**. The majority (91.20%) of maternal blood samples were collected after overnight fasting. Maternal mid-pregnancy weight, fasting glucose, and lipids profile were measured at a mean of 20.0 (SD = 4.0), 24.6 (SD = 1.4), and 20.5 (SD = 3.5) gestation weeks, respectively. Cord blood samples were stored for a median of 488 (Inter Quartile range [IQR] 394 to 707) days before analysis.

Table 8 Baseline characteristics table

Characteristics	Included participants (n=1,522)
Maternal baseline information	
Maternal age at enrolment (years)	29.50 ± 3.30
Ethnic Han	1,486 (97.70)
Primiparous	1,223 (80.35)
Spontaneous delivery	1,239 (81.41)
Early pregnancy cigarette exposure	436 (28.68)
Maternal metabolic profile	
GDM	181 (11.89)
Glucose(mmol/L)	4.25 ± 0.42
Gestational age of OGTT test (weeks)	25.60 (1.38)
Pre-pregnancy BMI (kg/m^2)	20.47 ± 3.85
Early gestational weight gain (kg)	4.21 ± 8.42
Total cholesterol (mmol/L)	5.47 ± 0.90
HDL-C(mmol/L)	2.07 ± 0.43
LDL-C(mmol/L)	3.06 ± 0.77
Triglycerides(mmol/L) *	1.71 (1.39-2.15)
Gestational age of blood sampling (weeks)*	19 (17-24)
Neonatal information	
Gestational age (days)*	275 (270-281)
Preterm delivery	66 (4.34)
Male	820 (53.88)
Birthweight (g)	3,203 ± 411
LGA	96 (6.31)
SGA	106 (6.96)
Cord blood insulin (μU/mL) *	7.43 (4.34-12.61)

Data are mean ± SD or n (%). *Median (Inter Quartile Range)
GDM, gestational diabetes mellitus; OGTT, the oral glucose tolerance test; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol ; LDL-C, low-density lipoprotein cholesterol; LGA, large-for-gestational age; SGA, small-for-gestational age.

Association between maternal metabolic parameters and birthweight

Table 9 presents the associations between five maternal main metabolic parameters (pre-pregnancy BMI, GWG, fasting glucose, HDL-C, and TG levels) and neonatal outcomes. Pre-pregnancy BMI (adjusted β = 29.25, 95%CI: 22.77 to 35.73 g per kg/m^2), GWG (adjusted β = 18.75, 95%CI: 13.06 to 24.43 g per Kg), fasting glucose (adjusted β = 84.32, 95%CI: 42.65 to 125.98 g per mmol/L), and triglycerides (adjusted β = 67.97, 95%CI: 42.38 to 93.55 g per mmol/L) were positively associated with birthweight. Higher maternal HDL-C was significantly associated with lower birthweight (adjusted β = 45.78, 95%CI: 5.59 to 85.97 g per mmol/L). There was no evidence of an association between maternal TC and LDL-C levels and birthweight (***Supplementary material S15***).

Elevated maternal pre-pregnancy BMI, early GWG, fasting blood glucose and triglycerides level were significantly associated with an increase in the odds of LGA. The risk was particularly high for fasting glucose (OR = 2.06, 95%CI 1.31 to 3.24). Higher pre-pregnancy BMI and triglycerides were significantly associated with lower odds of SGA. There was no evidence of an association between maternal HDL-C level and risk of LGA/SGA.

Association between maternal metabolic parameters and cord blood insulin

Maternal fasting glucose (adjusted β = 2.23, 95%CI: 0.89 to 3.57 μU/ml per mmol/L) and triglycerides levels (adjusted β = 0.88, 95%CI: 0.05 to 1.71 μU/ml per mmol/L) were significantly associated with higher cord blood insulin. Pre-pregnancy BMI, GWG, TC, and LD L-C levels did not show an association with cord blood insulin (**Table 9** and ***Supplementary material S15***).

Table 9 Multivariate analyses of maternal metabolic risk factors with birthweight and cord blood insulin level.

	Pre-pregnancy BMI(Kg/m^2)	Early GWG (Kg)	Glucose (mmol/L)	HDL-C (mmol/L)	TG (mmol/L)
Regression Coefficients(95%CI)					
Birthweight(g)$^{\Delta}$	**29.25 (22.77, 35.73)**	**18.75 (13.06, 24.43)**	**84.32 (42.65, 125.98)**	**-45.78 (-85.97, -5.59)**	**67.97 (42.38, 93.55)**
Cord blood insulin¶ (μU/mL)	0.20 (-0.02, 0.42)	0.08 (-0.11, 0.27)	**2.23 (0.89, 3.57)**	-0.81 (-2,10, 0.49)	**0.88 (0.05, 1.71)**
Odds Ratio (95%CI)					
LGA§	**1.24 (1.15, 1.32)**	**1.12 (1.04, 1.20)**	**2.06 (1.31, 3.24)**	0.78 (0.49, 1.26)	**1.30 (1.01, 1.68)**
SGA§	**0.86 (0.78, 0.94)**	0.94 (0.87, 1.00)	0.72 (0.44, 1.18)	0.99 (0.62, 1.56)	**0.53 (0.36, 0.78)**

Δ Adjusted for maternal age, ethnic group, parity, gestational age, neonatal sex, and early pregnancy cigarette exposures. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.

¶ Adjusted for maternal age, ethnic group, parity, gestational age, neonatal sex, early pregnancy cigarette exposures, delivery mode, and sample storage duration. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.

§ Adjusted for maternal age, ethnic group, parity, and early pregnancy cigarette exposures. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.

BMI, body mass index; GWG, gestational weight gain; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; LGA, large-for-gestational age; SGA, small-for-gestational age.

Association between maternal metabolic parameter Z-score and birthweight Z-score

Table 10 shows the estimates of the association between maternal metabolic parameter Z-Scores and birthweight Z-Score as well as cord blood insulin Z-Score, and subgroup estimates for boys and girls. Maternal pre-pregnancy BMI (adjusted β = 0.20, 95%CI 0.15 to 0.24) and early GWG (adjusted β = 0.17, 95%CI 0.12 to 0.22) Z-scores had the strongest association with birthweight Z-Score. Both maternal triglycerides (adjusted β = 0.12, 95%CI 0.08 to 0.16) and glucose (adjusted β = 0.08, 95%CI 0.04 to 0.12) Z-scores were also positively associated with birthweight Z-Scores. Maternal HDL-C Z-Score showed a statistically significant negative association with birthweight Z-Score in boys (adjusted β = -0.06, 95%CI -0.12 to -0.01) only. The association of maternal pre-pregnancy BMI Z-Score, GWG Z-Score, Glucose Z-Score as well as triglycerides Z-Score with birthweight Z-Score remained statistically significant after adjusting for all five maternal metabolic risk factors.

Table 10 Multivariate analyses of maternal metabolic parameter Z-Scores with birthweight Z-Score and cord blood insulin Z-Score

	Pre-pregnancy BMI Z-Score	GWG Z-Score	Glucose Z-Score	HDL-C Z-Score	TG Z-Score
Birthweight Z-Score					
Model 1 (All)	**0.20(0.15, 0.24)**	**0.17(0.12, 0.22)**	**0.08(0.04, 0.12)**	**-0.05(-0.09, -0.00)**	**0.12(0.08, 0.16)**
Boys	**0.18(0.12, 0.23)**	**0.18(0.10, 0.25)**	**0.07(0.02, 0.12)**	**-0.07(-0.12, -0.01)**	**0.14(0.09, 0.19)**
Girls	**0.22(0.15, 0.29)**	**0.16(0.09, 0.24)**	**0.09(0.02, 0.16)**	-0.03(-0.09, 0.04)	**0.10(0.03, 0.16)**
Model 2 (All)	**0.20(0.15, 0.24)**	**0.16(0.11, 0.22)**	**0.04(0.00, 0.09)**	0.01(-0.03, 0.06)	**0.07(0.03, 0.12)**
Cord blood insulin Z-Score					
Model 3 (All)	**0.10(0.05, 0.15)**	0.05(-0.01, 0.12)	**0.13(0.08, 0.18)**	-0.04(-0.09, 0.01)	**0.06(0.01, 0.11)**
Boys	**0.13(0.07, 0.20)**	0.04(-0.04, 0.13)	**0.14(0.08, 0.21)**	-0.04(-0.11, 0.03)	**0.08(0.02, 0.15)**
Girls	**0.08(0.00, 0.15)**	0.08(-0.02, 0.17)	**0.12(0.05, 0.19)**	-0.05(-0.12, 0.02)	0.06(-0.01. 0.13)
Model 4 (All)	**0.08(0.03, 0.14)**	0.05(-0.02, 0.11)	**0.11(0.06, 0.16)**	-0.00(-0.06, 0.05)	0.03(-0.02, 0.09)

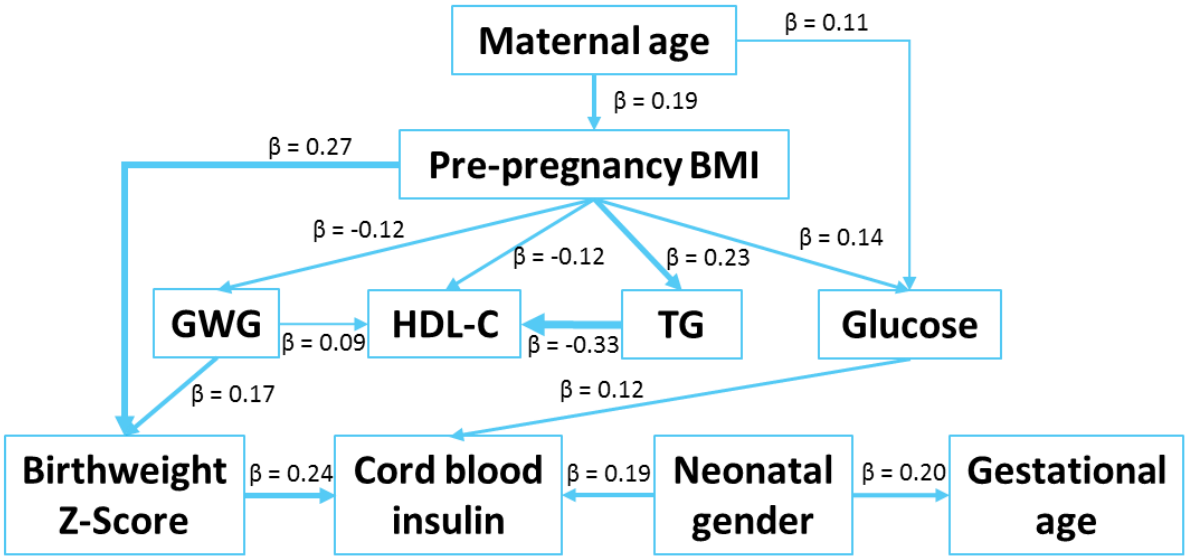
Model 1: Adjusted for maternal age, ethic group, parity, and early pregnancy cigarette exposures. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.
Model 2: Model 1 + pre-pregnancy BMI Z-Score + GWG Z-Score + Glucose Z-Score + HDL-C Z-Score + TG Z-Score + gestational age of maternal weight measurements during pregnancy.
Model 3: Adjusted for maternal age, ethic group, parity, early pregnancy cigarette exposures, gestational age, neonatal sex, delivery mode, and sample storage duration. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.
Model 4: Model 3 + pre-pregnancy BMI Z-Score + GWG Z-Score + Glucose Z-Score + HDL-C Z-Score + TG Z-Score + gestational age of maternal weight measurements during pregnancy.
BMI, body mass index; GWG, gestational weight gain; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides,

Association between maternal metabolic parameter Z-score and cord blood insulin Z-score

Maternal glucose Z-Score (adjusted β = 0.13, 95%CI 0.08 to 0.18) appears to be the most important contributor to cord blood insulin Z-Score in both boys and girls. Both maternal pre-pregnancy BMI (adjusted β = 0.10, 95%CI 0.05 to 0.15) and triglycerides (adjusted β = 0.06, 95%CI 0.01 to 0.11) Z-Scores showed positive associations with cord blood insulin Z-Score. No statistically significant association was observed between maternal early GWG and HDL-C Z-Scores with cord blood insulin Z-Score.

ABN analysis results for interdependent maternal metabolic parameters

Figure 7 shows the optimal summary DAGs inferred by ABN analysis (Technical details are provided in ***Supplementary file S13***). The adjusted regression coefficients (β) in the graph represent how much the dependent variable changes per unit increase in the independent variable. Maternal pre-pregnancy BMI appeared to be the most influential upstream factor for both maternal metabolic parameters in pregnancy (glycaemia: β = 0.14, 95%CI 0.09 to 0.19; early GWG: β = -0.12, 95%CI -0.17 to -0.06; triglycerides: β = 0.23, 95%CI 0.18 to 0.28; HDL-C: β = -0.12, 95%CI -0.17 to -0.07) and birthweight (β = 0.27, 95%CI 0.22 to 0.32). An indirect effect on neonatal insulin secretion was also observed. Our DAGs results showed that maternal glycaemia was associated with cord blood insulin (β = 0.12, 95%CI 0.07 to 0.17). Birthweight was also associated with cord blood insulin (β = 0.24, 95%CI 0.19 to 0.29). Neither triglycerides nor HDL-C were linked to birthweight or cord blood insulin.



β, standardized regression coefficient; BMI, body mass index; GWG, gestational weight gain; HDL-C, high-density-lipoprotein cholesterol; TG, triglycerides,

Figure 7 Additive Bayesian Network Graph

Discussion

To our knowledge, this is perhaps the first large prospective birth cohort study to map the metabolic network, and assess associations between maternal modifiable metabolic risk factors, birthweight, and insulin secretion in neonates. We showed that high maternal pre-pregnancy BMI appears the most influential upstream metabolic risk factor for both maternal and neonatal health. Maternal early GWG is directly associated with birthweight, but not neonatal insulin secretion. Maternal fasting glucose is significantly associated with increased neonatal insulin secretion. Although maternal low HDL-C and high triglycerides concentrations are significantly associated with elevated birthweight, our results demonstrated that these lipid pathways may not be meaningfully involved in the metabolic network pathway between mothers and neonates, and instead be a proxy measure for maternal metabolic health. These findings suggest: 1) the primary focus of weight management in clinical practice to prevent adverse pregnancy outcomes should start from preconception; 2) the observed association between maternal glucose and birthweight is likely to be partly mediated through elevated neonatal insulin secretion; 3) The pathogenic relationships of maternal glucose/triglycerides with birthweight/CBI need to be inferred with caution and evaluated in further studies.

Comparison with previous studies and potential mechanisms

The 'foetal origins' hypothesis proposed that intrauterine nutrient environment has a significant impact on developmental adaptations in foetal organs, and might even contribute to health outcomes throughout the life through permanent physiological, behavioural, and genetic changes (326). Recent evidence also demonstrated that maternal nutrition factors in preconception might disturb metabolic pathways and contribute to poor pregnancy outcomes and future cardiovascular disease via one-carbon metabolism (402). The 1-C metabolism drives

the synthesis of proteins, biogenic amines and lipids required for early growth, together with the synthesis and methylation of DNA and histones essential for the regulation of gene expression.

Our results are generally consistent with previous relevant evidence, but importantly we also provide new insights that differ from the conclusions of previous studies. The Hyperglycaemia and Adverse Pregnancy Outcome Study research group (HAPO) published a series of network analyses reporting that maternal metabolites (acylcarnitines, fatty acids, carbohydrates, and amino acids) during pregnancy are associated with BMI, fasting glucose, and insulin resistance in mothers (403) and birth size, growth, adiposity, and cord blood C-peptide in neonates (398, 404). Consistent with our findings, another study that looked at all metabolic parameters but not in the context of network analysis and restricted to women with GDM only (n = 357) reported that the number of altered maternal metabolic characteristics (pre-pregnancy BMI, fasting glycaemia, HbA1c, triglycerides, and HDL-C) are associated with incidence of LGA (405). However, none of those studies explored the inter-dependent relationships between maternal metabolic risk factors and compared the strength of their associations with neonatal conditions.

A robust systematic review published in 2013 concluded that maternal pre-pregnancy overweight/obesity is associated with an increased risk of LGA, and decreased risk of SGA (286). Our analyses demonstrated that maternal pre-pregnancy BMI is the most important contributor to increased birthweight, which is independent of maternal early GWG, glucose, and triglycerides levels during pregnancy. It is also worth noting that high maternal pre-pregnancy BMI is closely related to gestational metabolic disorders, namely, increased fasting glucose and triglycerides levels, therefore, further contributing to elevated birthweight and insulin secretion in offspring. Similar to our results, a small study containing 66 mother-

offspring pairs found that obese mothers might induce increased insulin secretion in offspring. Their results also showed a clear sexual dimorphism (boys have higher insulin secretion than girls) (406). In this study, the association between maternal pre-pregnancy BMI with cord blood insulin in boys seemed stronger than in girls (β [95%CI], boys 0.13 [0.07, 0.20] vs. girls 0.08 [0.00, 0.15]), but the difference was not statistically significance. On the other hand, we found that maternal early GWG is only statistically associated with birthweight, but not cord blood insulin, which suggests that the weight accumulation in the early pregnancy may indirectly affect neonatal metabolism through increased birthweight.

The HAPO trial, the biggest study anywhere on this, indicated that maternal glucose is strongly associated with increased birthweight and cord blood C-peptide levels (388). The recommendation from WHO (2013) also summarized that GDM is positively associated with the increased risk of LGA, and treatment of GDM could decrease the risk of LGA based on four RCTs (393). We observed a positive association between maternal fasting glucose and increased birthweight using classic multivariate linear regression analyses. However, the ABN results suggests that maternal fasting glucose is perhaps not directly linked with birthweight. When we entered cord blood insulin Z-Score in the regression model, the association between maternal glucose Z-Score and birthweight Z-Score decreased dramatically but remained statistically significant (β = 0.05, 95%CI 0.01 to 0.09). This suggests that maternal fasting glucose likely influences birthweight via increasing insulin secretion in foetus, in line with prior hypotheses.

Maternal circulating glucose transports freely across the placenta, and provides energy to satisfy the growth needs of the foetus (407). Two studies found a significant association between maternal fasting glucose at the end of second trimester and insulin/C-peptide in the cord blood (407, 408). Lawlor et al. reported that the de novo anabolic effects of cord blood insulin is key

for foetal fat deposition (407). At the same time, Tam et al. argued that the association of maternal hyperglycaemia with neonatal high blood C-peptide may not be mediated through macrosomia at birth and childhood obesity (408). Our results demonstrated that cord blood insulin may partly be explained by a response to the concentration of glucose transported from the maternal end. The increased cord blood insulin might stimulate adipose accumulation in neonates. Meanwhile, in addition to maternal fasting glucose level, elevated birthweight might also be a strong contributor for cord blood insulin (196, 409).

We recently published a systematic review which found that increased maternal triglycerides and decreased HDL-C are positively associated with high birthweight (392). Similar results were observed using multivariate regression analysis in this study. The association between maternal triglycerides and birthweight remain statistically significant even when adjusted for the other four metabolic risk factors. Additionally, Geraghty et al. reported that high maternal triglycerides throughout pregnancy is positively associated with sum of skinfold in neonates in 331 mother-child pair from the ROLO study (117). However, our ABN analysis now take us a step further by suggesting that both maternal HDL-C and triglycerides are likely to be measures of gestational metabolic disorder, and not themselves involved in the metabolic pathway that increases birthweight and cord blood insulin. Importantly, a Mendelian randomization study analysing data from 30,487 women in 18 studies concluded that genetically higher maternal fasting HDL-C/triglycerides was not potentially causally associated with higher birthweight (410). Thus, both detailed pathways analyses in this paper and genetic finding go against lipid pathways being directly relevant to birthweight.

Strengths and limitations

The major strengths of this study are the prospective design based on a relatively large sample size, standardization of strength of association for the comparison among maternal metabolic risk factors, and the use of powerful analytical tools for interpretation of multi-dimensional data. Given the practical constraints, maternal fasting glucose and triglycerides levels were measured only once during pregnancy. Therefore, we could not investigate the dynamic long-term influences of maternal metabolic risk factors in detail. The average pre-pregnancy BMI of included women and incidence of LGA/SGA babies in this study were significantly lower than for people living in the northern part of China. The relative healthiness of our cohort suggests that our results might underestimate the true impact of maternal metabolic disorders on neonatal health outcomes if extrapolated to this wider population. The pre-pregnancy weight was self-reported, which might potentially underestimate the true value. However, evidence suggest that utilization of self-reported or measured pre-pregnancy weight for pre-pregnancy BMI classification results in identical categorization for most women (411). In addition, due to lacking of dynamic data, the ABN analysis might have limited ability on exploring feedback loop. Therefore, the results of ABN, as with any observational analyses, need to be interpreted with a degree of caution.

Implication

Most current clinical guidelines on preconception and antenatal care only focus on weight management during pregnancy. Our results provide further important evidence on the clinical importance of maternal pre-pregnancy high BMI for both maternal and neonatal health outcomes. Interventions to reduce weight in overweight/obese women before conception to reduce adverse effects of high maternal pre-pregnancy BMI may need further investigation in

randomized trials. Recommendations on pre-pregnancy weight management is limited and ambiguous (28, 78, 412). None of the guideline on weight management in adults provides advice to women in child-bearing age to prevent adverse pregnancy outcomes. Only one public health guideline in UK mentions potential course of actions that could be taken by health professional to improve outcomes in women with a BMI equal or in excess of 30 kg/m^2 prior to pregnancy (412). Our results, if applied to wider communities, provide further evidence for public health measures at improving weight levels in women in general and particularly those of child-bearing age.

Conclusion

In conclusion, in this cohort study, high maternal pre-pregnancy BMI appeared to be the most influential upstream risk factor for gestational hyperglycaemia/hypertriglyceridemia in mothers and increased birthweight/insulin secretion in neonates. Our findings based on robust novel statistical methods highlights the impact of maternal pre-pregnancy BMI on maternal and neonatal metabolic outcomes. We also found that maternal hyperglycaemia was positively associated with elevated neonatal insulin secretion, suggesting this may be a key reason for foetal fat deposition. By contrast, maternal lipid levels do not appear to have meaningful independent associations with neonatal birthweight or cord blood insulin.

Chapter 5 Glucose, insulin and lipids in cord blood of neonates and their association with birthweight: differential metabolic risk of large-for-gestational-age and small-for-gestational-age babies

Abstract

Objectives

Babies born small-for-gestational-age (SGA) and large-for-gestational-age (LGA) are at similar risks of developing obesity, diabetes, and cardiovascular disease. Little is known whether SGA and LGA babies have similar metabolic profile at birth that lead to adverse health outcomes subsequently. We investigated the association of birthweight with cord blood metabolic parameters (glucose, lipids, and insulin).

Methods

Data of 1,522 newborns were obtained from the Born in Guangzhou Cohort study (BIGCS). Generalized additive model and multivariable linear regression model were used to explore the non-linear and linear relationships between birthweight and cord blood metabolic parameters, and to evaluate the differences of metabolic parameters Z-Scores among SGA, appropriate-for-gestational-age (AGA), and LGA babies.

Results

Birthweight Z-Score was linearly associated with increased cord blood insulin Z-Score (adjusted β = 0.32, 95%CI 0.26 to 0.37). Compared to AGA babies, neonates born SGA had significantly higher cord blood triglycerides Z-Score (adjusted mean difference [MD_{adj}] = 0.70, 95% CI 0.51 to 0.88) and lower cord blood insulin (MD_{adj} = -0.54, 95%CI -0.74 to -0.35), high-density-lipoprotein cholesterol (MD_{adj} = -0.36, 95%CI -0.55 to -0.16), and total cholesterol (MD_{adj} = -0.20, 95%CI -0.40 to -0.01) Z-Scores, while neonates born LGA had higher cord

blood insulin Z-Score (MD_{adj} = 0.48, 95%CI 0.27 to 0.68) and lower triglycerides Z-Scores (MD_{adj} = -0.24, 95%CI -0.43 to -0.04).

Conclusions

Our findings support the hypothesis that SGA and LGA babies are exposed to different intra-uterine environments, which may contribute to altered fat accumulation patterns with implications for the risk of metabolic dysfunction later in life. There is a need to consider the development of tailored intervention strategies to prevent metabolic dysfunction in adult life for these babies.

Manuscript (Under review)

Introduction

Babies who are small-for-gestational-age (SGA) and large-for-gestational-age (LGA) are at a higher risk of similar adverse health outcomes, such as infant mortality, subsequent life-long risk of obesity, type 2 diabetes, and cardiovascular disease (65-67). These adverse metabolic conditions have become major public health concerns globally. The World Health Organisation estimated that over 381 million children and adolescents, and more than 1.9 billion adults were overweight or obese in 2016 globally (1). In parallel with the increasing prevalence of obesity, there has been an accompanying rise in the prevalence of associated metabolic and cardiovascular diseases (46, 391). Obesity and its related conditions have become major global health concerns resulting in significant social and economic burden. While birthweight has been used as a marker for risk of adverse health outcomes in adults, it is still unclear why relatively low and high birthweights have similar adverse effects.

Fat accumulation is acknowledged as the common factor that mediates the effects of SGA and LGA on adult metabolic health (175). Meanwhile, metabolic dysfunction including hyperglycaemia, dyslipidaemia (altered cholesterol and triglycerides levels), hyperinsulinemia, and high body mass index (BMI) have been identified as major risk factors for diabetes and cardiovascular diseases (413, 414). The metabolic markers in cord blood could reflect the intrauterine environment or the initial metabolic status in the foetus, and may potentially be linked to subsequent metabolic dysfunction.

Evidence on the association of birthweight with foetal metabolism is limited and controversial. Lindsay et al. found that cord blood insulin was independently associated with increased birthweight in neonates of mothers with type 1 diabetes but not in mothers without type 1 diabetes (415). In contrast, Hou et al. observed a positive association in term neonates whose

mother did not have diabetes (416). Rodie V et al. reported that there was no evidence of the association between birthweight and foetal lipids (417), while Kelishadi R et al. observed elevated risk of high cord blood triglycerides levels in SGA and LGA babies (313). Most of these studies only focused on a specific population with small sample size (313, 415, 417), or ignored the potential non-linear relationship between birthweight and cord blood metabolic markers (313, 416).

Understanding the metabolic profile in low and high birthweight babies may provide new insights on the underlying metabolic mechanism in newborns, and help uncover strategies for timely and effective intervention to prevent subsequent metabolic diseases. Therefore, the aim of this study is to determine the associations of birthweight with insulin, glucose, and lipids concentrations in cord blood.

Methods

Study Design

This study was conducted in a subgroup of the Born in Guangzhou Cohort Study (BIGCS), an ongoing large-scale prospective birth cohort study, in whom relevant maternal and cord-blood metabolic parameters were assessed. The cohort details of BIGCS have been described previously (400). Briefly, pregnant women (< 20 gestation week) with Chinese nationality living in Guangzhou and who intended to deliver at one of the two Guangzhou Women and Children's Medical Centre (GWCMC) campuses were eligible for BIGCS. At recruitment, participants were asked to complete a semi-structured questionnaire (Q1) for demographic data collection. All women in BIGCS were offered a standard 2h 75g oral glucose tolerance test (OGTT) appointment at 22-28 weeks gestation.

To be eligible for this subgroup study, BIGCS women had to deliver at GWCMC with umbilical cord blood retained between January 2015 and June 2016. We excluded participants who: 1) were without available fasting blood samples at 14-27 weeks' gestation; 2) did not complete an OGTT test; 3) had type 1 or type 2 diabetes, thyroid dysfunction, hypertension, virus hepatitis and kidney diseases prior to pregnancy; 4) were without maternal demographic information. Among the eligible participants, a total number of 1,522 randomly selected mother-child pairs were finally included in this study (***Figure 6***). The sample size was designed based on a previous study (***Supplementary material S9***). A sample of 1,552 will give 80% power to detect a correlation coefficient of 0.07 at the 5% significance level (two-sided). Ethical approval for this study was granted by the GWCMC Ethics Committee.

Data and Biological Samples Collection

Maternal demographic data, including age, height, pre-pregnancy weight, parity, ethnicity, family and personal medical history, and cigarette exposures were self-reported. Cigarette exposures, including both active and passive smoking, was categorised as a binary variable (yes/no). Pre-pregnancy BMI was calculated by dividing weight in kilograms by height in meters squared. Women were classified to lean group (< 24 kg/m^2) and overweight group (≥ 24 kg/m^2) by pre-pregnancy BMI according to the recommendations of the China Obesity Task Force of the Chinese Ministry of Health (6).

Participants were asked to complete an OGTT test in the morning after overnight fasting. Plasma samples were taken at three time points: 0 minutes, 60 minutes and 120 minutes, and were sent to the clinical laboratory centre in GWCMC for immediate assays. Women were diagnosed as having gestational diabetes mellitus (GDM) if their OGTT results met or exceeded at least one threshold of the International Association of Diabetes and Pregnancy Study Groups

criteria (fasting plasma glucose ≥ 5.1 mmol/L, 1h glucose ≥ 10.0 mmol/L, and 2h glucose ≥ 8.5 mmol/L) (401).

Birthweight was measured to the nearest 50g using electronic scales by the attending midwives immediately after delivery. Newborns' information including sex, gestational age and delivery mode were obtained from routine medical records. Birthweight was standardized as birthweight Z-Score and birthweight percentile by gestational age and neonatal sex using INTERGROWTH-21st Newborn Size Standard and Tools (62). Babies with birthweight < 10th percentile and > 90th percentile for their gestational age and sex, were classified as SGA and LGA, respectively. Babies born before 37 weeks of pregnancy were defined as born pre-term.

Venous umbilical cord blood samples were collected by midwives at birth and were stored and delivered to Guangzhou Biobank on ice within 3 hours. In the pre-treatment centre, all samples were separated to serum and plasma immediately by centrifugation and were stored in Ethylenediaminetetraacetic acid (EDTA) tubes at -80℃in the Guangzhou Biobank until assays were carried out.

Metabolic Parameter Assays

The cord blood plasma samples were sent to a third-party medical laboratory for metabolic parameter assays. Samples were masked with the sample ID number, therefore, staff in pre-treatment centre, Guangzhou Biobank and medical laboratory were blinded to participants' information.

Cord blood insulin was measured on Roche Immunology Analyser (cobas 8000 e602) using electrochemiluminescence immunoassay. The assays of glucose, total cholesterol (TC, cholesterol oxidase method), high-density lipoprotein cholesterol (HDL-C, ELISA), low-density lipoprotein cholesterol (LDL-C, ELISA) and triglycerides (TG, enzymatic

measurements) were conducted on Roche Chemistry Analyser (cobas 8000 c702). Intra- and inter-day coefficients of variation (CVs) were consistently < 2% for all assays.

Statistical Analysis

Data were presented as mean ± standard deviation or median and interquartile range for describing symmetrical and skewed continuous variables respectively.

Lipids profile has been demonstrated to be stable in EDTA tubes at -80 °C for a period of at least 24 months. Pearson correlation was used to detect the influence of the long-term -80 °C storage in EDTA tubes on insulin and glucose concentrations. Concentration was then adjusted for storage duration using the regression model if a statistically significant association was detected (***Supplementary material S16***). All cord blood metabolic parameters (insulin, glucose, TC, HDL-C, LDL-C, and TG) were standardised as Z-Scores due to the different measurement units and scales. Cord blood insulin and TG concentrations were log-transformed prior to standardisation. The resultant adjusted regression coefficients represent differences in the mean of cord blood metabolic parameter Z-Scores on these scales per one unit increase in birthweight Z-Score. Multivariable linear regression analyses with birthweight Z-Score as the independent variable and Z-Score of cord blood parameters as dependent variables were adjusted for maternal age, parity, delivery mode, gestational age, baby's sex, smoking exposure and ethnic group. It is well known that maternal GDM/obesity and pre-term delivery can significantly influence birthweight and neonatal metabolic status, we therefore conducted subgroup analyses to understand how these conditions might influence the association between birthweight and cord blood metabolic parameters (286, 388). Subgroup analyses were performed among non-GDM/GDM, pre-term/term birth, and maternal lean/overweight groups. All statistical tests were two-tailed and a P-value less than 0.05 was considered statistically significant.

We also investigated the difference of cord blood metabolic parameter Z-Scores between SGA, appropriate-for-gestational-age (AGA), and LGA birthweight categories. The above analyses were carried out in Stata 14.0 (College Station, Texas, USA).

To further investigate the potential non-linear relationship between birthweight and cord blood metabolic parameter at both ends of the birthweight spectrum, the generalised additive model (GAM) was conducted as a post-hoc analysis. GAM was adjusted for maternal age, parity, delivery mode, gestational age, neonatal gender, cigarette exposures, and ethnic group. The results of GAM can help explain how cord blood metabolic parameter Z-Scores vary along with different birthweight percentiles. GAM analysis was performed in R 3.5.0 (The R Foundation for Statistical Computing).

Results

Baseline characteristics

***Table* 11** shows baseline characteristics for all participants and separately for SGA, AGA, and LGA groups. The mean age of all included women at recruitment was 29.5 years. The vast majority of them were ethnic Han Chinese (97.7%). Around 12% of women were diagnosed with GDM. Women with LGA babies were on average older, less likely to deliver spontaneously, be primipara, and have higher pre-pregnancy BMI and GDM incidence than SGA and AGA groups.

Table 11 Baseline characteristic table

Characteristics	Overall (n=1,522)	Birthweight parameters		
		SGA(n=105)	AGA(n=1,320)	LGA(n=96)
Maternal information				
Maternal age (years)	29.50 ± 3.30	28.93 ± 3.31	29.44 ± 3.25	30.97 ± 3.58
Ethic Han	1,486 (97.70)	103 (98.10)	1292(97.88)	91(94.79)
Primiparous	1,223 (80.35)	93 (88.57)	1,064 (80.55)	66 (68.75)
Spontaneous delivery	1,239 (81.41)	85 (80.95)	1,101 (83.35)	53 (55.21)
Pre-pregnancy BMI (kg/m^2)	20.47 ± 2.69	19.52 ± 2.09	20.41 ± 2.63	22.36 ± 3.15
GDM	181 (11.89)	9 (8.57)	154 (11.66)	18 (18.75)
Neonatal information				
Gestational age (days)*	275 (270-281)	276 (271-282)	275 (270-281)	276 (271-281)
Birthweight (g)	3,203 ± 411	2,603 ± 251	3,194 ± 334	3,982 ± 269
Male	820 (53.88)	46 (43.81)	730 (55.26)	44 (45.83)
Sample storage time (days)*	488(394-707)	519(389-695)	487 (395-707)	488 (394-707)
Cord blood metabolic parameters				
Insulin (μU/mL) *	7.43 (4.34-12.61)	5.64 (3.24-8.60)	7.39 (4.34-12.56)	11.53 (6.87-18.39)
Glucose (mmol/L)	4.81 ± 2.32	4.69 ± 2.32	4.90 ± 2.32	3.72 ± 2.10
TC (mmol/L)	1.72 ± 0.42	1.66 ± 0.39	1.73 ± 0.42	1.72 ± 0.51
HDL-C (mmol/L)	0.91 ± 0.28	0.83 ± 0.27	0.92 ± 0.28	0.92 ± 0.30
LDL-C (mmol/L)	0.61 ± 0.24	0.58 ± 0.24	0.61 ± 0.24	0.61 ± 0.31
TG (mmol/L) *	0.33 (0.27-0.41)	0.43 (0.34-0.52)	0.33 (0.27-0.41)	0.28 (0.23-0.36)

Data are presented as mean ± SD or n (%), *Median (Inter Quartile Range).
Abbreviation: SGA, small-for-gestational-age; AGA, appropriate-for-gestational-age; LGA, large-for-gestational-age; BMI, body mass index; GDM, gestational diabetes mellitus; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol, TG, triglycerides.

Multivariable linear regression model

***Table* 12** shows the relationship between birthweight Z-Score and cord blood metabolic parameter Z-Scores. After standardization and adjustment, birthweight Z-Score had a positive association with cord blood insulin Z-Score (adjusted β = 0.32, 95% CI 0.26 to 0.37), HDL-C Z-Score (adjusted β = 0.10, 95% CI 0.04 to 0.16), and TC Z-Score (adjusted β = 0.06, 95% CI 0.00 to 0.12); and an inverse association with cord blood TG Z-Score (adjusted β = -0.27, 95% CI -0.32 to -0.21). No statistically significant association was observed between birthweight Z-Score and cord blood glucose and LDL-C levels.

Compared to AGA babies, SGA babies were found to have much higher cord blood TG Z-Score (adjusted Mean Difference [MD] = 0.70, 95%CI 0.51 to 0.88) and lower cord blood insulin (adjusted MD = -0.54, 95%CI -0.74 to -0.35), TC (adjusted MD = -0.20, 95%CI -0.40 to -0.01), and HDL-C Z-Scores (adjusted MD = -0.36, 95%CI -0.55 to -0.16). Meanwhile, LGA babies had higher cord blood insulin Z-Score (adjusted MD = 0.48, 95%CI 0.27 to 0.68) and lower cord blood TG Z-Score (adjusted MD = -0.24, 95%CI -0.43 to -0.04) than AGA babies.

Table 12 Multivariable linear regression results of the association between birthweight and cord blood metabolic parameter Z-Scores

Cord blood metabolic parameter Z-Scores	Adjusted β* (95% CI)	Adjusted Mean Difference (95% CI) □		
		SGA	AGA	LGA
Insulin	**0.32 (0.26, 0.37)**	**-0.54 (-0.74, -0.35)**	Ref	**0.48 (0.27, 0.68)**
Glucose	0.01 (-0.04, 0.06)	-0.10 (-0.26, 0.07)	Ref	-0.08 (-0.25, 0.09)
TC	**0.06 (0.00, 0.12)**	**-0.20 (-0.40, -0.01)**	Ref	0.02 (-0.18, 0.23)
HDL-C	**0.10 (0.04, 0.16)**	**-0.36 (-0.55, -0.16)**	Ref	0.00 (-0.20, 0.21)
LDL-C	0.04 (-0.02, 0.10)	-0.14 (-0.34, 0.05)	Ref	0.03 (-0.17, 0.24)
TG	**-0.27 (-0.32, -0.21)**	**0.70 (0.51, 0.88)**	Ref	**-0.24 (-0.43, -0.04)**

*multivariable linear regression model account for the linear relationship between birthweight Z-Score and cord blood metabolic parameter Z-Scores. Adjusted for maternal age, parity, delivery mode, gestational age, neonatal gender, cigarette exposures, and ethnic group.

□multivariable linear regression model account for the adjusted mean difference of cord blood metabolic parameter Z-Scores between SGA/LGA groups and reference group (AGA). Adjusted for maternal age, parity, delivery mode, gestational age, neonatal gender, cigarette exposures, and ethnic group.

Abbreviation: SGA, small-for-gestational age; AGA, appropriate-for-gestational age; LGA, large-for-gestational age; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides, Ref, reference group

GAM analysis

Figure 8 shows the scatter plots and the results of GAM estimation of the association between birthweight percentile and standardised residuals of cord blood metabolic parameter Z-Scores. The association between birthweight percentile and cord blood insulin Z-Score ($p < 0.001$) was approximately linear in the graph. Birthweight percentile was also negatively associated with cord blood TG Z-Score ($p < 0.001$), and positively associated with cord blood HDL-C Z-Score ($p < 0.001$). The slope of observed associations diminished along with increasing birthweight percentile for TG and HDL. When examining the association between birthweight and TC Z-Score, there was a slight rise in the lowest 20th percentile but this was not statistically significant ($p = 0.09$). No association was evident between birthweight percentile with cord blood glucose ($p = 0.79$) or LDL-C Z-Scores ($p = 0.22$).

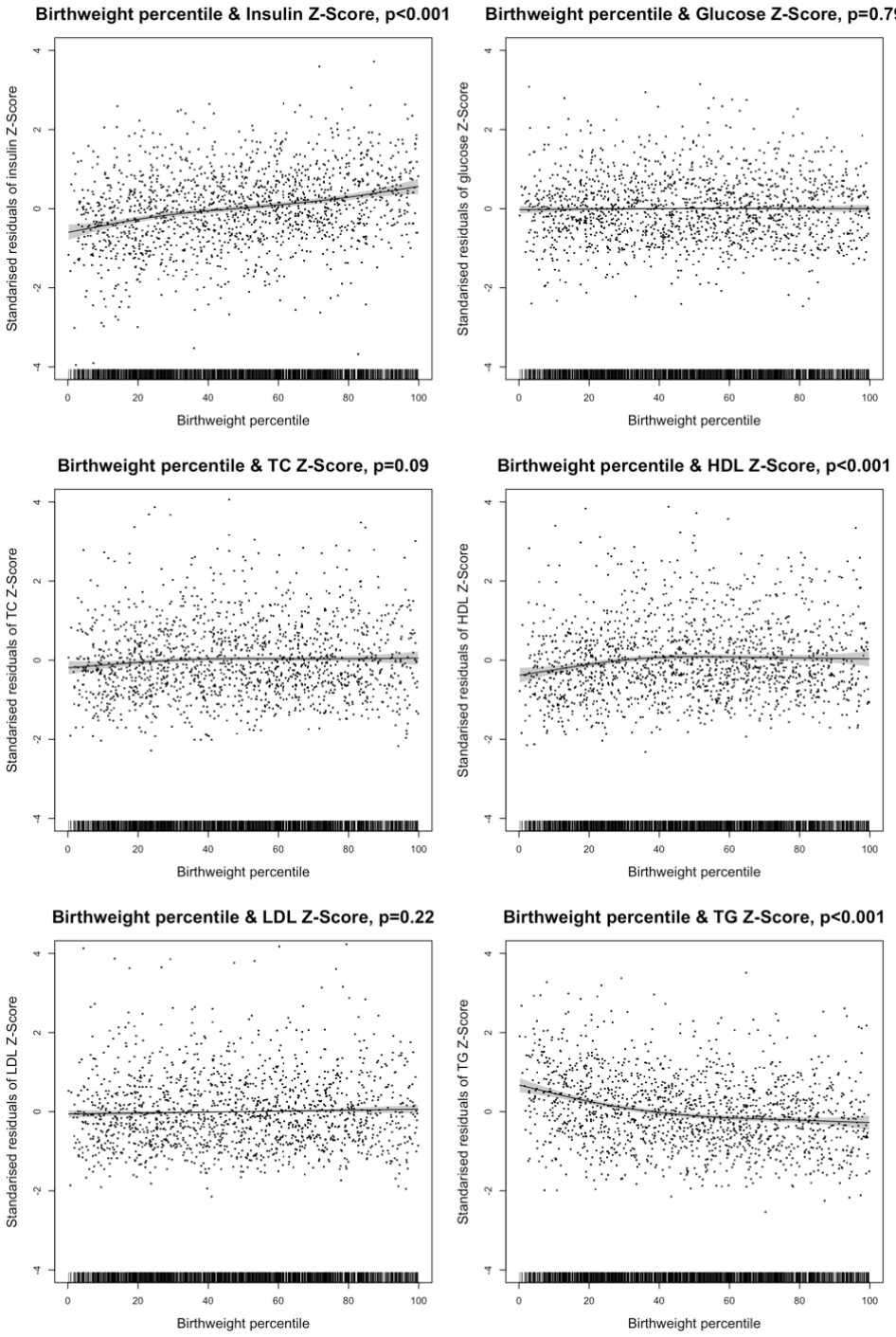


Figure 8 Results of Generalized Additive Model for the association between birthweight percentile and cord blood metabolic parameter Z-Scores

Subgroup analysis

Figure 9 shows the findings of our subgroup analyses for the associations between birthweight Z-Score and cord blood metabolic parameter Z-Scores. The relationships of birthweight Z-Scores and cord blood insulin/glucose Z-Scores were not influenced by GDM, maternal overweight status, or pre-term birth. The estimated effect sizes for HDL and TG Z-Scores in women with GDM were in the opposite direction to that observed in the non-GDM population. The estimated confidence intervals of effect sizes for HDL/TG Z-Scores in GDM group and non-GDM group did not overlap. In preterm birth neonates, we observed strong negative estimate of associations of birthweight Z-Scores with cord blood TC (β = -0.43, 95%CI -0.83 to -0.02) and LDL-C (β = -0.48, 95%CI -1.00 to 0.04) Z-Scores.

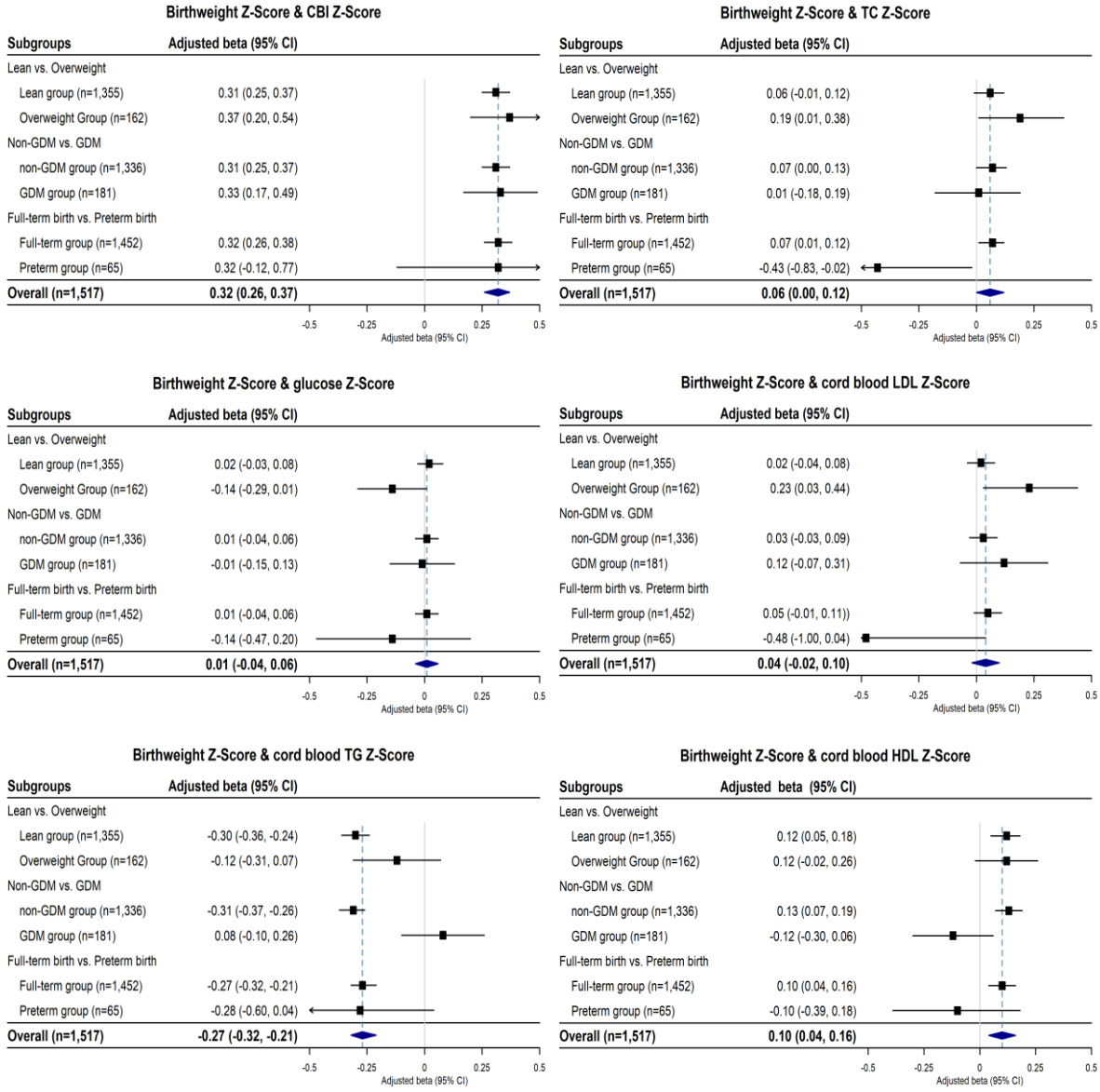


Figure 9 Results summary of the subgroup analyses

Discussion

This study of 1,522 mother-child pairs found that neonates born with low and high birthweight had different metabolic profiles. Increased birthweight was strongly associated with increased insulin and decreased triglycerides concentrations in cord blood. Compared to AGA, neonates born SGA had significantly lower cord blood insulin, TC, and HDL-C levels, and had higher cord blood triglycerides concentrations, while neonates born LGA had significantly higher cord blood insulin and lower triglycerides concentrations. Birthweight did not show an evident association with cord blood glucose and LDL-C concentrations.

Comparison with previous studies

Several studies have described the association between birthweight and cord blood insulin, but results are conflicting. Most of them observed elevated cord blood insulin in LGA neonates (416, 418-421), while some studies reported decreased or unchanged cord blood insulin levels in LGA neonates (422, 423). However, most these studies were small in number (415, 418-421, 423, 424) or failed to adjust for important confounding factors, such as gestational age and neonatal gender (415, 417, 418, 420, 421, 423, 424). Our study was the largest one that took into account clinically important confounders and demonstrated that birthweight was positively and linearly associated with cord blood insulin and that the estimates were consistent in GDM and non-GDM, lean and overweight, as well as preterm and full-term subgroups.

Evidence regarding the association between birthweight and cord blood lipids profile is inconsistent. Similar to our findings, Katragadda et al. and Hou et al. observed elevated TG levels in SGA or low birthweight neonates (416, 425), while Aletayeb et al. reported elevated TC, LDL-C, and TG levels in neonates born low or high birthweight (426). On the other hand, Kelishadi et al. found decreased HLD-C/LDL-C in LGA (313), and Rodie et al. and Kenchappa

et al. found there is no association between birthweight and cord blood lipids profile (417, 427). However, these studies were again limited by small sample size and failed to take account of relevant confounding factors. With appropriate design, our analysis showed that there was significant elevated cord blood TG and decreased TC and HDL-C levels in SGA, as well as decreased TG level in LGA. In the subgroup analysis, we found that birthweight was independently associated with increased cord blood HDL-C and decreased TG levels in neonates with non-GDM mothers, but not in neonates with GDM mothers. In addition, our results indicated that preterm birth neonates with lower birthweight might have higher cord blood cholesterol profile.

It is believed that newborns with low and high birthweight are at increased risk of neonatal hypoglycaemia (428, 429) based on previous studies, but the studies did not account for potential confounders such as caesarean section, parity, and ethnicity (422, 430-432). In our study, after adjusting for important confounding factors, we did not find an evident association between birthweight and cord blood glucose concentration (***Supplementary material S17***).

Potential mechanism

The vast majority of cord blood glucose is transported freely from the maternal end, and largely determines the foetal glycaemia and insulin level at birth (407). Insulin secretion is known to play a central role in foetal growth. Elevated insulin concentration increases glucose uptake in muscle and adipose tissue, blocks glycogenolysis and gluconeogenesis in the liver, and stimulate glycogen synthesis (433). Meanwhile, it also promotes the circulating free fatty acids (FFAs) and triacylglycerol uptake in adipose and muscle tissues, therefore lowering the plasma TG level (434). Through stimulating lipogenesis, glycogen, and protein synthesis, and

inhibiting lipolysis, glycogenolysis, and protein breakdown, insulin could also stimulate the storage of substrates (lipids, protein, and glycogen) in adipose, liver, and muscle tissues (433). Adipose tissue plays a central role in metabolism, buffering the daily influx of dietary fatty acids and providing storage for excess energy in the form of TG. When TG accumulation exceeds the buffer capacity of adipose tissue, ectopic fatty acid deposition in non-adipose tissue organs, such as the liver and muscle, occurs (435). Ectopic fatty acid deposition contributes to the development of insulin resistance and cardiovascular morbidity. One key factor that determines the buffer capacity of adipose tissue is adipocyte cell size and the capability of adipose tissue expansion, either through adipocyte hyperplasia or hypertrophy (435). Little is known about the exact determinants of expansion capacity, although genetic and developmental factors appear plausible (436). In this light, the intrauterine environment may also play an important role in the development of adipose tissue and its ability to expand in early life.

Compared to AGA, neonates born SGA, tend to experience intrauterine growth restriction (IUGR), and have deficits in skeletal muscle and absolute fat mass (175). Evidence indicated that IUGR could suppress β-cell replication, leading to diminished insulin production in the foetus (437). Rapid catch up growth experienced by most SGA babies in early postnatal life has been associated with increased risk of diabetes, obesity, and cardiovascular dysfunction in adulthood (438). We showed increased TG and low insulin concentrations within circulating blood, which could be linked to low intravascular lipolysis and decreased lipid deposition into adipose tissue. It could be speculated that this, in turn, results in smaller adipocytes postnatally, with decreased capacity for expansion during the catch-up growth period, thereby leading to ectopic fat deposition and future risk of cardio-metabolic diseases.

Interestingly, LGA babies have also been shown to have an increased risk of obesity, diabetes, and cardiovascular diseases in later life (175). The elevated insulin secretion we observed in

LGA babies, which could be attributed to the increased nutrients supply from the maternal side, is key for circulating lipid uptake and fat deposition in the foetus, mirrored by our finding of low TG concentrations (407). It is therefore possible that LGA babies are born with an already expanded adipose tissue with reduced buffer capacity, potentially contributing to elevated insulin secretion in neonates (439) and, compared to AGA, a higher risk of ectopic fatty acid deposition and the associated cardiometabolic complications in later life (66, 67). Our findings suggest that there is an optimal intrauterine environment driven by physiological insulin concentrations which result in AGA babies who have a lower cardiometabolic risk compared to both SGA and LGA babies. While the exact mechanisms require further investigation, parents of SGA and LGA babies should be provided with tailored professional advice on optimal feeding and weight development of their babies as early as possible to prevent rapid postnatal growth and the risk of obesity in later life (438).

Strengths and limitations

The major strength of this study is comprehensive analyses based on a large prospective cohort study, which allowed us to have well-recorded clinical data and sufficient statistical power to test our hypothesis. Given the practical constraints, we could not measure the fat mass distribution in neonates. Instead of the exact neonatal fat mass, we used birthweight percentile, a widely utilised measurement in clinics, as an approximate measurement to improve the generalizability of our results. In addition, we could only measure metabolic factors in cord blood instead of in neonates due to ethical reasons, which needs to be considered when interpreting our findings.

Conclusion

We found that LGA neonates have significantly higher cord blood insulin and lower TG levels, while SGA neonates have higher cord blood triglycerides and lower HDL-C/TC/insulin levels. No evident association was observed between birthweight and cord blood glucose level. Our findings suggest that the differential metabolic profile in SGA and LGA babies might be crucial for developing subsequent obesity, diabetes, and cardiovascular diseases. Differential intervention strategies may need to be developed to prevent life-long chronic metabolic conditions in children born SGA or LGA.

Chapter 6 Association of birthweight and cord blood triglyceride and cord blood proinflammatory cytokines: an Exploratory Study

Abstract

Objectives

To explore the association of birthweight and cord blood triglycerides levels with proinflammatory cytokines (IL-6, CRP, TNF-α) in cord blood.

Methods

We prospectively collected 222 umbilical cord blood samples from healthy newborns whose mothers have participated in the Born in Guangzhou Cohort Study (BIGCS). Triglycerides, TNF-α, IL-6, and CRP concentrations were measured in cord blood. Information of birthweight, neonatal sex, gestational age, parity, and delivery mode was obtained from clinical records. Generalized additive model and multivariable linear regression model were used to explore and quantify the non-linear and linear relationships between birthweight/cord blood triglyceride and cord blood pro-inflammatory cytokines. Adjusted covariates included gestational age, neonatal sex, delivery mode, parity, and sample storage duration.

Results

Birthweight Z-Score is negatively associated with Z-scores of cord blood CRP (β = -0.18, 95%CI -0.34 to -0.02), TNF-α (β = -0.15, 95%CI -0.32 to 0.02), and IL-6 (β = -0.17, 95%CI -0.33 to -0.00). Cord blood triglycerides Z-Score is positively associated with Z-Scores of cord blood CRP (β = 0.22, 95%CI 0.07 to 0.37) and IL-6 (β = 0.20, 95%CI 0.08 to 0.23), but not with cord blood TNF-α Z-Score. After further adjusting for cord blood triglycerides Z-Score, the estimated association strength between birthweight Z-Score and pro-inflammatory cytokines reduced and its 95% CI did not reach statistically significant levels. After further

adjusting for birthweight Z-Score, the associations between cord blood triglycerides and CRP/IL-6 remained statistically significant.

Conclusions

Our findings suggest that elevated cord blood triglycerides observed in babies with a lower birthweight percentile are associated with systemic low-grade inflammation, which might contribute to short- and long-term metabolic and cardiovascular dysfunctions. We did not observe increased pro-inflammatory cytokines in babies with higher birthweight percentile.

Manuscript

Introduction

Pro-inflammatory cytokines are a certain type of signalling protein produced predominantly by immune cells and are typically elevated in acute inflammatory responses and chronic low-grade inflammation (440). Elevated pro-inflammatory cytokines in chronic low-grade inflammation have been associated with insulin resistance, endothelial dysfunction, and hypertension (441). Compared to AGA babies, we found that SGA babies have significantly higher triglycerides and lower insulin levels in cord blood, while LGA babies have significantly higher insulin and lower triglycerides levels in cord blood (Chapter 5). Both lipotoxicity and expanded adipocytes could induce systemic low-grade inflammation, therefore contributing to the development of metabolic dysfunctions (193, 194, 204). Two studies found that cord blood IL-6 and CRP levels in SGA babies were higher than in AGA babies (442, 443), while the other two studies did not observe the difference (444, 445).

Therefore, we decided to conduct a small exploratory study to investigate whether altered birthweight/triglycerides levels would trigger low-grade inflammatory responses in neonates. The aim of this study is to assess the association of birthweight and triglycerides levels with pro-inflammatory cytokines (IL-6, CRP, TNF-α) in cord blood.

Methods

Study Design

This exploratory study was based on the Born in Guangzhou Cohort Study (BIGCS). The study design of BIGCS has been described in detail before. Chinese pregnant women living in Guangzhou who attended their first antenatal visit and intended to deliver at one of the two Guangzhou Women and Children's Medical Centre (GWCMC) were eligible for BIGCS. In

the main campus of GWCMC, we obtained (February - March 2017) 222 cord blood samples of healthy newborns, whose mothers have participated in BIGCS, for measuring triglycerides and pro-inflammatory cytokines (TNF-α, IL-6, and CRP) levels. Previous studies only compared the difference of proinflammatory cytokines between SGA and LGA babies, but did not explore the association of birthweight and cord blood triglycerides levels with cord blood pro-inflammatory cytokines. Therefore, we assumed a correlation estimate of 0.20. After using 'Fisher's z tests comparing one correlation to a reference value' tool, a sample of 194 will give 80% power to detect a correlation of 0.20 at 5% significance level (two-sided). We then assumed a 10% attrition rate due to missing data, thus giving a sample size of 216. Ethical approval for this study was granted by the GWCMC Ethics Committee.

Data and Biological Samples Collection

Information of birthweight, neonatal sex, gestational age, parity, and delivery mode was obtained from clinical records. Venous umbilical cord blood samples were collected by midwives at birth and were stored and delivered to Guangzhou Biobank on ice within 3 hours. In the pre-treatment centre, all samples were separated to serum and plasma immediately by centrifugation and were stored in Ethylenediaminetetraacetic acid (EDTA) tubes at -80℃in the Guangzhou Biobank until assays were carried out.

Metabolic Parameter Assays

Cord blood plasma samples were sent to a third-party medical laboratory for measuring triglycerides and proinflammatory cytokines (IL-6, CRP, and TNF-α). Samples were masked with the sample ID number, therefore, staff in pre-treatment centre, Guangzhou Biobank and medical laboratory were blinded to participants' information.

Cord blood TNF-α and IL-6 concentrations were measured on Bio-Plex 200 suspension array system. The assays of triglycerides (enzymatic measurements) and CRP (immunoturbidimetry) were conducted on Roche Chemistry Analyser (cobas 8000 c702). Intra-day CVs were consistently < 2% for all assays.

Statistical Analysis

Baseline characteristics were presented as mean ± standard deviation or median and interquartile range for describing symmetrical and skewed continuous variables respectively. Birthweight was standardised as birthweight Z-Score and birthweight percentile by gestational age and neonatal sex using INTERGROWTH-21st Newborn Size Standard and Tools (62). Cord blood triglycerides and proinflammatory cytokines (TNF-α, IL-6, and CRP) were standardised as Z-Scores due to the different measurement units and scales. Cord blood triglycerides, IL-6, and CRP concentrations were log-transformed prior to standardisation.

We first applied GAM to explore the potential non-linear relationship of birthweight and cord blood triglycerides with pro-inflammatory cytokines. Multivariable linear regression models were then used to quantify the associations of birthweight and cord blood triglycerides with pro-inflammatory cytokines. Covariates that were considered in GAM and multivariable linear regression model included gestational age, neonatal sex, delivery mode, parity, and sample storage duration. Descriptive analysis and multivariable linear regression analysis were conducted in Stata 14.0 (College Station, Texas, USA). GAM analysis was performed in R 3.5.0 (The R Foundation for Statistical Computing).

Results

Baseline characteristics

Table 13 shows the baseline characteristics of all neonates. Among 222 neonates, there are 17 babies born to SGA and 13 babies born to LGA. Thirteen of 222 neonates were born before the 37th week of gestation. About 80% of babies were spontaneous vaginal delivered. One hundred and fifteen of 222 neonates (51.80%) were the first child of their mothers. The average birthweight of these 222 newborns was 3183.29 gram.

Table 13 Baseline characteristics table

Characteristics	Mean ± SD
Gestational age (days)	273.26 ± 8.08
Spontaneous delivery$^{\psi}$	178 (80.18)
Male$^{\psi}$	123 (55.41)
Birthweight (g)	3183.29 ± 375.22
Sample storage time (days)	51.22 ± 12.28
Triglycerides (mmol/L) *	0.32 (0.26, 0.38)
IL-6 (pg/mL) *	3.14 (2.64, 3.65)
TNF-α (pg/mL)	36.88 ± 0.62
CRP (mg/L) *	0.17 (0.15, 0.19)

Data are mean ± SD, ψ n (%), * Median (Inter Quartile Range).
Abbreviation: SD, Standard deviation; IL-6, interleukin 6; TNF-α, Tumour necrosis factor alpha; CRP, C-reactive protein.

GAM analysis

Figure 10 shows the scatter plots and the results of GAM estimation of the association of birthweight percentile and triglycerides Z-Score with cord blood pro-inflammatory cytokine Z-Scores. After adjusting for gestational age, neonatal sex, delivery mode, parity, and sample storage duration, the associations of birthweight and cord blood triglyceride Z-Score with cord blood CRP, TNF-α, and IL-6 were all approximately linear in the graph.

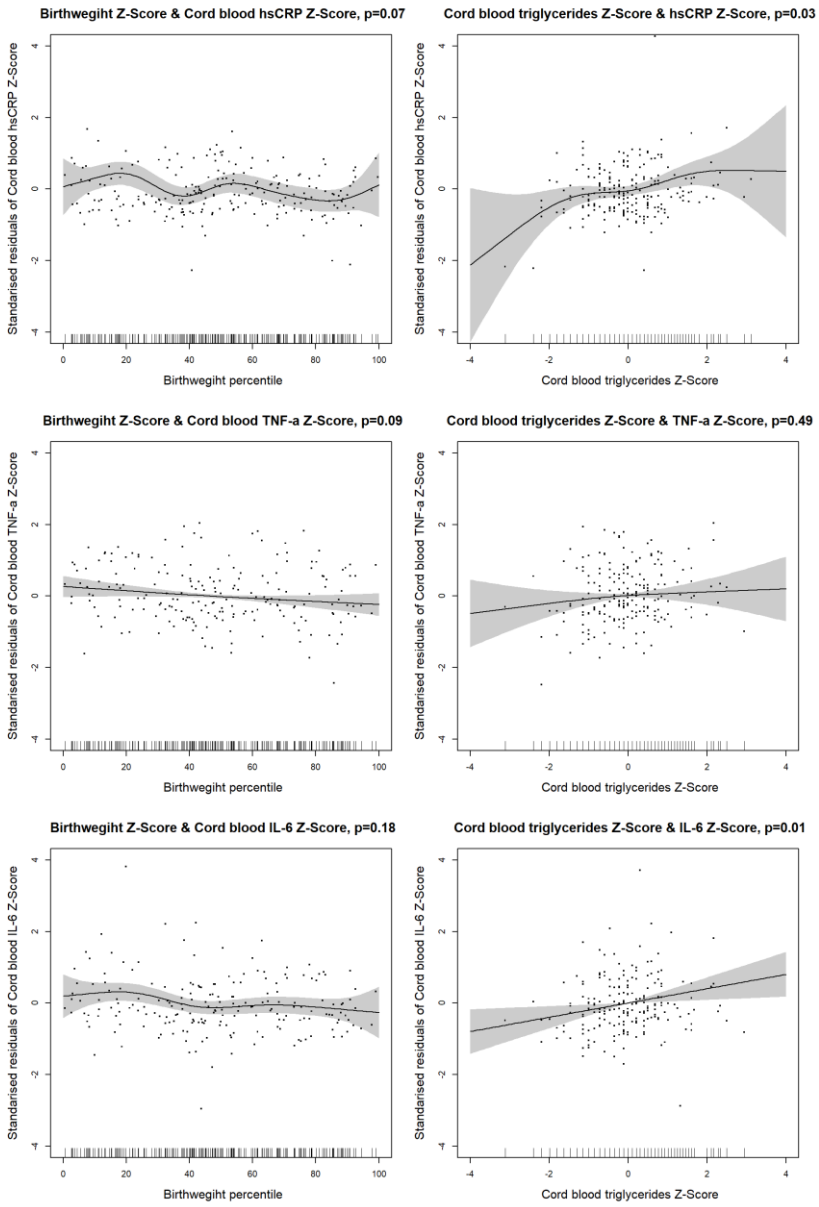


Figure 10 GAM scatter plots of the association of birthweight percentile and cord blood triglycerides Z-Score with cord blood pro-inflammatory cytokine Z-Scores

Multivariable linear regression model

***Table* 14** shows the estimated regression coefficients of the association of birthweight Z-Score and triglycerides Z-Score with cord blood pro-inflammatory factor Z-Scores. After adjusting for gestational age, neonatal sex, delivery mode, parity, and sample storage duration, the increased birthweight Z-Score is significantly associated with decreased CRP (β = -0.18, 95%CI -0.34 to -0.02) and IL-6 (β = -0.17, 95%CI -0.33 to -0.00) Z-Scores. When further adjusting for cord blood triglyceride Z-Score, the association between birthweight Z-Score and cord blood pro-inflammatory cytokines was attenuated. Cord blood triglycerides Z-Score is positively associated with cord blood CRP (β = 0.22, 95%CI 0.07 to 0.37) and IL-6 (β = 0.20, 95%CI 0.05 to 0.35) Z-Scores, and the results remained statistically significant when further adjusted for birthweight Z-Score (CRP: β = 0.20, 95%CI 0.04 to 0.35; IL-6: β = 0.18, 95%CI 0.03 to 0.33).

Table 14 Multivariable analysis of the association of birthweight and cord blood triglycerides Z-Scores with cord blood proinflammatory cytokine Z-Scores

Proinflammatory cytokine Z-Scores (β, 95%CI)	Birthweight Z-Score	Triglycerides Z-Score
Model 1		
CRP	**-0.18 (-0.34, -0.02)**	**0.22 (0.07, 0.37)**
TNF-α	-0.15 (-0.32, 0.02)	0.07 (-0.08, 0.23)
IL-6	**-0.17 (-0.33, -0.00)**	**0.20 (0.05, 0.35)**
Model 2		
CRP	-0.13 (-0.30, 0.03)	**0.20 (0.04, 0.35)**
TNF-α	-0.14 (-0.31, 0.03)	0.05 (-0.11, 0.21)
IL-6	-0.14 (-0.30, 0.03)	**0.18 (0.03, 0.33)**

Model 1: Adjusted for gestational age, neonatal sex, delivery mode, parity, and sample storage duration.

Model 2: For birthweight Z-Score, further adjusted for cord blood triglycerides Z-Score based on model 1; For cord blood triglycerides Z-Score, further adjusted for birthweight Z-Score based on model 1

Abbreviations: CI, confidence interval; CRP, C-reactive protein; TNF-α, Tumour necrosis factor alpha; IL-6, Interleukin 6.

Discussion

In this study, we found that the decreased birthweight was associated with increased CRP, TNF-α, and IL-6 levels but this effect was attenuated when further adjusted for triglycerides. Increased cord blood triglycerides were significantly associated with increased CRP and IL-6 levels in cord blood independent of birthweight.

Comparison with previous studies

Results from previous studies on the association between birthweight and cord blood pro-inflammatory cytokine are inconsistent. Two small studies showed that SGA babies have significantly higher levels of cord blood IL-6 and CRP than LGA babies (442, 443). Compared to AGA babies, Amarily et al. (n = 40) found a slightly higher IL-6 level in SGA babies (IL-6: $p = 0.001$; TNF-α: $p = 0.087$; CRP: $p = 0.005$) (442). Similar results were found in the study led by Lausten-Thoms et al. (n = 90, IL-6: $p = 0.002$; TNF-α: $p = 0.70$; CRP: $p = 0.042$) (443). In contract with their results, Matoba et al. (n = 493) and Linder et al. (n = 93 preterm infants) found there is no difference in cord blood IL-6 and TNF-α between SGA and AGA babies (444, 445).

Evidence from different age groups are also conflicting. In prepubertal children (n = 168), Galcheva et al. found that increased BMI is significantly associated with increased CRP level ($r = 0.39$, $p < 0.0001$) but not with IL-6 and TNF-α levels, while triglyceride was not associated with all three pro-inflammatory factors (446). In adolescents, Gobel et al. observed statistically positive associations between BMI and CRP ($r = 0.53$, $p < 0.01$), and between triglycerides and IL-6 ($r = 0.87$, $p < 0.001$), but findings were limited to obese children (n = 51) (447). Piche et al found that both BMI and triglycerides were significantly associated with increased CRP (BMI: $r = 0.60$, $p < 0.0001$; triglycerides: $r = 0.33$, $p < 0.01$) and IL-6 (BMI: $r = 0.49$, $p < 0.0001$;

triglycerides: r = 0.32, $p < 0.01$) levels, but not with TNF-α, in healthy postmenopausal women (n = 112) (448).

In contrast with the results in adolescents and adults, our results showed that decreased birthweight is associated with increased cord blood IL-6, CRP, and TNF-α levels, while increased cord blood triglycerides was significantly associated with increased CRP and IL-6 levels in cord blood. In this study, we took into account clinically important confounders, applied rigorous methodology, and examined if the association of birthweight and cord blood triglycerides with proinflammatory cytokines were independent to each other.

Potential mechanism

IL-6 acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine, which is produced by various cells with pleiotropic activity (449). Specifically, it induces the synthesis of CRP and has an inhibitory effect on TNF-α. The hepatic synthesis of CRP, stimulated by the increased level of IL-6, activates the complement system and promotes phagocytosis by macrophages, inducing the clearance of necrotic and apoptotic cells (450). TNF-α is a signalling cytokine that is predominantly produced by the macrophage. It plays an important role in the regulation of the immune cell (451). Increased TNF-α indicates infiltration of macrophage in tissues (e.g. fat mass).

In light with previous findings, there are two possible mechanisms to explain our results. Firstly, the fat mass and beta-cell deficiency in SGA babies induced by IUGR results in increased circulating triglycerides levels, leading to systemic low-grade inflammatory responses. Intrauterine growth restriction (IUGR) has been recognised as a major reason for SGA babies. SGA babies who experienced IUGR tend to have deficits in absolute fat mass, skeletal muscle, and beta-cells (175, 437), leading to insulin deficiency and increased circulating triglycerides

(Chapter 5). Our results showed that the elevated triglycerides in babies with relatively lower birthweight percentile are significantly associated with increased CRP and IL-6 levels, but not with TNF-α. It indicates that the lipotoxicity induced by the increased triglycerides might trigger systemic low-grade inflammation that is independent to local inflammation response (e.g. adipocytes). Unlike adolescents and adults, we did not observe elevated pro-inflammatory cytokines in babies with relatively higher birthweight percentile. Therefore, our results suggest that the expanded adipocytes in babies with a higher birthweight percentile does not induce low-grade inflammation at birth.

Secondly, in IUGR pregnancy, the pro-inflammatory cytokines produced by placenta may induce systematic low-grade inflammation in the foetus, which can influence foetal development systematically and results in decreased birthweight as well as increased triglycerides. It has been well known that maternal innate immune response during pregnancy has a critical role in spontaneous abortion, preterm delivery, and IUGR (452). However, it is still unknown whether babies experienced IUGR have higher circulating levels of proinflammatory cytokines at birth. Compared to AGA babies, Mullins observed an elevated cord blood IL-6 level, not CRP or TNF-α, in babies experienced foetal growth restriction (453). Systemic inflammation can expose the foetus to unfavourable conditions, therefore may potentially influence foetal morphogenesis. For example, Kristina et al. demonstrated that IL-1β can disrupt postnatal lung morphogenesis in the mouse (454). Evidence suggested that acute infection and inflammation is associated with changes in triglycerides and lipoproteins (455), but it remains unclear if chronic low-grade inflammation is independently associated with increased triglycerides or not.

Strengths and Limitations

The major strengths of this study includes rigorous statistical methods and consideration of adjusting for clinically important confounding factors. Although there is relatively small number of babies in this study, we have been able to show the trend of associations using appropriate methods.

Conclusion

We found that the increased cord blood triglycerides observed in babies with a lower birthweight percentile are independently associated with increased cord blood CRP and IL-6 levels, but not with TNF-α. We did not observe increased proinflammatory cytokines in babies with higher birthweight percentile. Our findings suggest that babies with lower birthweight percentile are at risk of systemic low-grade inflammation, which might contribute to short- and long-term metabolic and cardiovascular dysfunctions.

Chapter 7 Discussion

In this chapter, I will summarize and interpret the key findings of my research. After that, I will discuss the strengths and limitations of this project and potential future investigations. The research within this thesis aimed to address: 1) the association between maternal lipid levels during pregnancy and adverse birthweight outcomes; 2) the interdependency between maternal metabolic risk factors and their association with birthweight and cord blood insulin concentration; 3) the association between birthweight and cord blood metabolic parameters; 4) the association of birthweight and cord blood triglycerides with cord blood pro-inflammatory cytokine levels.

7.1 Summary of findings

Other than for gestational dyslipidaemia, there is substantial evidence for an association between other maternal metabolic risk factors and adverse neonatal health outcomes (100, 102-104, 106, 111, 112, 130). Therefore, the purpose of Chapter 3 was to synthesize previous epidemiological studies on the association of maternal lipid levels during pregnancy with birthweight and neonatal metabolic parameters through robust review methodology and analyses. This systematic review found that maternal circulating low HDL-C and high TG levels throughout pregnancy are associated with increased birthweight. No evidence of association was observed between maternal TC/LDL-C and birthweight. Among mothers with pre-pregnancy obesity, maternal HDL-C and TG levels during pregnancy were found to have a more profound impact on birthweight. The review hardly found any studies that investigated the association between gestational dyslipidaemia and other neonatal metabolic parameters at birth or in early childhood.

Since metabolic risk factors are often tightly linked to one another, exploring the interdependency between them is critical for causal inference. All studies we identified in the

systematic review utilized classical statistical methods, which has limited capacity to analyse complex interdependent variables. Meanwhile, a robust individual patient data meta-analysis concluded that diet and lifestyle interventions in pregnancy only achieved modest success in reducing GWG and had no effect on composite maternal and foetal outcomes. Understanding how maternal metabolic risk factors influence neonatal health outcomes, and then identifying key metabolic pathways that lead to adverse health outcomes in neonates is crucial for developing future tailored interventions during preconception and pregnancy period to improve maternal and neonatal outcomes. Chapter 4 aimed to quantify the interdependency between maternal metabolic risk factors and their association with birthweight and cord blood insulin concentration using Additive Bayesian Network analysis, a data-driven causal inference model. In this study, maternal pre-pregnancy BMI was demonstrated to be the most influential upstream risk factor for both maternal and neonatal metabolic conditions, compared to GWG, gestational hyperglycaemia, and gestational dyslipidaemia. The results also highlight the key role of maternal hyperglycaemia towards increasing neonatal hyperinsulinemia that could result in foetal fat deposition. In contrast with the results of systematic review, maternal lipid levels appear to be secondary to maternal pre-pregnancy obesity with no clear links to metabolic adverse outcomes in neonates.

Babies born either SGA or LGA have an increased risk of developing obesity, diabetes, and cardiovascular diseases. The ectopic fat deposition was believed to be the key common feature for developing metabolic dysfunctions in both SGA and LGA babies (175). Little is known about whether SGA and LGA babies have similar metabolic characteristics that result in ectopic fat deposition and subsequent metabolic condition s in later life. Insulin is known to play a central role in glucose, lipids, and adipocyte regulation generally (187). Our previous results also suggested that increased neonatal insulin secretion might be driven by both maternal

glycaemic level and increased birthweight. To address the metabolic profiles in SGA and LGA babies, Chapter 5 aimed to address the association of birthweight with cord blood glucose, lipids, and insulin levels. In this study, SGA babies were found to have significantly higher cord blood TG and lower HDL-C, TC, and insulin levels. Meanwhile, LGA babies were found to have significantly higher cord blood insulin and lower TG levels.

Thereafter, we conducted an exploratory study (Chapter 6) to examine whether the altered birthweight or triglycerides would trigger low-grade inflammatory responses in neonates, which could lead to the development of systemic metabolic dysfunctions. This study found that increased cord blood TG levels and decreased birthweight are linearly associated with increased cord blood proinflammatory cytokines, specifically, IL-6 and CRP levels.

7.2 Interpretation and implications of findings

Research in context

It is well established that maternal high pre-pregnancy BMI, excessive GWG, and gestational hyperglycaemia are associated with an increased risk of a series of metabolic dysfunctions in offspring (100, 102-104, 106, 111, 112, 130). In the general population, people with obesity or diabetes were often found to have dyslipidaemia, which has been found to accelerate the progression of metabolic dysfunctions (263, 456). In 1985, Knopp et al. first proposed that fatty acids in maternal triglycerides might cross the placenta and contribute to foetal fat deposition (360). Results of subsequent several studies indicated that the altered maternal lipid levels during pregnancy might be a neglected strong determinant of foetal growth and that it may be as important as maternal glycaemic status (118, 166, 367). Existing systematic reviews concluded that maternal lipid levels during pregnancy are associated with an increased incidence of gestational diabetes, pre-eclampsia, and preterm delivery (108, 110, 331, 457). However, evidence regarding the association of maternal lipid levels during pregnancy with birthweight and metabolic dysfunctions in neonates was controversial. We first estimated the association of maternal lipid levels during pregnancy with neonatal birthweight and metabolic dysfunctions systematically. Data from 42 longitudinal studies firstly show that maternal HDL-C and TG levels throughout pregnancy are inversely and positively associated with neonatal birthweight respectively. However, due to the nature of the included studies, we were unable to demonstrate if gestational dyslipidaemia is an independent risk factor for increased birthweight. The vast majority of previous studies focused only on one specific maternal metabolic trait or on the number of metabolic disorders, but neglected the underlying interacted effect of the natural metabolic network. To address the interdependency of maternal metabolic risk factors and their association with birthweight and cord blood insulin level appropriately, a traditional

multivariable regression model followed by the Additive Bayesian Network (ABN) methods were used in Chapter 4. The results of the multivariable regression model are in line with our previous findings in Chapter 3. In contrast to the conclusion of our systematic review, the results of ABN demonstrated that the altered maternal lipid profiles during pregnancy are likely to be measures of gestational metabolic dysfunctions, and not themselves involved in the pathway relevant to adverse birthweight and cord blood insulin levels. Although this result challenges the conclusions of most previous observational studies, it is consistent with the result of a large Mendelian randomizations study (another causal inference design) (410). Meanwhile, ABN indicated that maternal high pre-pregnancy BMI is the most influential upstream risk factor for both maternal and neonatal health outcomes, while gestational weight gain seems only moderately associated with increased birthweight but not with cord blood insulin. Similar results were also found in the latest individual-patient-data meta-analysis published in The Journal of the American Medical Association (JAMA) (458). This meta-analysis began with the association between GWG and adverse pregnancy outcomes, but ultimately emphasized the importance of pre-pregnancy BMI (458). To the best of our knowledge, our study is the first one to date that weighs all maternal key metabolic risk factors systematically. The conflicting results of these two models also indicate the importance of using appropriate methods in future research to address the causal relationship in complex causal pathways.

In adults, people with a higher BMI often have higher lipid levels and impaired glucose tolerance and are at a higher risk of metabolic dysfunctions (e.g. insulin resistance and high blood pressure) (459, 460). The metabolic profiles in neonates are largely unknown in previous literature. We hypothesised that babies with a higher birthweight may have metabolic profiles similar to those of adults. Neonates born to either SGA or LGA are at a higher risk of developing metabolic dysfunction subsequently, but the underlying mechanisms remain unclear. Ectopic

fat deposition is thought to be a common pathway to further metabolic dysfunctions for both SGA and LGA babies (175). Similar to obese adults, it is believed that the lipid accumulation in LGA babies might exceed the buffering capacity of adipocyte, therefore contributing to the development of metabolic dysfunctions (461). In Chapter 5, we observed an elevated cord blood insulin level, but not triglycerides levels, in LGA babies. The results show that the expanded fat mass in LGA babies, in the presence of insulin, buffers lipid accumulation in adipocytes. It indicates that moderately expanded adipose tissue might play an important role in preventing adverse metabolic health outcomes by absorbing oversupplied nutrients. The ectopic fat deposition in SGA babies is attributed to catch-up-growth previously (462, 463). However, it is hard to explain why SGA babies with moderate catch-up-growth still show impaired insulin sensitivity in their childhood (176, 464). Previous in vivo evidence proposed that IUGR universally experienced by SGA babies may be linked to reduced pancreatic β-cell mass, leading to insulin deficiency and glucose insensitivity (465). The results in Chapter 5 supports this hypothesis. Apart from the reduced pancreatic β-cell replication, SGA babies are often deficit in skeletal muscle and absolute fat mass, which could partly be explained by low nutrition utilization rate induced by insulin deficiency. In SGA babies with or without catch-up growth, impaired glucose sensitivity in their subsequent life has been frequently observed (176). In light of this, the amount of fat mass impaired by IUGR may partially determine its expansion capacity in early life. Therefore, it is possible that lipotoxicity together with the impaired capacity of adipocyte expansion results in ectopic fat deposition, leading to further metabolic dysfunctions in SGA babies.

Chapter 6 further explored whether increased birthweight or triglycerides would induce low-grade inflammation. The results indicate that elevated triglycerides observed in SGA babies are strongly associated with increased cord blood CRP and IL-6 levels, which might contribute to

the development of metabolic dysfunctions. In LGA babies, the expanded adipose tissue does not induce low-grade inflammatory responses. The findings in Chapter 6 further emphasises that the differential metabolic risk of LGA and SGA babies would contribute to their short- or long-term metabolic health in different ways.

In summary, maternal pre-pregnancy BMI and gestational hyperglycaemia are the most critical metabolic risk factors for neonatal insulin secretion and fat deposition, whereas the differential insulin, lipids, and proinflammatory cytokines observed in SGA and LGA babies may contribute to future metabolic dysfunctions through different mechanisms. The potential mechanism pathway is summarised in ***Figure 11***.

Implication to practice

Although it is already known that maternal pre-pregnancy BMI is associated with both maternal and neonatal health conditions, most current clinical recommendations on maternal weight management focus only on antenatal period (28, 78, 83). People used to believe that weight management during pregnancy may help to improve adverse pregnancy outcomes. However, our results and other recent robust studies demonstrate that pre-pregnancy BMI is the most influential metabolic risk factors for adverse pregnancy outcomes, while gestational weight gain may not have a profound impact as previously expected (303, 458). We are missing an optimal opportunity to prevent subsequent adverse health outcomes in both mothers and babies. This situation is partially because of the gaps in services for women in child-bearing age and lack of contact with health professionals in the preconception period. This gap could be filled by prenatal consultation in general practices or health education and promotion delivered by public health professionals in communities. Our results, if applied to wider communities, provide further evidence for public health measures at improving weight management in

women in general and particularly those of child-bearing age. Moreover, unlike previous evidence, our results indicate that maternal lipid levels during pregnancy are not involved in the pathway towards birthweight and neonatal insulin secretion. Although maternal lipid levels in pregnancy are secondary to pre-pregnancy BMI, it can potentially be a clinical marker of maternal metabolic status for predicting birthweight.

Although babies born either SGA or LGA are linked to adverse health outcomes, there is no specific guidelines or recommendations on how to feed them. The metabolic profiles in SGA and LGA babies are largely unknown in current literature. Therefore, it could not reliably inform clinical practice and recommendations to avoid subsequent metabolic dysfunctions in babies. We are the first study to date that addressed the metabolic profiles of SGA and LGA babies in a detailed and rigorous way. The robust evidence that we provided would help to uncover tailored intervention strategies to prevent subsequent metabolic dysfunctions in SGA and LGA babies.

7.3 Strengths and limitations

In the systematic review, a comprehensive search was conducted to capture the majority of studies on the association between maternal lipid levels during pregnancy and adverse birthweight outcomes. We also took special care on handling missing data by contacting the authors to minimize reporting bias. Since the results of included studies were reported in various formats, a novel and thorough approach was used to utilize every piece of existing evidence. The inclusion of longitudinal data allowed us to address the temporal association between maternal lipid levels in three trimesters and neonatal outcomes. The major limitation of the systematic review was the substantial heterogeneity of included studies on settings, populations, and covariates adjustments, which was addressed by the subgroup analysis. Due to the nature of the individual study, it is not feasible to explore whether the effects of gestational dyslipidaemia are independent of other important maternal metabolic factors, such as maternal pre-pregnancy BMI, gestational hyperglycaemia, as well as GWG. Therefore, we conducted a primary study to address this issue.

The study in Chapter 4 is based on a large birth cohort study, the prospective design of which allows us to investigate the temporal relationship between maternal metabolic risk factors and neonatal outcomes. The two-way analytic approaches, multivariable regression model (hypothesis-driven) and additive Bayesian Network analysis (data-driven), were used to address the independent association between maternal metabolic risk factors and neonatal outcomes appropriately. Given the practical constraints, the maternal fasting glucose and triglycerides levels were measured only once in the second trimester. The one-point measurement does not allow us to investigate the dynamic long-term influences of maternal metabolic risk factors and limit our ability in exploring feedback loop between nodes in Bayesian Network analysis. The information on maternal pre-pregnancy BMI was self-reported,

which might potentially underestimate the true value. However, evidence suggest that utilization of self-reported or measured pre-pregnancy weight for pre-pregnancy BMI classification results in identical categorization for most women (466).

The major strength of the Chapter 5 is the comprehensive analyses based on longitudinal data. In this study, the utilization of generalized additive model allows us to investigate the non-linear relationships between birthweight and cord blood metabolic parameters. Given the practical constraints, we could not measure the distribution of fat mass in neonates. Instead of fat mass, birthweight percentile was used as an approximate measurement to improve the generalizability of our results. The relatively small sample size is the major limitation of the exploratory study. However, we were still able to observe the direction of the associations suggesting inflammatory pathway may play a role in the long-term metabolic risk observed in SGA babies.

7.4 Future investigations

Missed opportunity for prevention of adverse pregnancy outcomes in preconception period

In my study, pre-pregnancy BMI was found to be the most influential upstream risk factor for both maternal and neonatal metabolic health conditions. Therefore, interventions should focus more on tackling weight management in the preconception period. However, very few previous trials have attempted to target the preconception period for improving maternal weight management. Future intervention studies on weight management of women in child-bearing age are needed to prevent subsequent adverse pregnancy outcomes. Before implementing pre-pregnancy weight management interventions, it is critical to understand the potential barriers and facilitators among women and health professionals in the communities to develop appropriate strategies. It is likely to be a multifaceted intervention involving women with overweight/obesity, public health professionals, general practices, clinicians, community staffs, and policymakers at different stages. The culture differences and ethical issues in the barriers to implementing weight management need to be taken into consideration. In addition to assessing the effects of pre-pregnancy weight management on preventing adverse pregnancy outcomes, the cost-effectiveness of the intervention strategy should also be considered.

Future research on maternal lipid levels during pregnancy

Although we found that maternal lipid levels during pregnancy may not have a causal relationship with neonatal metabolic conditions at birth, we still do not know whether maternal lipid levels will have a profound long-term influence on neonates in childhood/adulthood through epigenetic programming. A two-steps Mendelian Randomization study might be needed in the future to examine the potential epigenetic programming effects of maternal lipid

levels during pregnancy on neonatal metabolic status. Meanwhile, maternal lipid levels can be a potential clinical marker for maternal metabolic condition or for predicting neonatal size, due to its strong correlation with increased risk of gestational diabetes, preeclampsia, and increased birthweight. Therefore, it is still worthwhile to add maternal lipid levels during pregnancy to the clinical prediction models for adverse pregnancy outcomes in future studies.

Tailored intervention strategies for prevention of further metabolic dysfunctions in babies born SGA and LGA

Based on the differential metabolic profiles that we found in babies born SGA and LGA, tailored interventions strategies for preventing further metabolic dysfunctions in babies with low or high birthweight is needed. Although breastfeeding if preferable for all babies, special care might be needed for improving metabolic profile of SGA or LGA babies to prevent subsequent metabolic dysfunctions, especially for those babies who can not get or take human milk. Omega-3 fatty acids supplements, such Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), may help to improve lipid profiles, therefore might be a potential intervention for babies born SGA (467). A recent study published in the Journal of Clinical Investigation identified a bioactive component named alkylglycerols in human breast milk that could maintain beige adipose tissue (BeAT) and prevent the transdifferentiation from BeAT to white adipose tissue (468). Therefore, it might be worthwhile to explore the association between the duration of breastfeeding and childhood obesity in babies born LGA for future research.

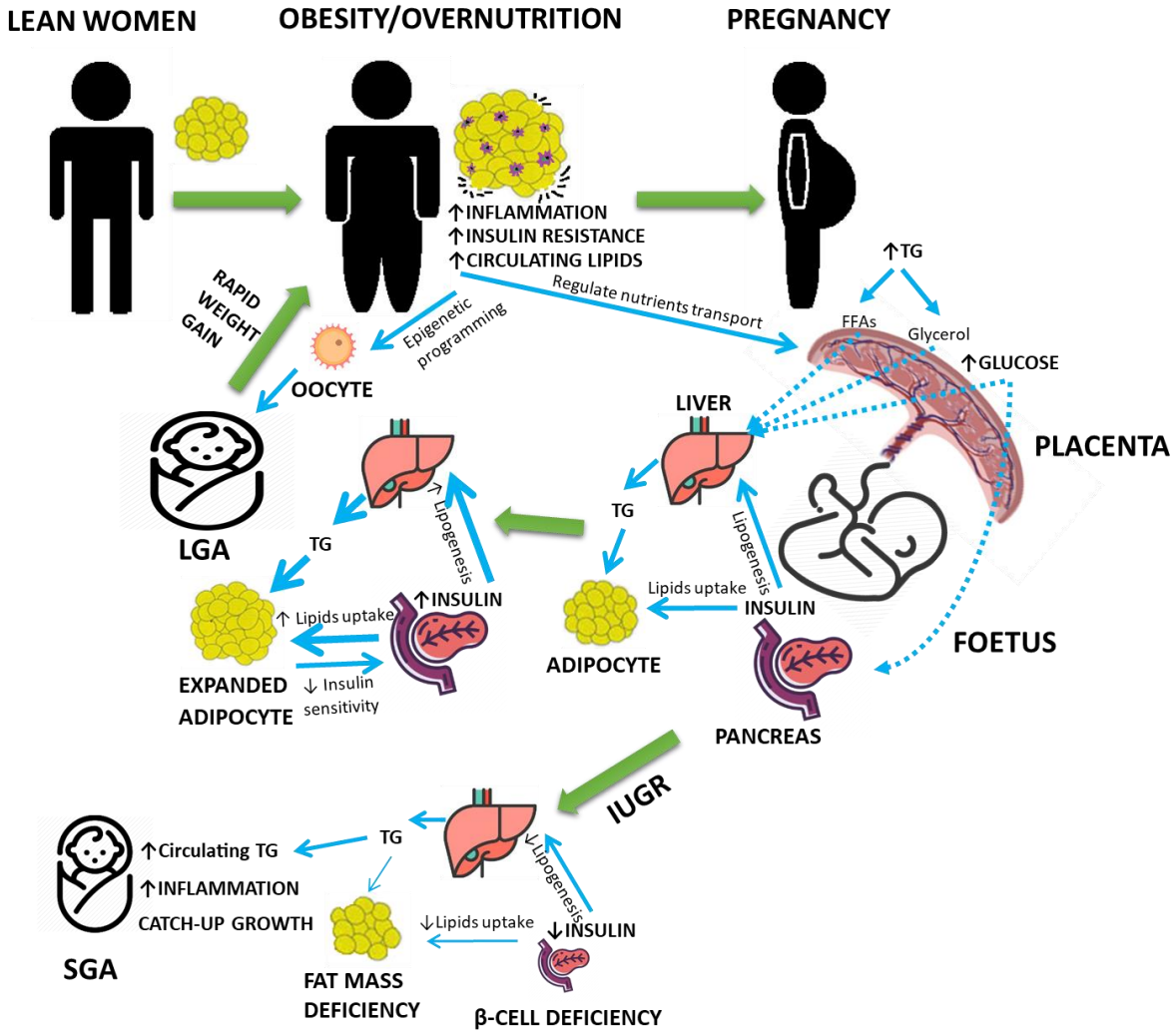


Figure 11 Diagrammatic portrayal of metabolic components in mothers and babies.

The enlarged adipose tissue in women with obesity could induce systemic low-grade inflammation, insulin resistance, and increased circulating lipid levels, especially triglycerides (TG). All these may jointly influence the development and production of the oocyte in the pre-conception period through epigenetic programming. Pregnant women with obesity are often found to have increased TG and glucose levels. The quality and quantity of nutrients transported through the placenta are also regulated by maternal obesity. The de novo synthesized insulin in foetus induce lipogenesis in the liver using nutrients transported from the maternal side and promote lipids uptake in the adipocyte. The expanded adipocyte become less insulin sensitivity thereafter inducing a state of hyperinsulinemia in large-for-gestational-age (LGA) babies. The adipose buffering capacity of LGA babies is close to saturation point, so if they experience rapid growth, they have a higher risk of obesity in adulthood. In intrauterine growth restriction (IUGR) pregnancy, the deficiency of fat mass and β-cell could jointly induce low insulin secretion and high circulating TG levels. The increased circulating TG, low-grade inflammatory response, and catch-up growth observed in SGA babies, may jointly contribute to the development of metabolic dysfunctions in future.

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Supplementary material

S1 Systematic review: Sample search in MEDLINE

1. exp Lipids/ or lipid$.mp.

2. lipoprotein$.mp. or exp Lipoproteins/

3. exp Fatty Acids/ or fat* acids.mp.

4. triglycerides.mp. or exp Triglycerides/

5. exp Lipoproteins, VLDL/ or exp Cholesterol, VLDL/ or VLDL.mp.

6. LDL.mp. or exp Cholesterol, LDL/ or exp Lipoproteins, LDL/

7. IDL.mp. or exp Lipoproteins, IDL/

8. exp Lipoproteins, HDL/ or exp Cholesterol, HDL/ or HDL.mp.

9. exp Cholesterol/ or cholesterol.mp. or exp Cholesterol Esters/

10. hyperlipid?emia$.mp. or exp Hyperlipidemias/

11. dyslipid?emia$.mp. or exp Dyslipidemias/

12. hypertriglycerid?emia$.mp. or exp Hypertriglyceridemia/

13. hypercholesterol?emia.mp. or exp Hypercholesterolemia/

14. metabolic.mp.

15. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14

16. exp Maternal Health/ or maternal.mp.

17. exp Pregnanes/ or pregnan*.mp.

18. exp Pregnancy/ or gestation*.mp.

19. gravidity.mp. or exp Gravidity/

20. mother$.mp. or exp Mothers/

21. 16 or 17 or 18 or 19 or 20

22. (birth weight or birthweight).mp. or exp Birth Weight/ or exp Infant, Low Birth Weight/

23. overweight.mp. or exp Obesity/ or exp Overweight/ or exp Body Weight/

24. (SGA or Small for gestational age).mp. or exp Infant, Small for Gestational Age/

25. (LGA or Large for gestational age).mp.

26. exp Fetal Macrosomia/ or macrosomia.mp.

27. exp "Growth and Development"/ or exp Growth/ or (growth or development).mp. or exp Fetal Growth Retardation/ or exp Fetal Development/ or exp Child Development/

28. weight gain.mp. or exp Weight Gain/

29. (hyperglyc?emia or hypoglyc?emia).mp. or exp Hyperglycemia/ or exp Hypoglycemia/

30. (insulin* or hyperinsulinism or IR).mp. or exp Insulin/ or exp Insulin Resistance/ or exp Hyperinsulinism/

31. exp Glucose Intolerance/ or glucose.mp. or exp Glucose/ or exp Glucose Metabolism Disorders/

32. skinfold thickness.mp. or exp Skinfold Thickness/

33. (monocyte chemoattractant protein-1 or MCP-1).mp.

34. (interleukin 6 or IL-6).mp.

35. exp Tumor Necrosis Factor-alpha/ or tumour necrosis factor-alpha.mp.

36. exp 11-beta-Hydroxysteroid Dehydrogenase Type 1/ or HSD1.mp.

37. exp Leptin/ or leptin.mp.

38. exp Inflammation/ or inflammat*.mp.

39. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38

40. (neonatal or fetal or foetal or fetus or foetus or infant or offspring or new born).mp. or exp Infant/

41. 15 and 21 and (39 and 40)

42. (animal or mouse or mice or rodent or sheep or mutton or pig or hoggory or hog or swine or rabbit$).mp.

43. 41 not 42

44. cohort studies/ or longitudinal studies/ or follow-up studies/ or prospective studies/ or retrospective studies/ or cohort.ti,ab. or longitudinal.ti,ab. or prospective.ti,ab. or retrospective.ti,ab.

45. "randomized controlled trial".pt.

46. (random$ or placebo$ or single blind$ or double blind$ or triple blind$).ti,ab.

47. (retraction of publication or retracted publication).pt.

48. or/44-47

49. (animals not humans).sh.

50. ((comment or editorial or meta-analysis or practice-guideline or review or letter or journal correspondence) not "randomized controlled trial").pt.

51. (random sampl$ or random digit$ or random effect$ or random survey or random regression).ti,ab. not "randomized controlled trial".pt.

52. or/49-51

53. 48 not 52

54. 43 and 53

S2 Systematic review: Data extraction form

A. Reference information

1. ID number
2. Title
3. Author
4. Journal
5. Publication Year
6. Language
7. Sponsor

B. Study design

1. Study design
2. Setting
3. Locations
4. Data collection

C. Participants

1. Eligibility criteria (source and methods of selection of participants)
2. Matching criteria (if applicable)
    a. Matching criteria
    b. Attempts were made within the design or analysis to balance the comparison groups for potential confounders (YES/NO).
    c. The groups are comparable at baseline, including all major confounding and prognostic factors (YES/NO).
3. Sample Size
    a. Number of both exposed and unexposed groups

b. Report numbers of individuals at each stage of study

c. Give reasons for non-participation at each stage (YES/NO)

d. Does the size of samples have enough power to detect the difference of primary outcomes? (YES/NO)

4. Demographic, clinical and social characteristics

a. Age

b. Ethnicity

c. Pre-pregnant BMI/weight

d. Marital status

e. Education

f. Other potential confounders information

D. Follow-up

1. Enrolment time

2. Length of follow-up

a. Length of follow-up (average and total amount)

b. All groups were followed up for an equal length of time (or analysis was adjusted to allow for differences in length of follow-up)

3. Methods of follow-up

4. Lost to follow-up

a. Attrition rate in each group

b. How many participants in each group were no outcome data available? (number & proportion)

c. Does it comparable? (YES/NO)

E. Exposure

1. Definition of exposures

2. When did they take samples

3. Exposure measurement

F. Outcomes

1. Primary outcomes (definition and measurement)

2. Secondary outcomes (definition and measurement)

G. Statistical methods

1. Statistical methods, including those used to control for confounding

2. Describe any methods used to examine subgroups and interactions

3. How missing data were addressed

4. Explain how lost to follow-up was addressed

5. Describe any sensitivity analysis

H. Results

1. Number of outcomes events or summary measures over time

2. Give unadjusted estimates and, if applicable, confound der-adjusted estimates and their precision (e.g. 95% confidence interval). Make clear which confounders were adjusted for and why they were included

3. Report category boundaries when continuous variables were categorized

4. Alpha value and beta value

I. Limitations

1. Interpretation

Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.

2. Generalizability (external validity)

J. Other notes

S3 Systematic review: Newcastle-Ottawa Scale

Selection

1. Representativeness of exposed cohort population

1) Truly representative of the average, community-dwelling target pregnant women ★

2) Somewhat representative of the average, community-dwelling target pregnant women★

3) Selected group of pregnant women, e.g. only certain socio-economic groups/areas

4) No description of the derivation of the cohort

2. Selection of the unexposed cohort

1) Drawn from the same source as the exposed cohort★

2) Drawn from a different source

3) No description of the derivation of the unexposed cohort

3. Ascertainment of exposures

1) Laboratory diagnosed ★

2) Secure record (e.g. health care/clinical record) ★

3) Written self-report

4) Other/ no description

4. Demonstration that outcome of interest was not present at start of study

1) Yes★

2) No

Comparability

1. Comparability of cohort based on the design or analysis

1) Study controls for

① Outcomes measured at delivery: gestational age ★

② Outcomes measured over 1 month after delivery: neonatal age ★

2) Study controls for any two of additional factors (e.g. neonatal gender, maternal age, parity, socio-economic level, cigarette exposures, delivery mode and so on) ★

Outcome

1. Assessment of outcomes

1) Independent blind assessment★

2) Record linkage★

3) Self-report

4) Other/ no description

2. Was follow up long enough for outcomes to occur

1) Yes, if the study follow their subjects until outcomes occur★

2) No, if the study follow their subjects until outcomes occur

3. Adequacy of follow up of cohorts

1) Complete follow up : all subjects accounted for★

2) Subjects lost to follow up unlikely to introduce bias: number lost <= 20%, or description of those lost suggesting no different from those followed★

3) Follow up rate <80% and no description of those lost

4) No statement

S4 Systematic review: Quality assessment form

Study ID	Selection				Comparability		Outcome			Overall Score
	A1	A2	A3	A4	B1	B2	C1	C2	C3	
Harmon et al.2011	0	1	1	1	0	0	0	1	1	5
Son et al.2010	0	1	1	1	0	0	0	1	1	5
Di et al.2005	0	1	1	1	0	0	0	1	1	5
Schaefer-Graf et al.2008	0	1	1	1	0	0	0	1	1	5
Slagjana et al.2014	0	1	1	1	0	0	0	1	1	5
Zhou et al.2012	0	1	1	1	0	0	0	1	1	5
Zawiejska et al.2008	0	1	1	1	0	0	0	1	1	5
Emet et al.2013	0	1	1	1	0	0	0	1	1	5
Schaefer-Graf et al.2011	0	1	1	1	0	0	0	1	1	5
Mossayebi et al.2014	0	1	1	1	0	0	0	1	1	5
Swierzewska et al.2015	0	1	1	1	0	0	0	1	1	5
Ortega et al.1996	0	1	1	1	0	0	0	1	1	5
Alberti-Fidanza et al.1995	0	1	1	1	0	0	1	1	0	5
Charles et al. 2016	0	1	1	1	0	0	0	1	1	5
Wang et al.2015	0	1	1	1	1	0	0	1	1	6
Ahmad et al.2006	0	1	1	1	1	0	0	1	1	6

Whyte et al. 2013	0	1	1	1	0	0	1	1	1	6
Vinod et al. 2011	0	1	1	1	1	0	0	1	1	6
Olmos et al.2014	0	1	1	1	1	0	0	1	1	6
Knopp et al.1992	0	1	1	1	1	0	0	1	1	6
Nolan et al.1995	0	1	1	1	1	0	0	1	1	6
Friis et al.2012	0	1	1	1	1	0	0	1	1	6
Lei et al.2016	0	1	1	1	1	0	0	1	1	6
Kitajima et al.2001	0	1	1	1	1	0	0	1	1	6
Couch et al.1998	0	1	1	1	0	0	1	1	1	6
Brockerhoff 1986	0	1	1	1	0	0	1	1	1	6
Retnakaran et al.2012	0	1	1	1	1	1	0	1	1	7
Hou et al.2014	0	1	1	1	1	1	0	1	1	7
Laleh et al.2013	0	1	1	1	1	1	0	1	1	7
Liu et al.2016	0	1	1	1	1	0	1	1	1	7
Brunner et al.2013	0	1	1	1	1	0	1	1	1	7
Knopp et al.1985	0	1	1	1	1	0	1	1	1	7
Geraghty et al.2016	0	1	1	1	1	1	0	1	1	7
Jin et al.2016	0	1	1	1	1	1	0	1	1	7
Robin et al. 2007	0	1	1	1	1	1	0	1	1	7
Ye et al.2015	0	1	1	1	1	1	1	1	1	8
Crume et al.2015	0	1	1	1	1	1	1	1	1	8
Hwang et al.2015	0	1	1	1	1	1	1	1	1	8
Kulkarni et al.2013	1	1	1	1	0	1	1	1	1	8
Vrijkotte et al.2012	0	1	1	1	1	1	1	1	1	8

Kramer et al.2014	0	1	1	1	1	1	1	1	1	8
Vrijkotte et al. 2011	0	1	1	1	1	1	1	1	1	8
Clausen et al.2005	1	1	1	1	0	1	1	1	1	8
Mathews et al.2003	0	1	1	1	1	1	1	1	1	8
Sommer et al.2015	1	1	1	1	1	1	1	1	1	9

S5 Systematic review: Data analysis for birthweight

Data summary

S7.1 Table Results summary of the association of maternal lipid levels with birthweight throughout pregnancy

Maternal lipids	Trimester	Negative associations	No direction	Positive associations	Total
TC	The first trimester	1	1	2(1)	4
	The second trimester	1	4	7(2)	12
	The third trimester	3(1)	12	8(3)	23
HDL-C	The first trimester	2(1)	0	0	2
	The second trimester	6(2)	4	1	11
	The third trimester	11(6)	6	1	18
LDL-C	The first trimester	1	0	1	2
	The second trimester	1	5	2	8
	The third trimester	2	5	7(3)	15
TG	The first trimester	0	1	4(3)	5
	The second trimester	0	2	10(8)	12
	The third trimester	3(1)	4	20(14)	27
VLDL	The first trimester	0	0	0	0
	The second trimester	0	0	0	0
	The third trimester	0	1	1	2
FFAs	The first trimester	0	1	0	1

	The second trimester	0	0	1	1
	The third trimester	0	3	4(2)	7

1. This table summarised the results distribution of studies that reported the association of maternal lipid levels with birthweight throughout pregnancy;
2. Number in this table represent the number of studies;
3. 'No direction' means that the number of studies reported statistically insignificant results without its direction, as well as the number of studies did not report their results;
4. Number in the bracket means the number of studies reported statistically significant results;
5. Abbreviation: Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), triglycerides (TG) and free fatty acids (FFAs).

Total cholesterol (TC)

S7.2 Table Results summary of the association of maternal TC level with birthweight

ID	Population	Countries	Sample size	Tri.	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors							
												a	b	c	d	e	f	g	h
Vinod et al.2011(1)	Normal weight	USA	65	1	Crude β	-19.33	-120.03	81.36	ND	SLR	6	×	×	×	×	×	×	×	×
Vinod et al.2011(2)	Overweight/obese	USA	71	1	Crude β	58.00	-67.86	183.87	ND	SLR	6	×	×	×	×	×	×	×	×
Vrijkotte et al.2011	General	Netherlands	2,052	1	Crude β	11.82	-10.00	33.65	ND	Univariate analyses	8	√	√	×	×	×	×	√	×
Vrijkotte et al.2011	General	Netherlands	2,052	1	Adjusted β	**22.67**	**4.00**	**41.33**	ND	MLR	8	√	√	√	√	√	×	√	×
Nolan et al.1995	General	Australia	388	1	ND	ND			ND	ND	6	ND	ND	ND	ND	ND	ND	×	ND
Liu et al.2016	General	China	1,546	2	r	0.02			0.518	Partial correlation	7	×	×	×	×	×	×	×	×
Vinod et al.2011(1)	Normal weight	USA	71	2	Crude β	-50.27	-112.24	11.69	ND	SLR	6	×	×	×	×	×	×	×	×
Vinod et al.2011(2)	Overweight/obese	USA	71	2	Crude β	3.87	-91.02	98.75	ND	SLR	6	×	×	×	×	×	×	×	×
Mathews et al.2003	General	UK	733	2	Adjusted β	**30.10**	**1.21**	**58.90**	ND	MLR	8	√	√	×	×	×	×	√	×
Crume et al.2015	General	USA	804	2	Adjusted β	17.79	-11.82	47.39	0.200	MLR	8	√	√	√	×	×	×	√	×
Kulkarni et al.2013	non-GDM	India	631	2	Adjusted β	**39.07**	**10.57**	**67.58**	ND	MLR	8	×	√	√	√	×	×	√	×

ID	Population	Countries	Sample size	Tri.	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors							
												a	b	c	d	e	f	g	h
Geraghty et al.2016	non-GDM	UK	331	2	Adjusted β	27.87	-17.89	73.63	ND	MLR	7	√	√	×	√	√	×	×	×
Whyte et al. 2013	General	Ireland	189	2	ND	ND			ND	ND	6	ND	ND	ND	ND	ND	ND	×	ND
Wang et al.2015	General	China	636	2	ND	ND			ND	Partial correlation	6	√	√	×	×	×	×	×	×
Di et al.2005	OGTT+	Italy	83	2	ND	ND			ND	ND	5	ND	ND	ND	ND	ND	ND	×	ND
Olmos et al.2014	GDM	Chile	279	2	ND	ND			ND	ND	6	ND	ND	ND	ND	ND	ND	×	ND
Mossayebi et al.2014	General	Iran	154	3	r	**0.50**			**<0.001**	Pearson correlation	5	×	×	×	×	×	×	√	×
Charles et al. 2016	General	Multiple	1062	3	r	**-0.103**			**<0.0001**	Pearson correlation	4	×	×	×	×	×	×	×	×
Ahmad et al. 2006	non-GDM	Malaysia	246	3	r	**0.16**			**0.021**	Univariate analyses	6	√	×	×	×	×	×	√	×
Kitajima et al.2001	OGTT +	Japan	146	3	r	0.01			0.990	SLR	6	×	×	×	×	×	×	√	×
Vinod et al.2011(1)	Normal weight	USA	69	3	Crude β	-46.40	-118.05	25.24	ND	SLR	6	×	×	×	×	×	×	×	×
Vinod et al.2011(2)	Overweight/obese	USA	70	3	Crude β	15.47	-89.10	120.03	ND	SLR	6	×	×	×	×	×	×	×	×
Sommer et al.2015	General	Norway	699	3	Crude β	-4.20	-39.40	31.00	ND	SLR	9	×	×	×	×	×	×	√	×

ID	Population	Countries	Sample size	Tri.	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors							
												a	b	c	d	e	f	g	h
Sommer et al.2015	General	Norway	699	3	Adjusted β	-6.10	-37.50	25.20	ND	MLR	9	√	√	√	×	×	×	√	×
Mathews et al.2003	General	UK	537	3	Adjusted β	11.10	-18.00	40.30	ND	MLR	8	√	√	×	×	×	×	√	×
Ye et al.2015	non-GDM	China	1,243	3	Adjusted β	9.10	-6.40	24.60	ND	MLR	8	√	√	√	√	√	√	√	×
Kulkarni et al.2013	non-GDM	India	631	3	Adjusted β	**54.34**	**24.85**	**83.88**	ND	MLR	8	×	√	√	√	×	×	√	×
Geraghty et al.2016	non-GDM	UK	331	3	Adjusted β	24.85	-9.39	59.09	ND	MLR	7	√	√	×	√	√	×	×	×
Couch et al.1998	General	USA	40	3	p	ND			>0.05	Pearson correlation	6	×	×	×	×	×	×	×	×
Ortega et al.1996	General	Spain	292	3	p	ND			>0.05	Student t test	5	×	×	×	×	×	×	√	×
Swierzewska et al.2015	General	Poland	136	3	p	ND			>0.05	MLR	5	ND	ND	ND	ND	ND	ND	×	ND
Emet et al.2013	General	Turkey	801	3	p	ND			0.616	Pearson correlation	5	×	×	×	×	×	×	×	×
Friis et al.2012	General	German	207	3	p	ND			>0.05	MLR	6	√	×	×	×	×	×	×	×
Retnakaran et al.2012	non-GDM	Canada	472	3	p				0.500	Analysis of variance for continuous variables	7	×	×	×	×	×	×	×	×
Schaefer-Graf et al.2011	non-GDM	German	190	3	p	ND			>0.05	Pearson correlation	5	×	×	×	×	×	×	√	×
Son et al.2010	GDM	Korea	104	3	p	ND			>0.05	ND	5	ND	ND	ND	ND	ND	ND	√	ND
Crume et al.2015	General	USA	804	3	ND	ND			ND	MLR	8	√	√	√	×	×	×	√	×
Slagjana et al.2014	non-GDM	Yugoslavia	200	3	ND	ND			ND	ND	5	ND	ND	ND	ND	ND	ND	×	ND
Olmos et al.2014	GDM	Chile	279	3	ND	ND			ND	ND	6	ND	ND	ND	ND	ND	ND	×	ND
Schaefer-Graf et al.2008	GDM	German	150	3	ND	ND			ND	Spearman correlation	5	×	×	×	×	×	×	×	×

ID	Population	Countries	Sample size	Tri.	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors							
												a	b	c	d	e	f	g	h
Robin et al. 2007	General	American	957	2		Adjusted MD(g)			p	MLR	7	√	√	√	×	×	×	√	×
					High-TC group (n=100)	Ref group			Ref group										
					Mid-TC group(n=757)	29			0.47										
					Low-TC group(n=100)	-150			0.001										

The bold font represents statistically significant results.

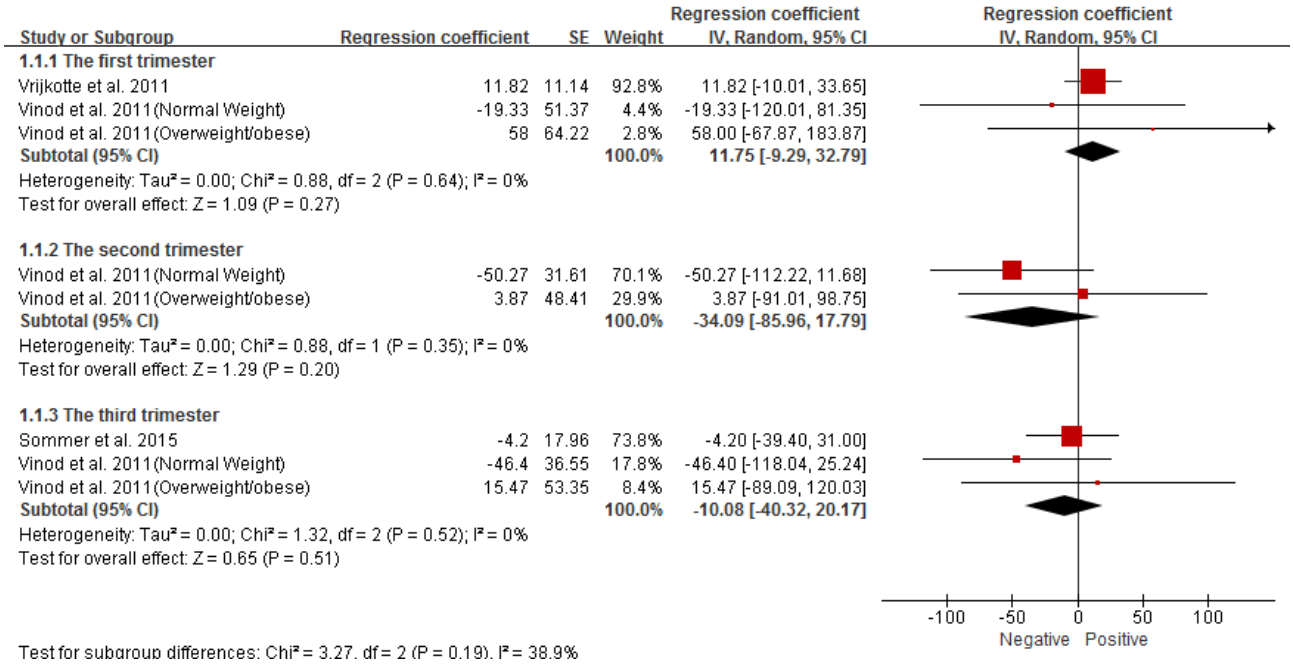
r: Correlation coefficients; β: regression coefficients.

Confounding factors: a. Gestational age; b. Neonatal gender; c. Maternal age; d. Pre-pregnancy BMI; e. Gestational weight gain; f. Maternal glucose level; g. pre-term birth; h. Maternal lipid levels.

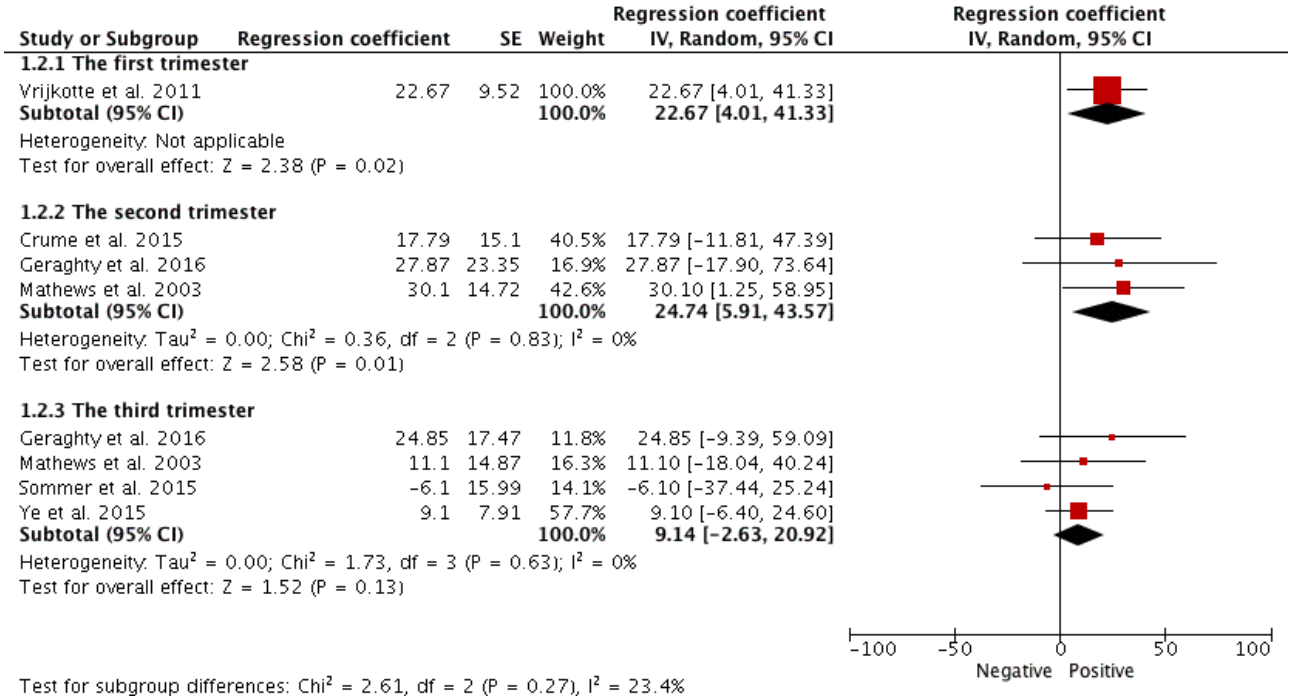
Abbreviation: Trimesters(Tri.), Gestational diabetes mellitus(GDM), Positive screenes of Oral Glucose Tolerance test(OGTT+), Confidence interval(CI), Not documented(ND), Simple linear regression(SLR), Multiple linear regression(MLR), United Kingdom(UK), Mean difference(MD), Reference(Ref).

Meta-analysis

S7.1 Figure Overall meta-analysis of crude regression coefficients for the association between maternal TC levels and birthweight throughout pregnancy

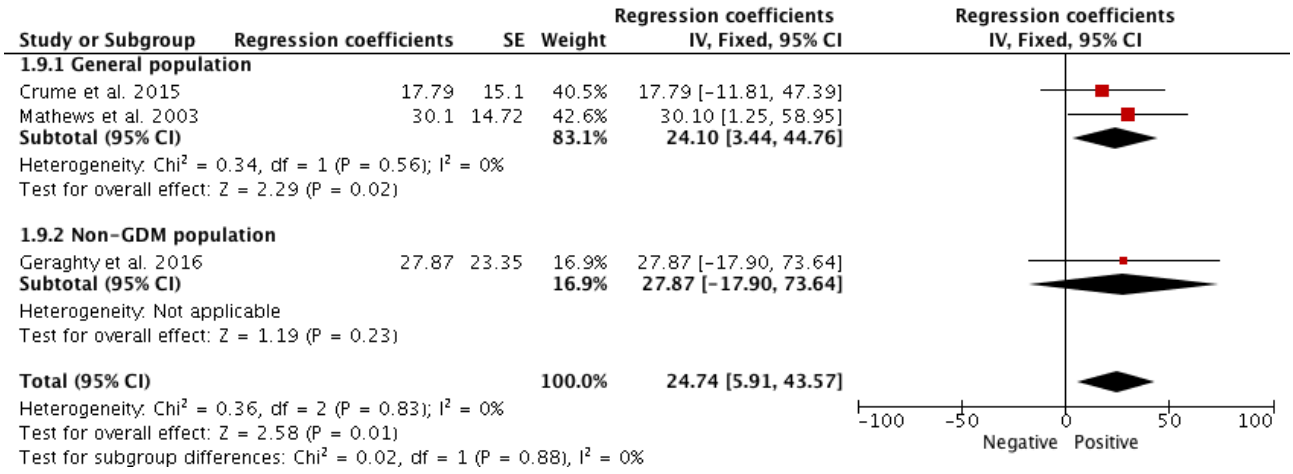


S7.2 Figure Overall meta-analysis of adjusted regression coefficients for the association between maternal TC levels and birthweight throughout pregnancy

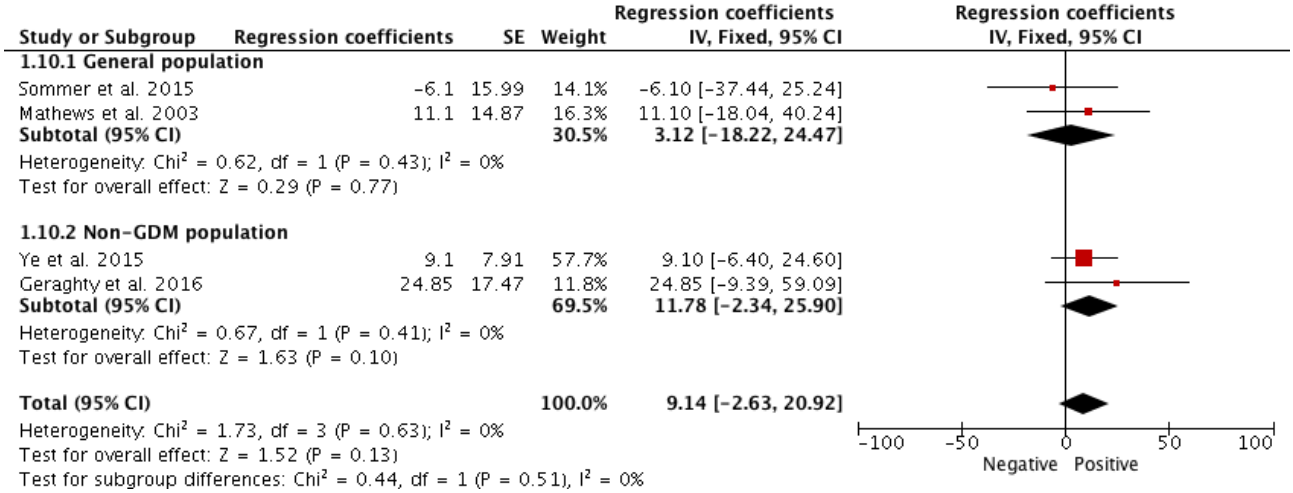

Study or Subgroup
Regression coefficient
SE
Weight
Regression coefficient IV, Random, 95% CI
Regression coefficient IV, Random, 95% CI
1.2.1 The first trimester
Vrijkotte et al. 2011 22.67 9.52 100.0% 22.67 [4.01, 41.33]
Subtotal (95% CI) 100.0% 22.67 [4.01, 41.33]
Heterogeneity: Not applicable
Test for overall effect: Z = 2.38 (P = 0.02)
1.2.2 The second trimester
Crume et al. 2015 17.79 15.1 40.5% 17.79 [-11.81, 47.39]
Geraghty et al. 2016 27.87 23.35 16.9% 27.87 [-17.90, 73.64]
Mathews et al. 2003 30.1 14.72 42.6% 30.10 [1.25, 58.95]
Subtotal (95% CI) 100.0% 24.74 [5.91, 43.57]
Heterogeneity: Tau² = 0.00; Chi² = 0.36, df = 2 (P = 0.83); I² = 0%
Test for overall effect: Z = 2.58 (P = 0.01)
1.2.3 The third trimester
Geraghty et al. 2016 24.85 17.47 11.8% 24.85 [-9.39, 59.09]
Mathews et al. 2003 11.1 14.87 16.3% 11.10 [-18.04, 40.24]
Sommer et al. 2015 -6.1 15.99 14.1% -6.10 [-37.44, 25.24]
Ye et al. 2015 9.1 7.91 57.7% 9.10 [-6.40, 24.60]
Subtotal (95% CI) 100.0% 9.14 [-2.63, 20.92]
Heterogeneity: Tau² = 0.00; Chi² = 1.73, df = 3 (P = 0.63); I² = 0%
Test for overall effect: Z = 1.52 (P = 0.13)
Test for subgroup differences: Chi² = 2.61, df = 2 (P = 0.27), I² = 23.4%
-100
-50
0
50
100
Negative
Positive

Subgroup analysis

S7.3 Figure Adjusted regression coefficient _ General vs. non-GDM _ the 2nd trimester _ Random effect model

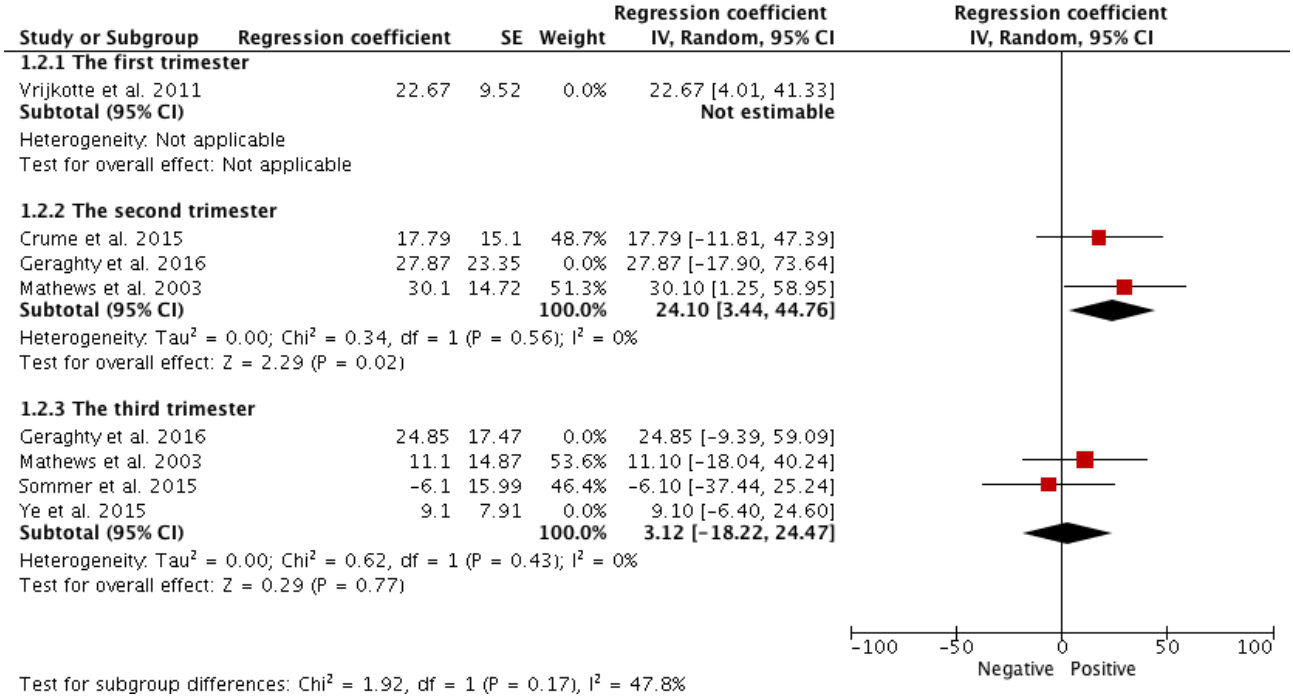


S7.4 Figure Adjusted regression coefficient _ General vs. non-GDM _ the 3rd trimester _ Random effect model

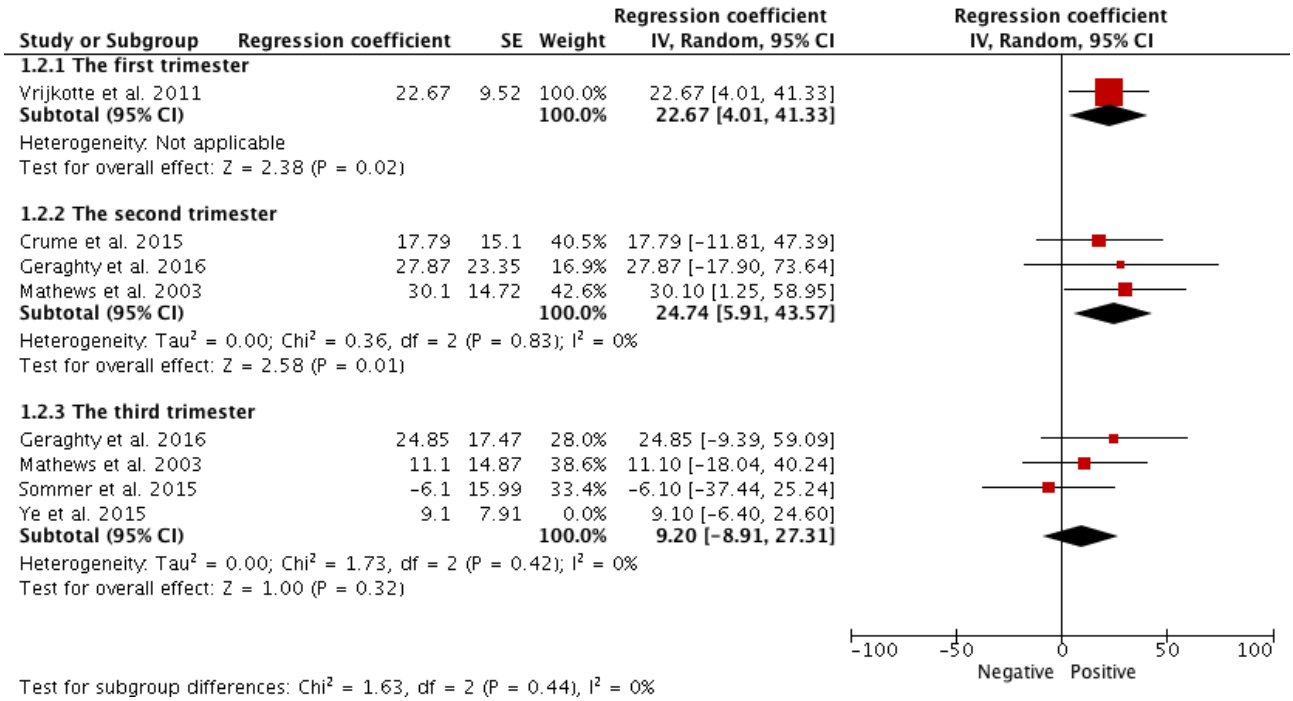


Sensitivity analysis

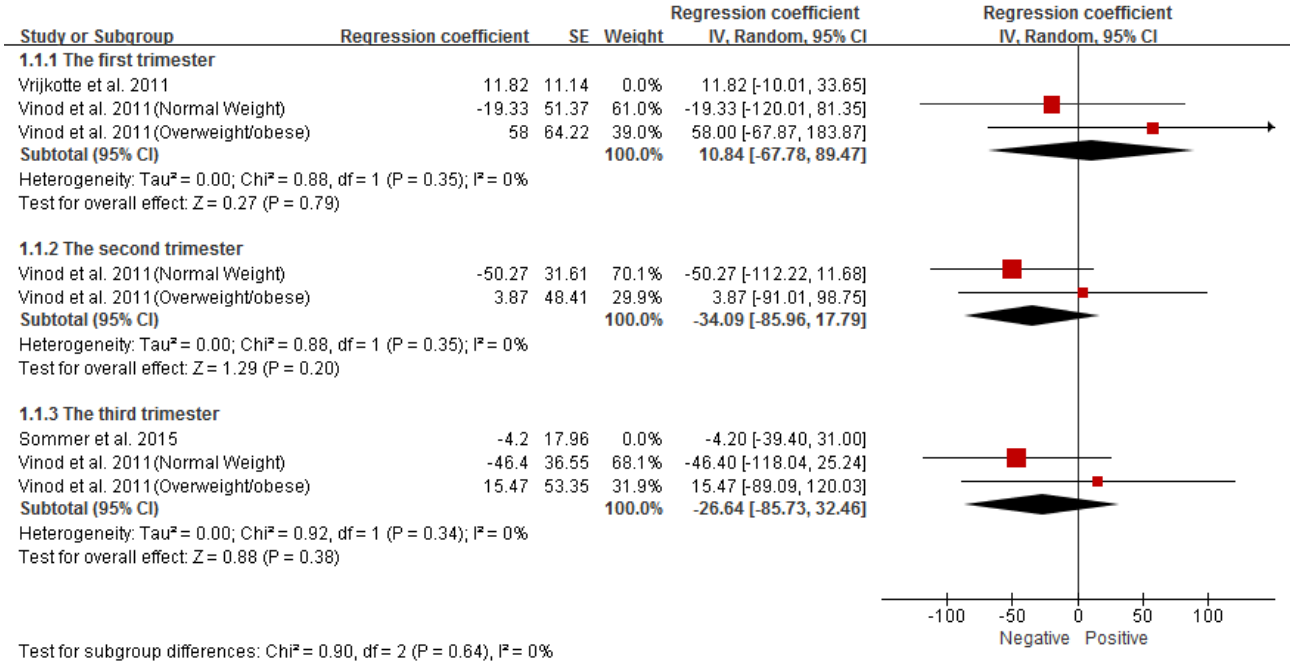
S7.5 Figure Adjusted regression coefficients_ exclude studies control for pre-pregnancy BMI or gestational weight gain



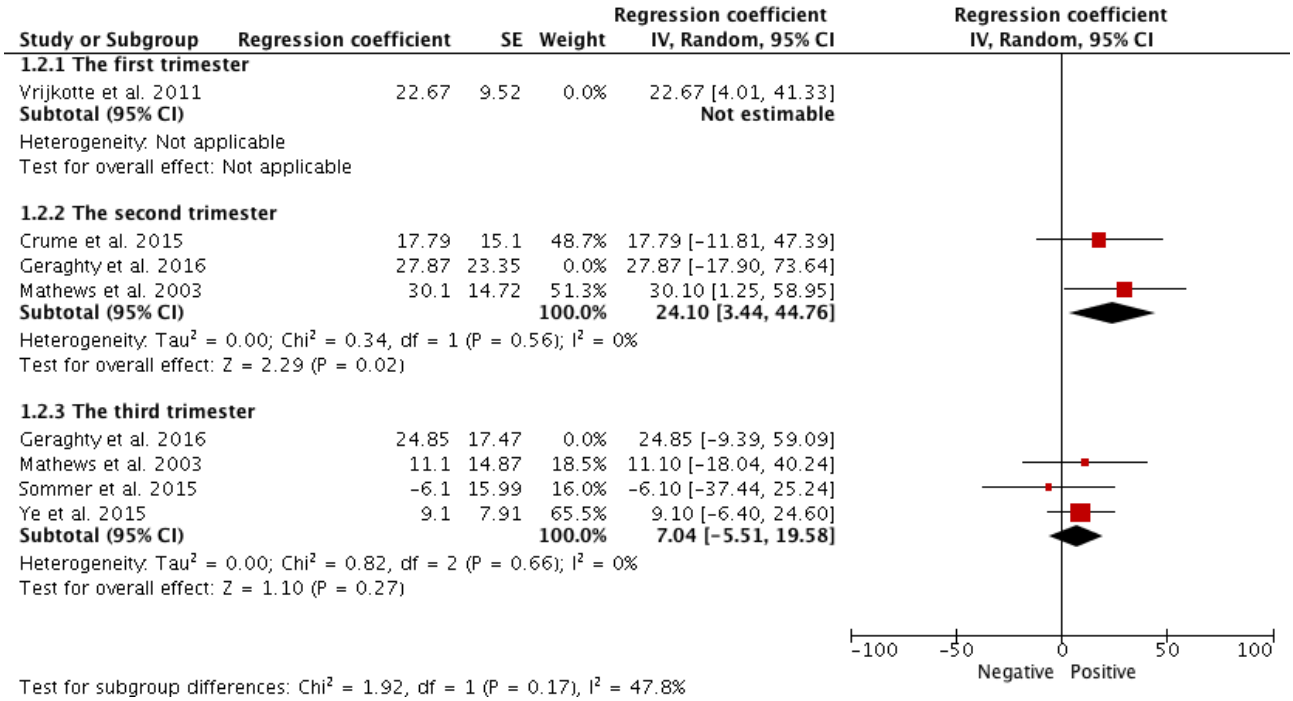
S7.6 Figure Adjusted regression coefficients_ exclude studies control for maternal glucose level



S7.7 Figure Crude regression coefficients_ exclude studies control for pre-term birth



S7.8 Figure Adjusted regression coefficients_ exclude studies that did not control for pre-term birth



High-Density lipoprotein Cholesterol (HDL-C)

S7.3 Table Results summary of the association of maternal HDL-C level with birthweight

ID	Population	Countries	Sample size	Tri.	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors							
												a	b	C	d	e	f	g	h
Vinod et al.2011(1)	normal weight	USA	65	1	Crude β	-81.21	-300.02	137.61	ND	SLR	6	×	×	×	×	×	×	×	×
Vinod et al.2011(2)	Overweight/obese	USA	71	1	Crude β	**-309.36**	**-603.69**	**-15.03**	ND	SLR	6	×	×	×	×	×	×	×	×
Wang et al.2015	General	China	636	2	r	**-0.12**			**0.010**	Partial correlation	6	√	√	×	×	×	×	×	×
Liu et al.2016	General	China	1,546	2	r	-0.01			0.701	Partial correlation	7	×	×	×	×	×	×	×	×
Vinod et al.2011(1)	Normal weight	USA	71	2	Crude β	-158.55	-340.57	23.48	ND	SLR	6	×	×	×	×	×	×	×	×
Vinod et al.2011(2)	Overweight/obese	USA	71	2	Crude β	**-286.16**	**-545.63**	**-26.68**	ND	SLR	6	×	×	×	×	×	×	×	×
Crume et al.2015	General	USA	804	2	Adjusted β	-20.88	-109.69	67.93	0.600	MLR	8	√	√	√	×	×	×	√	×
Kulkarni et al.2013	non-GDM	India	631	2	Adjusted β	17.57	-11.64	46.77	ND	MLR	8	×	√	√	√	×	×	√	×
Geraghty et al.2016	non-GDM	UK	331	2	Adjusted β	-1236.25	-3322.95	850.45	ND	MLR	7	√	√	×	√	√	×	×	×
Whyte et al. 2013	General	Ireland	189	2	ND	ND			ND	ND	6	ND	ND	N	ND	N	ND	×	ND
Di et al.2005	OGTT+	Italy	83	2	ND	ND			ND	ND	5	ND	ND	N	ND	N	ND	×	ND
Zawiejska et al. 2008	GDM	Poland	357	2	ND	ND			ND	ND	5	ND	ND	N	ND	N	ND	×	ND
Olmos et al.2014	GDM	Chile	279	2	ND	ND			ND	ND	6	ND	ND	N	ND	N	ND	×	ND
Knopp et al.1985	General	USA	248	3	r	-0.06			>0.05	Spearman	7	√	√	×	×	×	×	√	×
Mossayebi et al.2014	General	Iran	154	3	r	**-0.47**			**<0.00**	Pearson correlation	5	×	×	×	×	×	×	√	×
Charles et al. 2016	General	Multiple	1062	3	r	**-0.139**			**<0.00**	Pearson correlation	4	×	×	×	×	×	×	×	×

Vinod et al.2011(1)	Normal weight	USA	69	3	Crude β	-139.21	-332.85	54.43	ND	SLR	6	×	×	×	×	×	×	×	×
Vinod et al.2011(2)	Overweight/obese	USA	70	3	Crude β	**-386.70**	**-681.03**	**-92.37**	ND	SLR	6	×	×	×	×	×	×	×	×
Sommer et al.2015	General	Norway	699	3	Crude β	**-98.90**	**-188.10**	**-9.60**	ND	SLR	9	×	×	×	×	×	×	√	×
Retnakaran et al.2012	non-GDM	Canada	472	3	Crude β	-120.54	-244.42	3.35	ND	SLR	7	×	×	×	×	×	×	√	×
Sommer et al.2015	General	Norway	699	3	Adjusted β	**-105.40**	**-183.80**	**-27.00**	ND	MLR	9	√	√	√	×	×	×	√	×
Friis et al.2012	General	German	207	3	Adjusted β	**-170.00**	**-329.00**	**-9.00**	**0.040**	MLR	6	√	×	×	×	×	×	×	×
Crume et al.2015	General	USA	804	3	Adjusted β	-43.31	-128.33	41.71	0.300	MLR	8	√	√	√	×	×	×	√	×
Retnakaran et al.2012	non-GDM	Canada	472	3	Adjusted β	-57.16	-189.42	75.09	ND	MLR	7	√	√	√	√	√	√	√	√
Kulkarni et al.2013	non-GDM	India	631	3	Adjusted β	-8.89	-38.72	20.95	ND	MLR	8	×	√	√	√	×	×	√	×
Ye et al.2015	non-GDM	China	1,243	3	Adjusted β	**-69.50**	**-110.00**	**-28.20**	ND	MLR	8	√	√	√	√	√	√	√	×
Geraghty et al.2016	non-GDM	UK	331	3	Adjusted β	30.00	-114.85	174.84	ND	MLR	7	√	√	×	√	√	×	×	×
Emet et al.2013	General	Turkey	801	3	p	ND			0.754	Pearson correlation	5	×	×	×	×	×	×	×	×
Couch et al.1998	General	USA	40	3	p	ND			>0.05	Pearson correlation	6	×	×	×	×	×	×	×	×
Swierzewska et al.2015	General	Poland	136	3	p	ND			>0.05	MLR	5	ND	ND	N	ND	N	ND	×	ND
Son et al.2010	GDM	Korea	104	3	p	ND			>0.05	ND	5	ND	ND	N	ND	N	ND	√	ND
Slagjana et al.2014	non-GDM	Yugoslavi	200	3	ND	ND			ND	ND	5	ND	ND	N	ND	N	ND	×	ND
Olmos et al.2014	GDM	Chile	279	3	ND	ND			ND	ND	6	ND	ND	N	ND	N	ND	×	ND

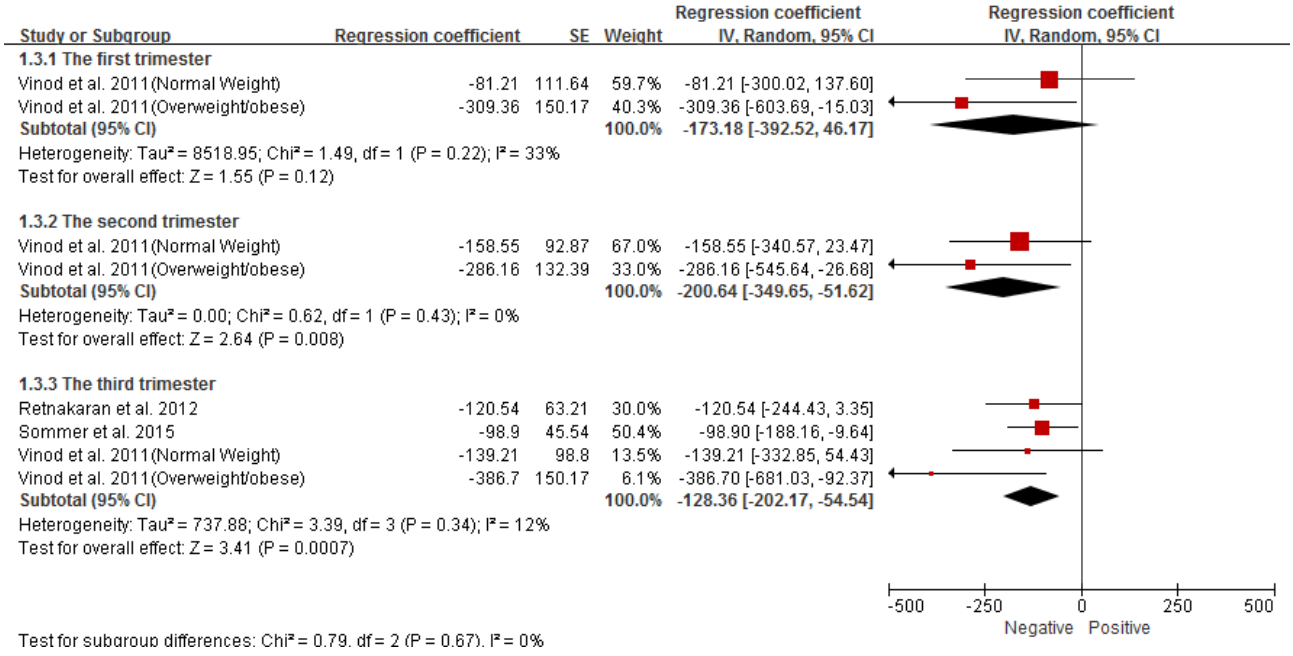
The bold font represents statistically significant results. r: Correlation coefficients; β: regression coefficients.

Confounding factors: a. Gestational age; b. Neonatal gender; c. Maternal age; d. Pre-pregnancy BMI; e. Gestational weight gain; f. Maternal glucose level; g. pre-term birth; h. Maternal lipid levels.

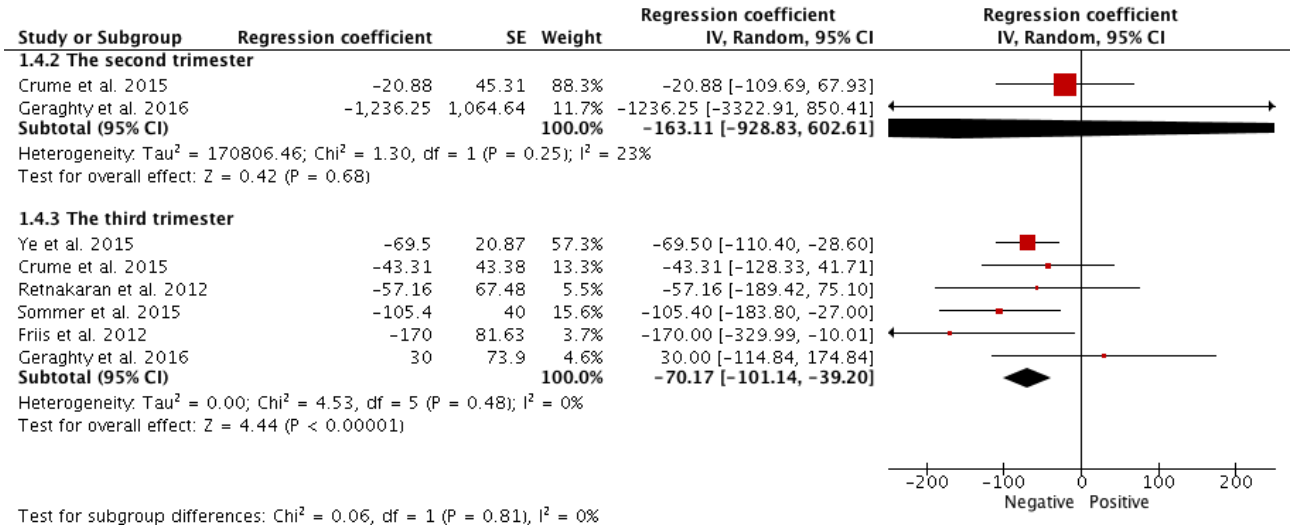
Abbreviation: Trimesters(Tri.), Gestational diabetes mellitus(GDM), Positive screenes of Oral Glucose Tolerance test(OGTT+), Confidence interval(CI), Not documented(ND), Simple linear regression(SLR), Multiple linear regression(MLR), United Kingdom(UK).

Meta-analysis

S7.9 Figure Overall meta-analysis of crude regression coefficients for the association between maternal HDL-C levels and birthweight throughout pregnancy

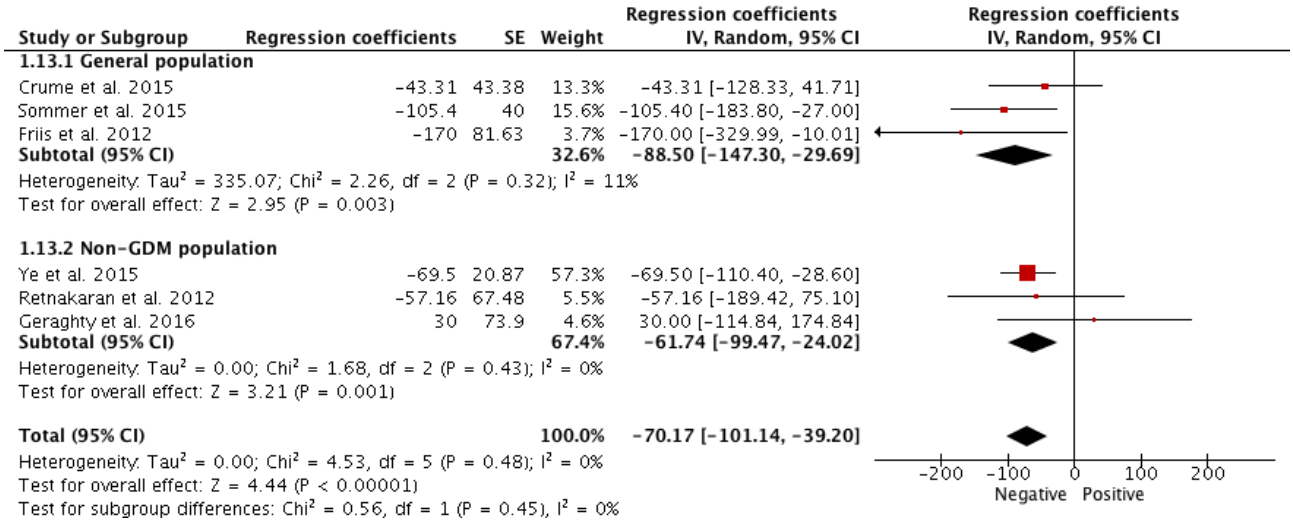


S7.10 Figure Overall meta-analysis of adjusted regression coefficients for the association between maternal HDL-C levels and birthweight throughout pregnancy



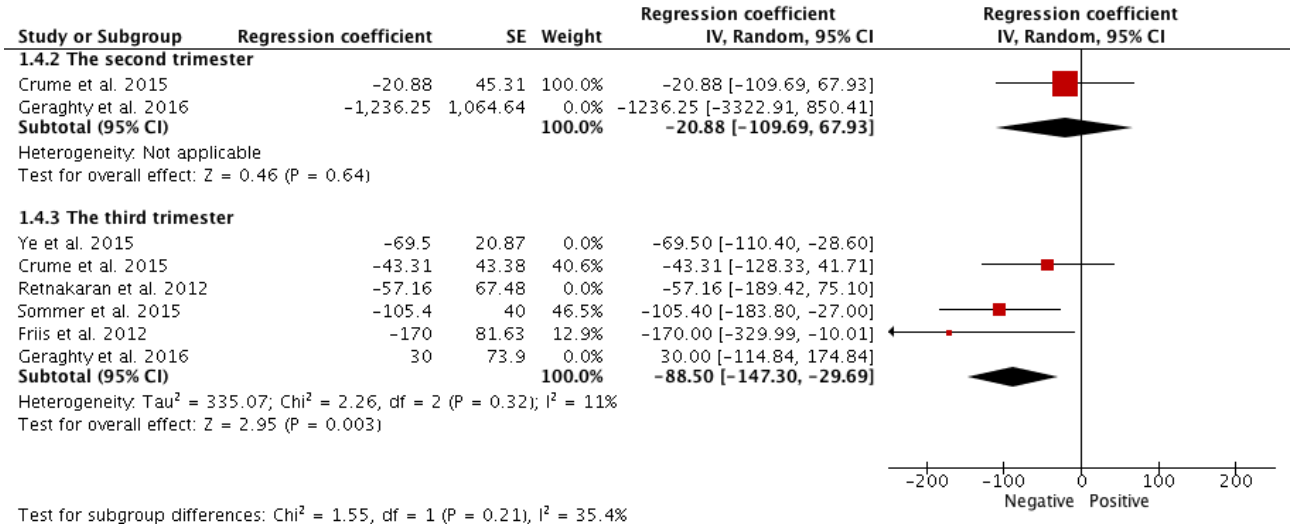
Subgroup analysis

S7.11 Figure Adjusted regression coefficient_ General vs. non-GDM_ the 3rd trimester_ Random effect model

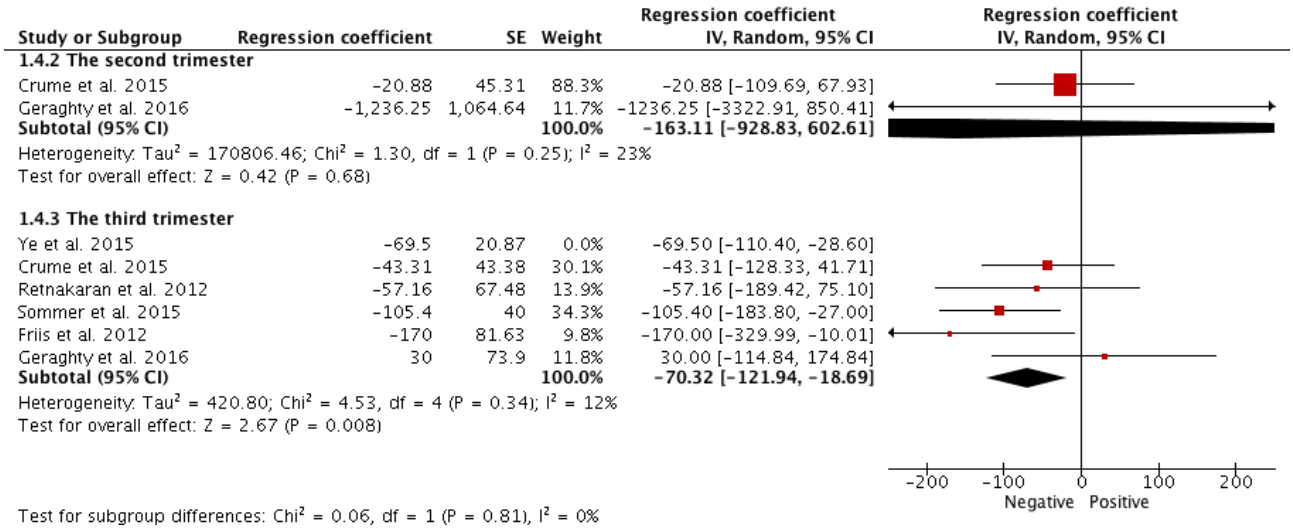


Sensitivity analysis

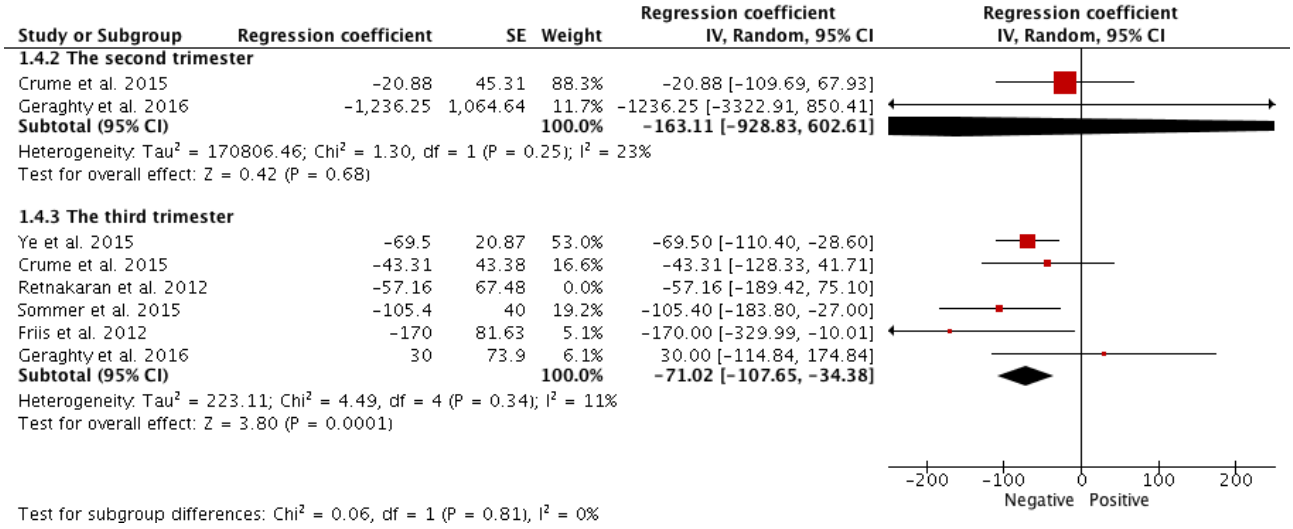
S7.12 Figure Adjusted regression coefficients_ exclude studies control for pre-pregnancy BMI or gestational weight gain



S7.13 Figure Adjusted regression coefficients_ exclude studies control for maternal glucose level



S7.14 Figure Adjusted regression coefficients_ exclude studies control for pre-term birth



Low-Density lipoprotein Cholesterol (LDL-C)

S7.4 Table Results summary of the association of maternal LDL-C level with birthweight

ID	Population	Countries	Sample size	Tri.	Reported	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding							
												A	b	c	d	e	f	g	h
Vinod et al.2011(1)	Normal weight	USA	65	1	Crude β	-34.80	-152.92	83.32	ND	SLR	6	×	×	×	×	×	×	×	×
Vinod et al.2011(2)	Overweight/obe	USA	71	1	Crude β	108.28	-42.76	259.31	ND	SLR	6	×	×	×	×	×	×	×	×
Liu et al.2016	General	China	1,546	2	r	-0.01			0.843	Partial correlation	7	×	×	×	×	×	×	×	×
Vinod et al.2011(1)	Normal weight	USA	71	2	Crude β	-58.00	-133.52	17.51	ND	SLR	6	×	×	×	×	×	×	×	×
Vinod et al.2011(2)	Overweight/obe	USA	71	2	Crude β	34.80	-83.32	152.92	ND	SLR	6	×	×	×	×	×	×	×	×
Geraghty et al.2016	non-GDM	UK	331	2	Adjusted β	18.39	-38.44	75.21	ND	MLR	7	√	√	×	√	√	×	×	×
Wang et al.2015	General	China	636	2	ND	ND			ND	Partial correlation	6	√	√	×	×	×	×	×	×
Whyte et al. 2013	General	Ireland	189	2	ND	ND			ND	ND	6	ND	ND	N	ND	ND	ND	×	ND
Di et al.2005	OGTT+	Italy	83	2	ND	ND			ND	ND	5	ND	ND	N	ND	ND	ND	√	ND
Olmos et al.2014	GDM	Chile	279	2	ND	ND			ND	ND	6	ND	ND	N	ND	ND	ND	×	ND
Knopp et al.1985	General	USA	248	3	r	0.01			>0.05	Spearman	7	√	√	×	×	×	×	√	×
Mossayebi et al.2014	General	Iran	154	3	r	0.40			<0.001	Pearson correlation	5	×	×	×	×	×	×	√	×
Charles et al. 2016	General	Multiple	1062	3	r	**0.001**			**<0.0001**	Pearson correlation	4	×	×	×	×	×	×	×	×
Retnakaran et al.2012	non-GDM	Canada	472	3	Crude β	-15.22	-55.49	25.05	ND	SLR	7	×	×	×	×	×	×	√	×
Vinod et al.2011(1)	Normal weight	USA	69	3	Crude β	-50.27	-131.60	31.06	ND	SLR	6	×	×	×	×	×	×	×	×
Vinod et al.2011(2)	Overweight/obe	USA	70	3	Crude β	38.67	-79.45	156.79	ND	SLR	6	×	×	×	×	×	×	×	×
Ye et al.2015	non-GDM	China	1,243	3	Adjusted β	35.40	10.10	60.80	ND	MLR	8	√	√	√	√	√	√	√	×
Retnakaran et al.2012	non-GDM	Canada	472	3	Adjusted β	-6.79	-46.98	33.39	ND	MLR	7	√	√	√	√	√	√	√	√
Geraghty et al.2016	non-GDM	UK	331	3	Adjusted β	19.97	-24.34	64.27	ND	MLR	7	√	√	×	√	√	×	×	×
Emet et al.2013	General	Turkey	801	3	p	ND			0.440	Pearson correlation	5	×	×	×	×	×	×	×	×
Couch et al.1998	General	USA	40	3	p	ND			>0.05	Pearson correlation	6	×	×	×	×	×	×	×	×

Swierzewska et al.2015	General	Poland	136	3	p	ND	>0.05	MLR	5	ND	ND	N	ND	ND	ND	×	ND
Sommer et al.2015	General	Norway	699	3	ND	ND	ND	ND	9	ND	ND	N	ND	ND	ND	×	ND
Slagjana et al.2014	non-GDM	Yugoslavia	200	3	ND	ND	ND	ND	5	ND	ND	N	ND	ND	ND	×	ND
Son et al.2010	GDM	Korea	104	3	ND	ND	ND	ND	5	ND	ND	N	ND	ND	ND	√	ND
Olmos et al.2014	GDM	Chile	279	3	ND	ND	ND	ND	6	ND	ND	N	ND	ND	ND	×	ND

The bold font represents statistically significant results.

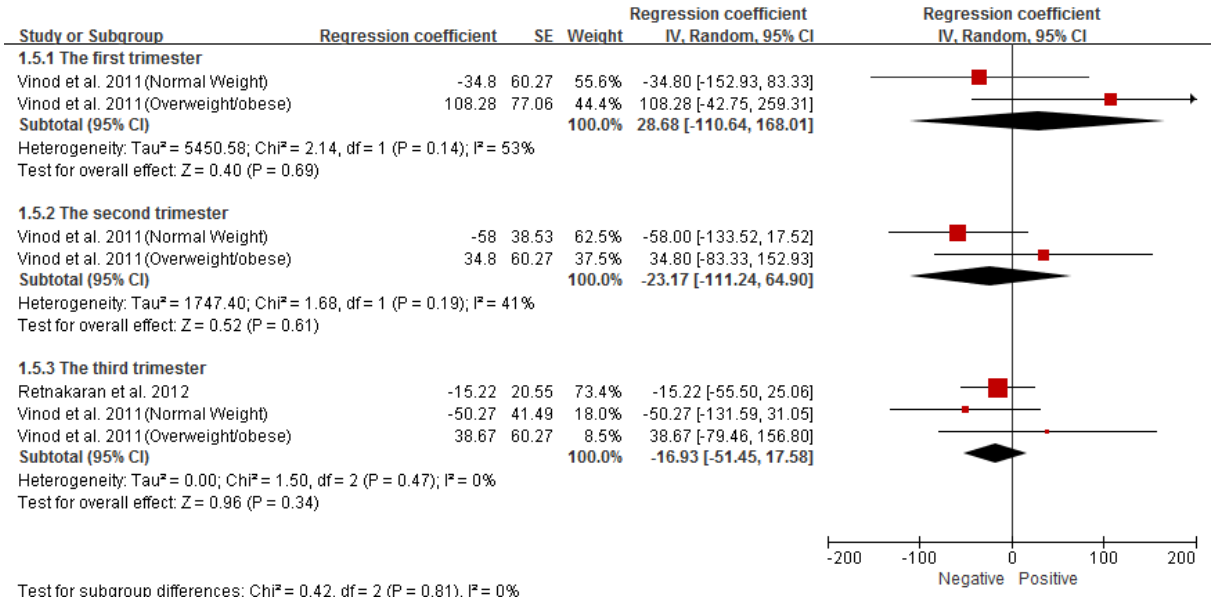
r: Correlation coefficients; β: regression coefficients.

Confounding factors: a. Gestational age; b. Neonatal gender; c. Maternal age; d. Pre-pregnancy BMI; e. Gestational weight gain; f. Maternal glucose level; g. pre-term birth; h. Maternal lipid levels.

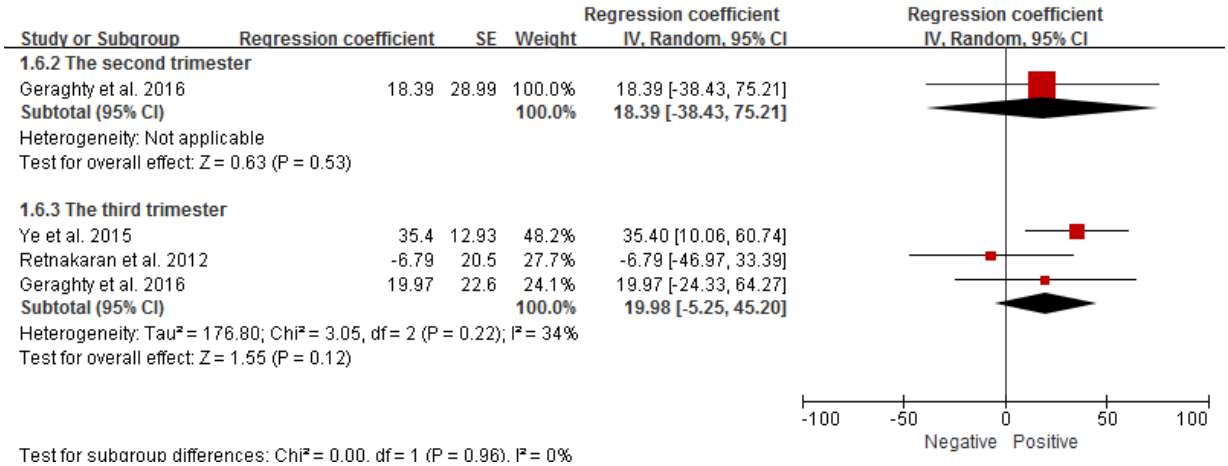
Abbreviation: Trimesters(Tri.), Gestational diabetes mellitus(GDM), Positive screenes of Oral Glucose Tolerance test(OGTT+), Confidence interval(CI), Not documented(ND), Simple linear regression(SLR), Multiple linear regression(MLR), United Kingdom(UK).

Meta-analysis

S7.15 Figure Overall meta-analysis of crude regression coefficients for the association between maternal LDL-C levels and birthweight throughout pregnancy

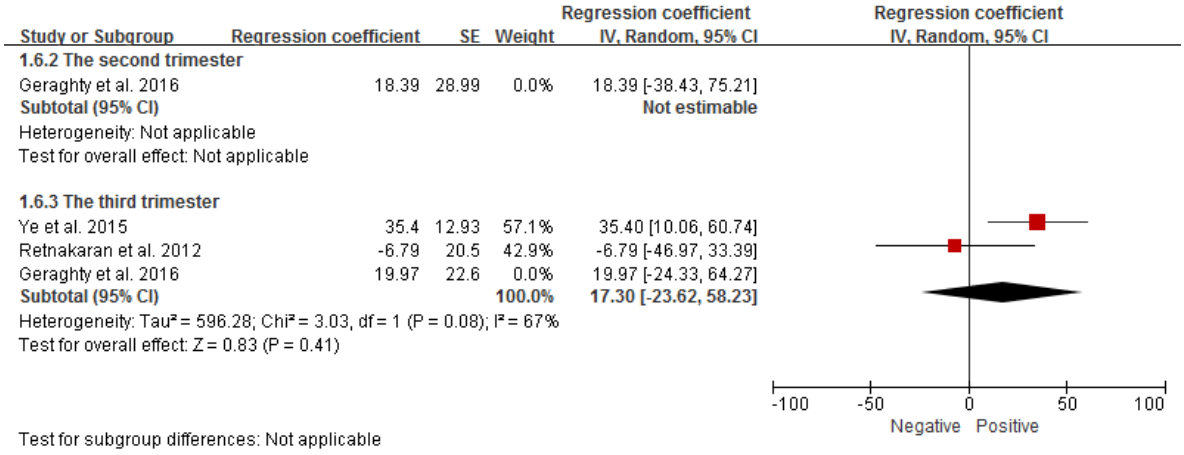


S7.16 Figure Overall meta-analysis of adjusted regression coefficients for the association between maternal LDL-C levels and birthweight throughout pregnancy

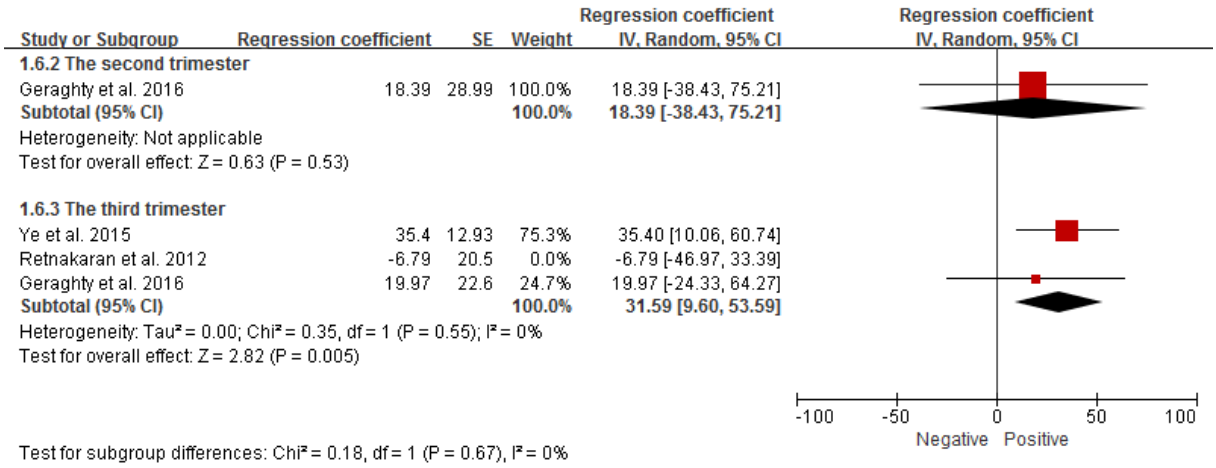


Sensitivity analysis

S7.17 Figure Adjusted regression coefficients_ exclude studies that did not control for pre-term birth



S7.18 Figure Adjusted regression coefficients_ exclude studies that did not control for other maternal lipid levels



Triglycerides (TG)

S7.5 Table Results summary of the association of maternal TG level with birthweight

ID	Population	Countries	Sample size	Tri.	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors							
												a	b	c	d	e	f	g	h
Nolan et al.1995	General	Australia	388	1	r	**0.12**			**0.020**	Univariate analyses	6	√	√	×	×	×	×	×	×
Vinod et al.2011(1)	Normal weight	USA	65	1	Crude β	**132.86**	**13.11**	**252.62**	ND	SLR	6	×	×	×	×	×	×	×	×
Vinod et al.2011(2)	Overweight/obese	USA	71	1	Crude β	124.00	-40.10	288.11	ND	SLR	6	×	×	×	×	×	×	×	×
Vrijkotte et al.2011	General	Netherlands	2,052	1	Crude β	**47.14**	**12.42**	**81.87**	ND	Univariate analyses	8	√	√	×	×	×	×	√	×
Vrijkotte et al.2011	General	Netherlands	2,052	1	Adjusted β	**86.72**	**56.13**	**117.30**	ND	MLR	8	√	√	√	√	√	×	√	×
Harmon et al.2011	non-GDM	USA	38	1	p	ND			>0.05	Pearson correlation	5	×	×	×	×	×	×	×	×
Liu et al.2016	General	China	1,546	2	r	**0.10**			**<0.001**	Partial correlation	7	×	×	×	×	×	×	×	×
Wang et al.2015	General	China	636	2	r	**0.19**			**<0.01**	Partial correlation	6	√	√	×	×	×	×	×	×
Di et al.2005	OGTT+	Italy	83	2	r	**0.30**			**<0.05**	SLR	5	×	×	×	×	×	×	×	×

ID	Population	Countries	Sample size	Tri.	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors							
												a	b	c	d	e	f	g	h
Zawiejska et al. 2008	GDM	Poland	357	2	r	**0.14**			**<0.01**	SLR	5	×	×	×	×	×	×	×	×
Vinod et al.2011(1)	Normal weight	USA	71	2	Crude β	**97.43**	**4.29**	**190.57**	ND	SLR	6	×	×	×	×	×	×	×	×
Vinod et al.2011(2)	Overweight/obese	USA	71	2	Crude β	**132.86**	**4.24**	**261.49**	ND	SLR	6	×	×	×	×	×	×	×	×
Crume et al.2015	General	USA	804	2	Adjusted β	7.97	-44.19	60.13	0.700	MLR	8	√	√	√	×	×	×	√	×
Kulkarni et al.2013	non-GDM	India	631	2	Adjusted β	14.76	-13.34	42.86	ND	MLR	8	×	√	√	√	×	×	√	×
Hwang et al.2015	non-GDM	Korea	1,011	2	Adjusted β^	**7125.42**	**1693.49**	**12557.35**	**0.002**	MLR	8	√	√	√	×	√	×	×	×
Whyte et al. 2013	General	Ireland	189	2	p	+			**<0.05**	SLR	6	×	×	×	×	×	×	×	×
Geraghty et al.2016	non-GDM	UK	331	2	p	ND			>0.1	MLR	7	√	√	×	√	√	×	×	×
Olmos et al.2014	GDM	Chile	279	2	ND	ND			ND	ND	6	ND	ND	ND	ND	ND	ND	×	ND

ID	Population	Countries	Sample size	Tri.	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors							
												a	b	c	d	e	f	g	h
Mossayebi et al.2014	General	Iran	154	3	r	**0.68**			**<0.001**	Pearson correlation	5	×	×	×	×	×	×	√	×
Charles et al. 2016	General	Multiple	1062	3	r	**-0.014**			**<0.0001**	Pearson correlation	4	×	×	×	×	×	×	×	×
Son et al.2010	GDM	Korea	104	3	r	0.17			0.070	ND	5	×	×	×	×	×	×	√	×
Ahmad et al. 2006	non-GDM	Malaysia	246	3	r	0.12			0.057	Univariate analyses	6	√	×	×	×	×	×	√	×
Couch et al.1998(1)	non-GDM	USA	20	3	r	**0.46**			**<0.05**	Pearson correlation	6	×	×	×	×	×	×	×	×
Slagjana et al.2014	non-GDM	Yugoslavia	200	3	r	0.16			0.077	Correlation analysis	5	×	×	×	×	×	×	×	×
Olmos et al.2014(1)	GDM-normal weight	Chile	128	3	r	0.12			0.158	SLR	6	×	×	×	×	×	×	×	×
Olmos et al.2014(2)	GDM-overweight	Chile	105	3	r	**0.42**			**<0.001**	SLR	6	×	×	×	×	×	×	×	×

ID	Population	Countries	Sample size	Tri.	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors							
												a	b	c	d	e	f	g	h
Olmos et al.2014(3)	GDM-obese	Chile	46	3	r	**0.47**			**<0.001**	SLR	6	×	×	×	×	×	×	×	×
Kitajima et al.2001	OGTT +	Japan	146	3	r	**0.22**			**0.009**	SLR	6	×	×	×	×	×	×	√	×
Knopp et al.1992(1)	OGTT-	USA	521	3	r	**0.09**			**≤0.05**	Spearman correlation	6	×	×	×	×	×	×	×	×
Knopp et al.1992(2)	OGTT+ plus GDM	USA	264	3	r	**0.16**			**≤0.01**	Spearman correlation	6	×	×	×	×	×	×	×	×
Vinod et al.2011(1)	Normal weight	USA	69	3	Crude β	79.72	-8.99	168.42	ND	SLR	6	×	×	×	×	×	×	×	×
Vinod et al.2011(2)	Overweight/obese	USA	70	3	Crude β	**168.29**	**52.97**	**283.61**	ND	SLR	6	×	×	×	×	×	×	×	×
Sommer et al.2015	General	Norway	699	3	Crude β	48.80	-14.80	112.40	ND	SLR	9	×	×	×	×	×	×	√	×
Retnakaran et al.2012	non-GDM	Canada	472	3	Crude β	61.11	-1.18	123.40	ND	SLR	7	×	×	×	×	×	×	√	×
Sommer et al.2015	General	Norway	699	3	Adjusted β	**94.40**	**37.80**	**150.90**	ND	MLR	9	√	√	√	×	×	×	√	×

ID	Population	Countries	Sample size	Tri.	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors							
												a	b	c	d	e	f	g	h
Retnakaran et al.2012	non-GDM	Canada	472	3	Adjusted β	-1.59	-70.67	67.49	ND	MLR	7	√	√	√	√	√	√	√	√
Brunner et al.2013	General	German	208	3	Adjusted β	-47.83	-138.75	43.09	>0.05	MLR	7	√	√	×	√	√	√	×	×
Friis et al.2012	General	German	207	3	Adjusted β	**94.00**	**2.00**	**187.00**	**0.046**	MLR	6	√	×	×	×	×	×	×	×
Mossayebi et al.2014	General	Iran	154	3	Adjusted β	**464.13**	**370.24**	**558.02**	ND	MLR	5	×	√	×	×	×	×	√	×
Crume et al.2015	General	USA	804	3	Adjusted β	17.71	-24.01	59.44	0.400	MLR	8	√	√	√	×	×	×	√	×
Geraghty et al.2016	non-GDM	UK	331	3	Adjusted β	**111.18**	**8.48**	**213.87**	ND	MLR	7	√	√	×	√	√	×	×	×
Ye et al.2015	non-GDM	China	1,243	3	Adjusted β	**25.20**	**7.90**	**42.60**	ND	MLR	8	√	√	√	√	√	√	√	×
Kulkarni et al.2013	non-GDM	India	631	3	Adjusted β	**36.27**	**4.32**	**68.23**	ND	MLR	8	×	√	√	√	×	×	√	×
Hwang et al.2015	non-GDM	Korea	1,011	3	Adjusted β^	**11609.12**	**6177.20**	**17041.05**	**<0.0001**	MLR	8	√	√	√	×	√	×	×	×

ID	Population	Countries	Sample size	Tri.	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors							
												a	b	c	d	e	f	g	h
Swierzewska et al.2015	General	Poland	136	3	p	ND			>0.05	MLR	5	ND	ND	ND	ND	ND	ND	×	ND
Emet et al.2013	General	Turkey	801	3	p¶	+			**0.033**	Pearson correlation	5	×	×	×	×	×	×	×	×
Schaefer-Graf et al.2011	non-GDM	German	190	3	p	ND			>0.05	Pearson correlation	5	×	×	×	×	×	×	√	×
Couch et al.1998(2)	GDM	USA	20	3	p	ND			>0.05	Pearson correlation	6	×	×	×	×	×	×	×	×
Schaefer-Graf et al.2008	GDM	German	150	3	p	ND			>0.05	Spearman correlation	5	×	×	×	×	×	×	×	×

The bold font represents statistically significant results.

^ Maternal TG level was log-transformed

¶ Exposure of this study is change in maternal TG level from the first trimester to the third trimester

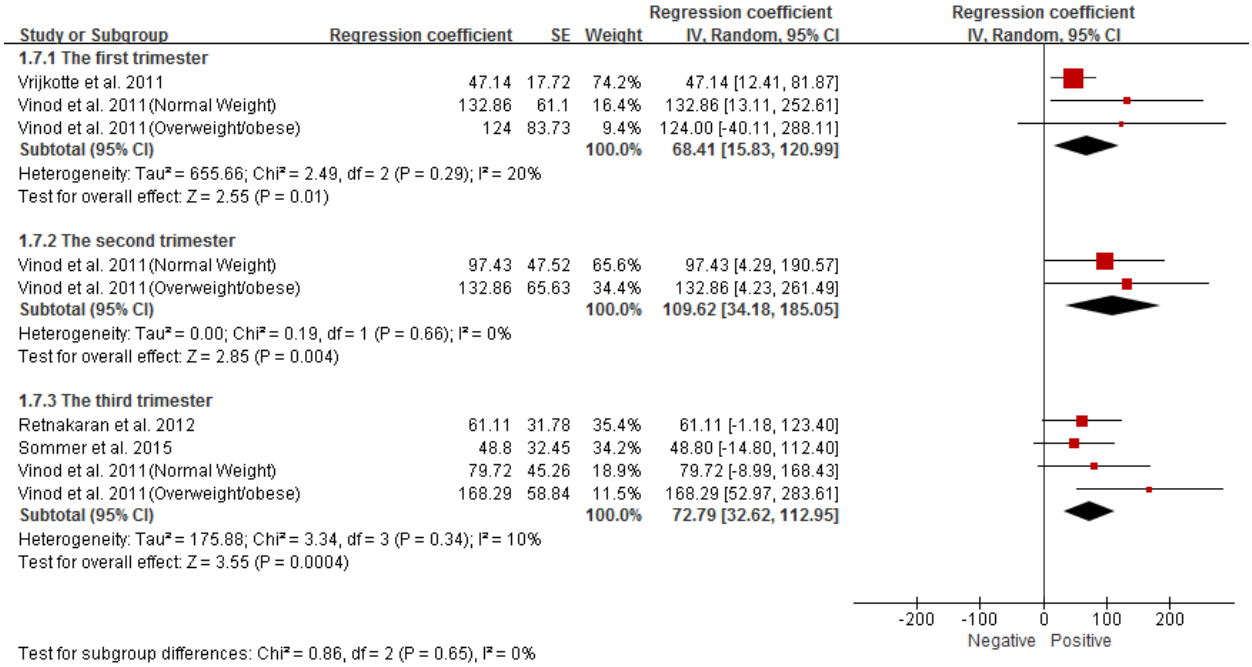
r: Correlation coefficients; β: regression coefficients.

Confounding factors: a. Gestational age; b. Neonatal gender; c. Maternal age; d. Pre-pregnancy BMI; e. Gestational weight gain; f. Maternal glucose level; g. pre-term birth; h. Maternal lipid levels.

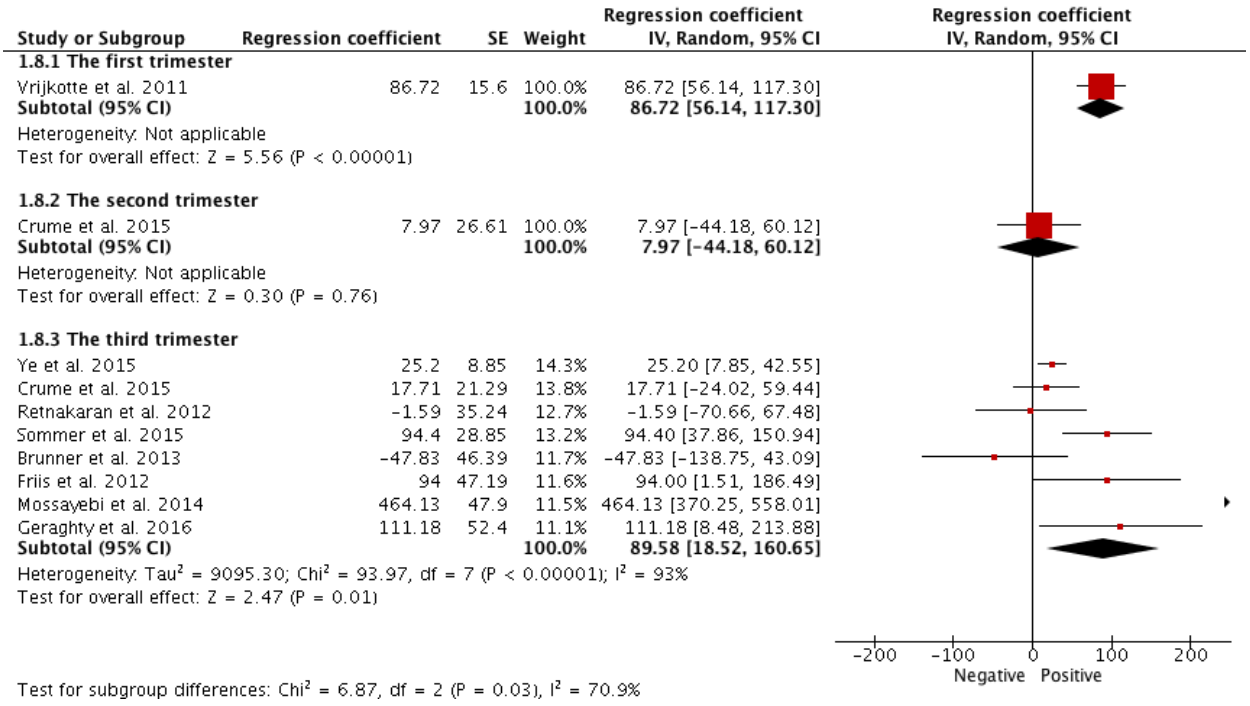
Abbreviation: Trimesters(Tri.), Gestational diabetes mellitus(GDM), Positive screenes of Oral Glucose Tolerance test(OGTT+), Confidence interval(CI), Not documented(ND), Simple linear regression(SLR), Multiple linear regression(MLR), United Kingdom(UK).

Meta-analysis

S7.19 Figure Overall meta-analysis of crude regression coefficients for the association between maternal TG levels and birthweight throughout pregnancy

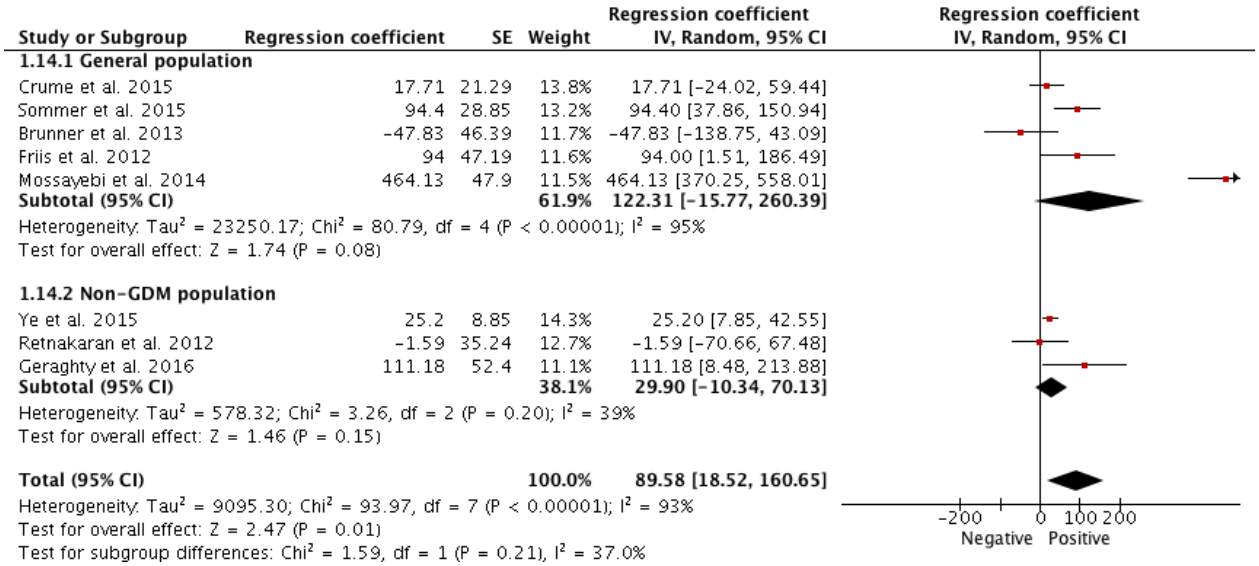


S7.20 Figure Overall meta-analysis of adjusted regression coefficients for the association between maternal TG levels and birthweight throughout pregnancy



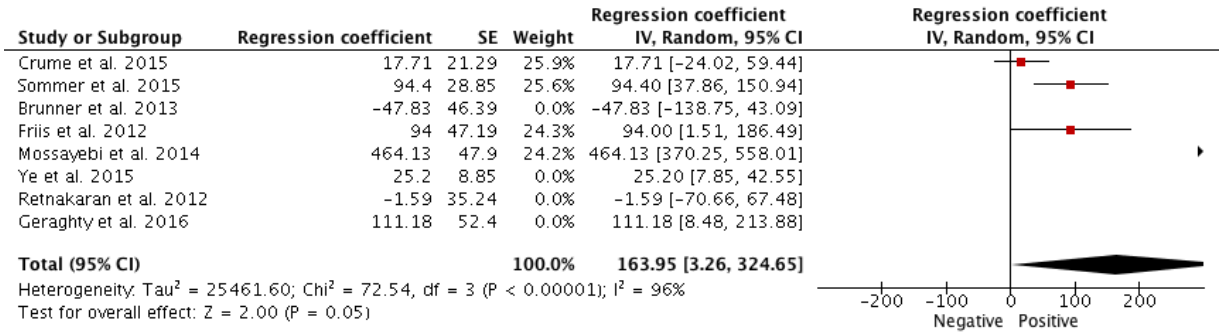
Subgroup analysis

S7.21 Figure Adjusted regression coefficient_ General vs. non-GDM_ the 3rd trimester_ Random effect model

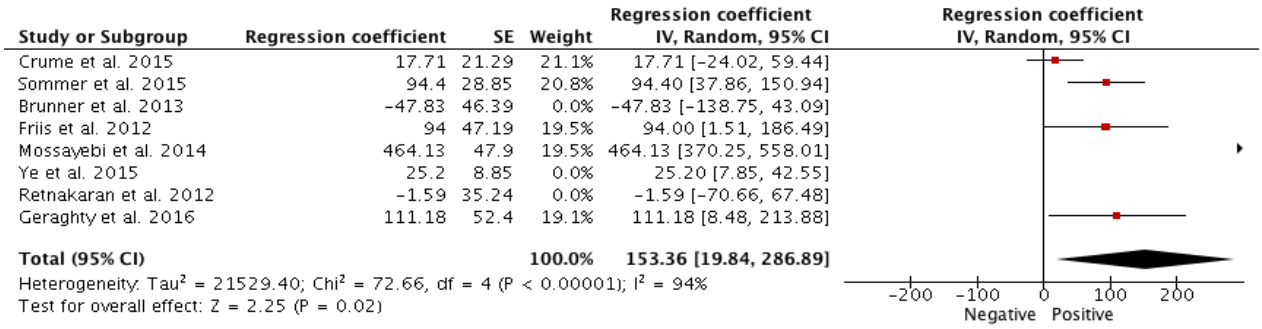


Sensitivity analysis

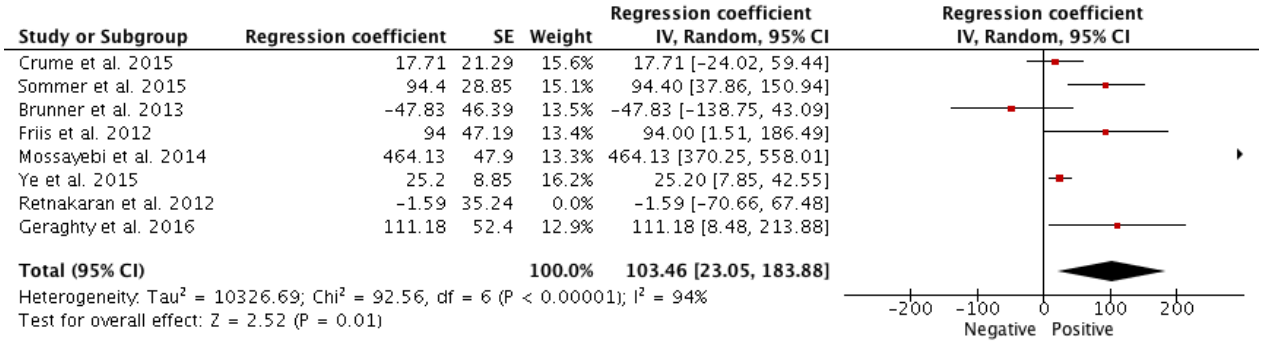
S7.22 Figure Adjusted regression coefficients_ the 3rd trimester_ exclude studies control for pre-pregnancy BMI or gestational weight gain



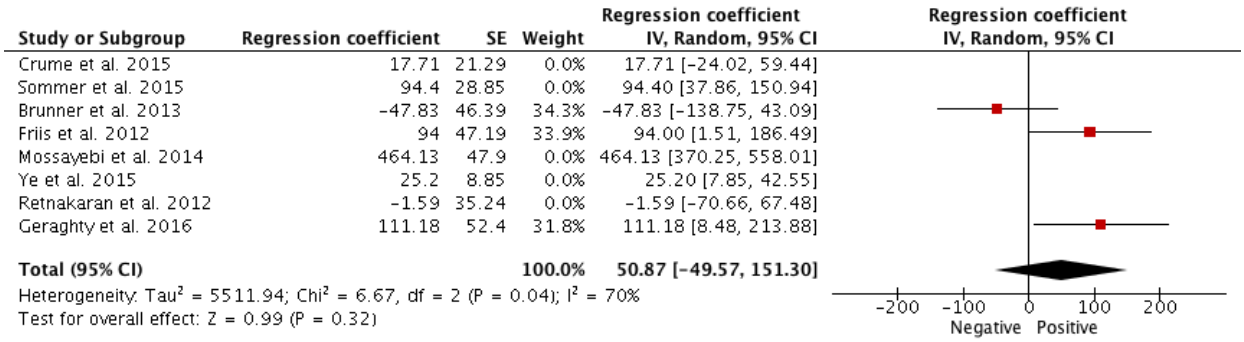
S7.23 Figure Adjusted regression coefficients_ the 3rd trimester_ exclude studies control for maternal glucose level



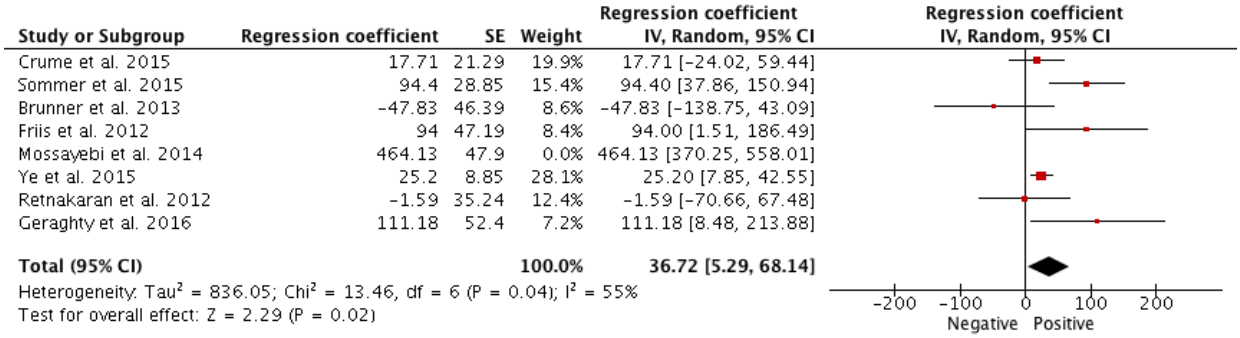
S7.24 Figure Adjusted regression coefficients_ the 3rd trimester_ exclude studies control for other maternal lipid levels



S7.25 Figure Adjusted regression coefficients_ the 3rd trimester_ exclude studies control for pre-term birth



S7.26 Figure Adjusted regression coefficients_ the 3rd trimester_ exclude studies that did not control for gestational age



Free Fatty Acids (FFAs)

S7.6 Table Results summary of the association of maternal FFAs levels with birthweight

ID	Population	Countries	Sample size	Tri.	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors								FFAs' unit
												a	b	c	d	e	f	g	h	
Harmon et al.2011	non-GDM	USA	38	1	p	ND			>0.05	Pearson correlation	5	×	×	×	×	×	×	×	×	μEq/L
Crume et al.2015	General	USA	804	2	Adjusted β	0.06	-0.12	0.24	0.500	MLR	8	√	√	√	×	×	×	√	×	mg/dL
Crume et al.2015	General	USA	804	3	Adjusted β	**0.21**	**0.01**	**0.41**	**0.030**	MLR	8	√	√	√	×	×	×	√	×	mg/dL
Knopp et al.1985	General	USA	248	3	r	0.002			>0.05	Spearman correlation	7	√	√	×	×	×	×	√	×	μmol/L
Kitajima et al.2001	OGTT +	Japan	146	3	r	0.03			0.730	SLR	6	×	×	×	×	×	×	√	×	mEq/dL
Schaefer-Graf et al.2008	GDM	German	150	3	r	**0.27**			**0.002**	Spearman correlation	5	×	×	×	×	×	×	×	×	μmol/L
Couch et al.1998	General	USA	40	3	p	ND			>0.05	Pearson correlation	6	×	×	×	×	×	×	×	×	mg/dL
Friis et al.2012	General	German	207	3	p	ND			>0.05	MLR	6	√	×	×	×	×	×	×	×	ND
Schaefer-Graf et al.2011	non-GDM	German	190	3	p	ND			>0.05	Pearson correlation	5	×	×	×	×	×	×	√	×	μmol/L

The bold font represents statistically significant results.

r: Correlation coefficients; β: regression coefficients.

Confounding factors: a. Gestational age; b. Neonatal gender; c. Maternal age; d. Pre-pregnancy BMI; e. Gestational weight gain; f. Maternal glucose level; g. pre-term birth; h. Maternal lipid levels.

Abbreviation: Trimesters(Tri.), Gestational diabetes mellitus(GDM), Positive screenes of Oral Glucose Tolerance test(OGTT+), Confidence interval(CI), Not documented(ND), Simple linear regression(SLR), Multiple linear regression(MLR).

Very Low-density lipoprotein cholesterol (VLDL)

S7.7 Table Results summary of the association of maternal VLDL-C levels with birthweight

ID	Population	Countries	Sample size	Trimester	Reported measures	Effect size	p	Statistical methods	Quality	The control of confounding factors							
										a	b	c	d	e	f	g	h
Couch et al.1998	General	USA	40	3	p	ND	>0.05	Pearson correlation	6	×	×	×	×	×	×	×	×
Knopp et al.1985	General	USA	248	3	r	0.03	>0.05	Spearman correlation	7	√	√	×	×	×	×	√	×

r: Correlation coefficients

Confounding factors: a. Gestational age; b. Neonatal gender; c. Maternal age; d. Pre-pregnancy BMI; e. Gestational weight gain; f. Maternal glucose level; g. pre-term birth; h. Maternal lipid levels.

Abbreviation: Not documented(ND).

S6 Systematic review: Data analysis for Large for gestational age (LGA)

Total cholesterol (TC)

S8.1 Table Results summary of the association of maternal TC levels with LGA

Study ID	Population	Countries	Sample size	Trimesters	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors					
												a	b	c	d	e	f
Jin et al.2016	non-GDM	China	934	1	ND	ND			ND	ND	7	ND	ND	ND	ND	×	ND
Vrijkotte et al.2012	non-GDM	Netherlands	4,008	1	Crude OR	1.10	0.97	1.25	ND	Logistic regression	8	×	×	×	×	×	×
Vrijkotte et al.2012	non-GDM	Netherlands	4,008	1	Adjusted OR	1.08	0.95	1.22	ND	MLOR	8	√	√	×	×	×	×
Jin et al.2016	non-GDM	China	934	2	ND	ND			ND	ND	7	ND	ND	ND	ND	√	ND
Di et al.2005	OGTT+	Italy	83	2	ND	ND			ND	ND	5	ND	ND	ND	ND	×	ND
Mossayebi et al.2014	General	Iran	82	3	Crude OR*	**13.30**	**2.80**	**62.50**	ND	Chi-squared test	5	×	×	×	×	√	×
Mossayebi et al.2014	General	Iran	82	3	Adjusted OR*	1.10	0.20	8.10	ND	MLOR	5	√	√	×	√	√	√
Ye et al.2015	non-GDM	China	1,204	3	Adjusted OR	1.04	0.94	1.15	ND	MLOR	8	√	√	√	√	√	×
Jin et al.2016	non-GDM	China	934	3	Adjusted OR	0.98	0.81	1.11	0.715	MLOR	7	√	√	√	×	√	×
Hou et al.2014	non-GDM	China	2,790	3	Adjusted OR¶	1.08	0.75	1.56	ND	MLOR	7	√	√	×	×	√	×

Schaefer-Graf et al.2008	GDM	German	150	3	p	ND		>0.05	MLOR	5	√	√	√	√	×	×
Laleh et al.2013	GDM	Iran	112	3	p	ND		>0.05	ANCOVA	7	√	√	×	×	×	×
Kitajima et al.2001	OGTT +	Japan	146	3	ND	ND		ND	ND	6	ND	ND	ND	ND	√	ND
Retnakaran et al.2012	non-GDM	Canada	472	3	ND	ND		ND	ND	7	ND	ND	ND	ND	√	ND
Ahmad et al. 2006	non-GDM	Malaysia	246	3	ND	ND		ND	ND	6	ND	ND	ND	ND	√	ND
					mmol/L	***Reference***	***LGA***	**p**									
Slagjana et al.2014	non-GDM	Yugoslavia	200	3	$\bar{x}$±SD	6.5±1.4 (AGA)	6.0±1.0	>0.05	Student t test	5	×	×	×	×	×	×
Son et al.2010	GDM	Korea	104	3	$\bar{x}$±SD	5.8±1.1 (non-LGA)	5.5±0.9	0.352	Student t test	5	×	×	×	×	√	×
Hou et al.2014	non-GDM	China	2,790	3	Median (IQR)	6.30 (AGA) (5.62, 7.10)	6.18 (5.49,7.04)	**0.017**	Mann-Whitney U test	7	×	×	×	×	√	×

The bold font represents statistically significant results.

* Result was calculated by comparing the highest quartile with the lowest quartile maternal TC level

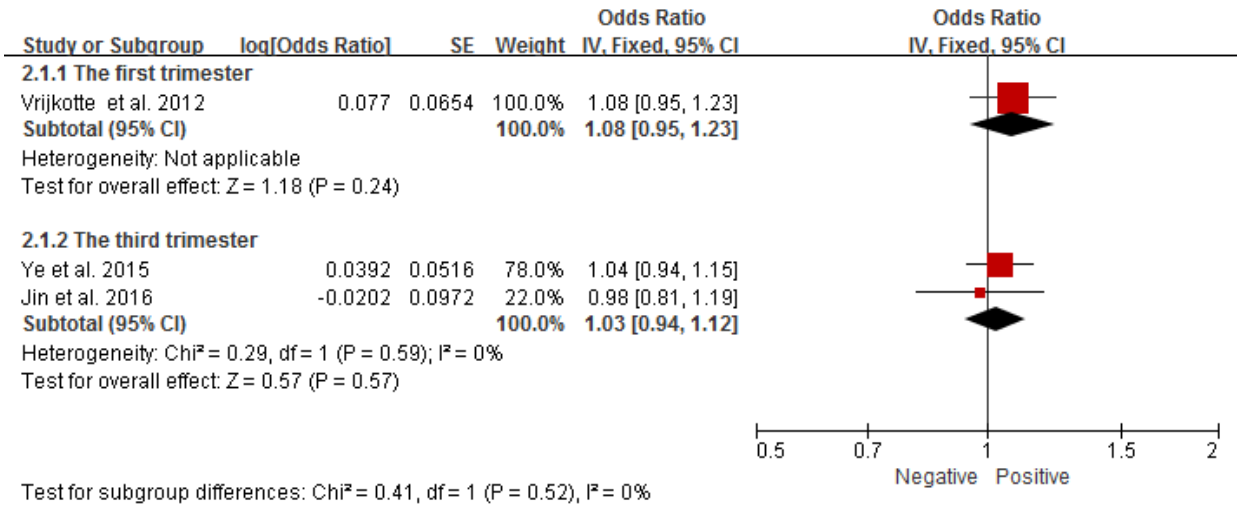
¶ Result was calculated by comparing the highest tertile with the lowest tertile maternal TC level

Confounding factors: a. Maternal age; b. Pre-pregnancy BMI; c. Gestational weight gain; d. Maternal glucose level; e. pre-term birth; f. Maternal lipid levels.

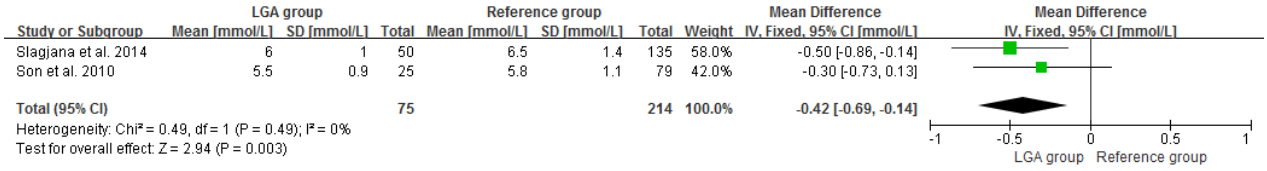
Abbreviation: Gestational diabetes mellitus(GDM), Positive screenes of Oral Glucose Tolerance test(OGTT+), Confidence interval(CI), Not documented(ND), Multiple logistic regression(MLOR), Analysis of covariance(ANCOVA), Standard deviation (SD), Interquartile range(IQR) and Appropriate for gestational age(AGA).

Meta-analysis

S8.1 Figure Meta-analysis of adjusted odds ratio for the association between maternal TC levels and LGA



S8.2 Figure Meta-analysis for mean difference of maternal TC levels between LGA and reference groups in the third trimester



High-density lipoprotein cholesterol (HDL-C)

S8.2 Table Results summary of the association of maternal HDL-C levels with LGA

Study ID	Countries	Population	Sample size	Trimesters	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors					
												a	b	c	d	e	f
Jin et al.2016	China	non-GDM	934	1	ND	ND			ND	ND	7	ND	ND	ND	ND	×	ND
Lei et al.2016	China	General	5,535	2	Crude OR^	**0.75**	**0.63**	**0.89**	ND	Logistic regression	6	×	×	×	×	×	×
Jin et al.2016	China	non-GDM	934	2	ND	ND			ND	ND	7	ND	ND	ND	ND	√	ND
Di et al.2005	Italy	OGTT+	83	2	ND	ND			ND	ND	5	ND	ND	ND	ND	×	ND
Mossayebi et al.2014	Iran	General	82	3	Crude OR*	**0.06**	**0.01**	**0.29**	ND	Chi-squared test	5	×	×	×	×	√	×
Retnakaran et al.2012	Canada	non-GDM	472	3	Crude OR	0.89	0.69	1.15	ND	Logistic regression	7	×	×	×	×	√	×
Ye et al.2015	China	non-GDM	1,204	3	Adjusted OR	**0.62**	**0.47**	**0.82**	ND	MLOR	8	√	√	√	√	√	×
Retnakaran et al.2012	Canada	non-GDM	472	3	Adjusted OR	0.99	0.70	1.39	ND	MLOR	7	√	√	√	√	√	√
Jin et al.2016	China	non-GDM	934	3	Adjusted OR	0.79	0.52	1.21	0.281	MLOR	7	√	√	√	×	√	×
Mossayebi et al.2014	Iran	General	82	3	Adjusted OR*	1.67	0.19	14.29	ND	MLOR	5	√	√	×	√	√	√
Hou et al.2014*	China	non-GDM	2,790	3	Adjusted OR¶	0.81	0.64	1.04	ND	MLOR	7	√	√	×	×	√	×
Laleh et al.2013	Iran	GDM	112	3	p	ND			>0.05	ANCOVA	7	√	√	×	×	×	×
					mmol/L	***Reference***		***LGA***									

Hou et al.2014	China	non-GDM	2,790	3	Median (IQR)	**1.76 (AGA) (1.52, 2.05)**	**1.70 (1.48, 1.95)**	**0.000**	Mann-Whitney U test		7	×	×	×	×	√	×
Slagjana et al.2014	Yugoslavia	non-GDM	200	3	$\bar{x}$±SD	**1.6±0.4(non-LGA)**	**1.3±0.4**	**0.001**	Student t test		5	×	×	×	×	×	×
Son et al.2010	Korea	GDM	104	3	$\bar{x}$±SD	1.7±0.5(non-LGA)	1.6±0.3	0.232	Student t test		5	×	×	×	×	√	×

The bold font represents statistically significant results.

^ Results was calculated with self-defined cut-off point: 1.3 mmol/L

* Result was calculated by comparing the highest quartile with the lowest quartile maternal HDL-C level

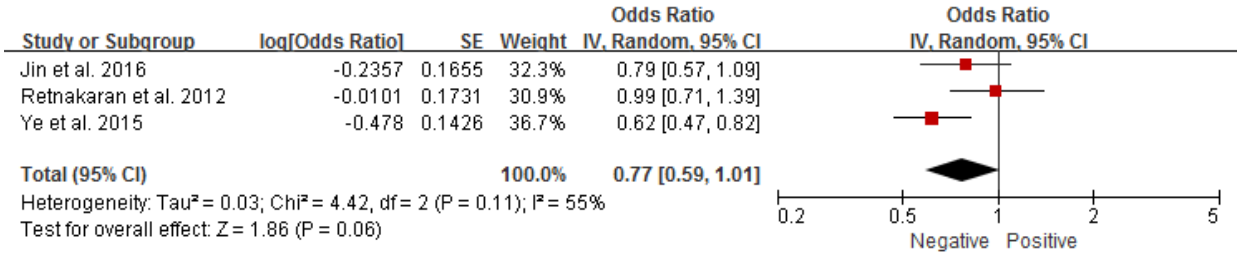
¶ Result was calculated by comparing the highest tertile with the lowest tertile maternal HDL-C level

Confounding factors: a. Maternal age; b. Pre-pregnancy BMI; c. Gestational weight gain; d. Maternal glucose level; e. pre-term birth; f. Maternal lipid levels.

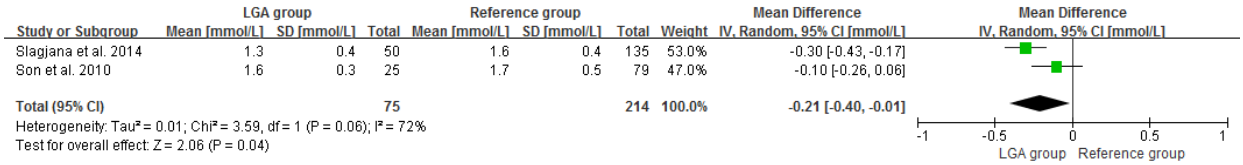
Abbreviation: Gestational diabetes mellitus(GDM), Positive screenes of Oral Glucose Tolerance test(OGTT+), Confidence interval(CI), Not documented(ND), Multiple logistic regression(MLOR), Analysis of covariance(ANCOVA), Standard deviation (SD), Interquartile range(IQR) and Appropriate for gestational age(AGA).

Meta-analysis

S8.3 Figure Meta-analysis of adjusted odds ratio for the association between maternal HDL-C levels and LGA in the third trimester

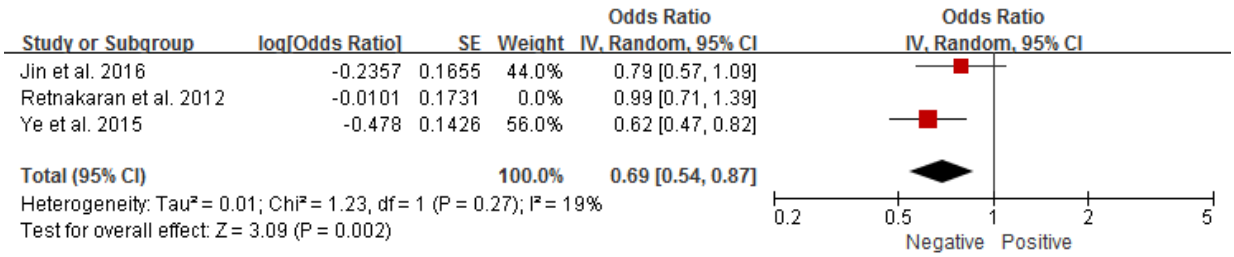


S8.4 Figure Meta-analysis for mean difference of maternal HDL-C levels between LGA and reference groups in the third trimester



Sensitivity analysis

S8.5 Figure Sensitivity analysis _ Adjusted odds ratio _ Exclude study adjust for other maternal lipid levels



Low-density lipoprotein cholesterol (LDL-C)

S8.3 Table Results summary of the association of maternal LDL-C levels with LGA

Study ID	Countries	Population	Sample size	Trimesters	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors					
												a	b	c	d	e	f
Jin et al.2016	China	non-GDM	934	1	ND	ND			ND	ND	7	ND	ND	ND	ND	×	ND
Jin et al.2016	China	non-GDM	934	2	ND	ND			ND	ND	7	ND	ND	ND	ND	√	ND
Di et al.2005	Italy	OGTT+	83	2	ND	ND			ND	ND	5	ND	ND	ND	ND	×	ND
Retnakaran et al.2012	Canada	non-GDM	472	3	Crude OR	0.80	0.61	1.05	ND	Logistic regression	7	×	×	×	×	√	×
Mossayebi et al.2014	Iran	General	82	3	Crude OR*	**5.80**	**1.50**	**22.60**	ND	Chi-squared test	5	×	×	×	×	√	×
Mossayebi et al.2014	Iran	General	77	3	Adjusted OR*	0.80	0.10	4.40	ND	MLOR	5	√	√	×	√	√	√
Hou et al.2014	China	non-GDM	2,790	3	Adjusted OR¶	0.83	0.59	1.17	ND	MLOR	7	√	√	×	×	√	×
Ye et al.2015	China	non-GDM	1,204	3	Adjusted OR	**1.25**	**1.06**	**1.47**	ND	MLOR	8	√	√	√	√	√	×
Jin et al.2016	China	non-GDM	934	3	Adjusted OR	0.93	0.78	1.11	0.418	MLOR	7	√	√	√	×	√	×
Retnakaran et al.2012	Canada	non-GDM	472	3	Adjusted OR	0.98	0.72	1.34	ND	MLOR	7	√	√	√	√	√	√
Laleh et al.2013	Iran	GDM	112	3	p	ND			>0.05	ANCOVA	7	√	√	×	×	×	×
Son et al.2010	Korea	GDM	104	3	ND	ND			ND	ND	5	ND	ND	ND	ND	√	ND
					mmol/L	***Reference***	***LGA***										

Hou et al.2014	China	non-GDM	2,790	3	Median (IQR)	**3.07 (AGA) (2.47, 3.74)**	**2.95 (2.30, 3.65)**	0.003	Mann-Whitney U test	7	×	×	×	×	√	×
Slagjana et al.2014	Yugoslavia	non-GDM	200	3	$\bar{x}$±SD	3.5±1.2	3.8±1.0	>0.05	Student t test	5	×	×	×	×	×	×

The bold font represents statistically significant results.

* Result was calculated by comparing the highest quartile with the lowest quartile maternal LDL-C level

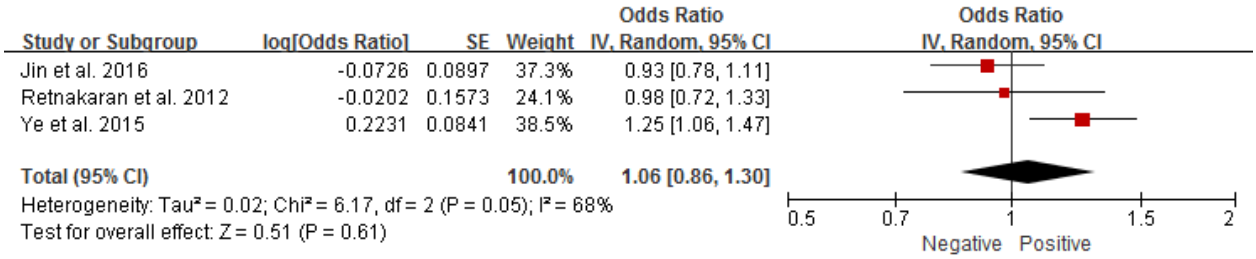
¶ Result was calculated by comparing the highest tertile with the lowest tertile maternal LDL-C level

Confounding factors: a. Maternal age; b. Pre-pregnancy BMI; c. Gestational weight gain; d. Maternal glucose level; e. pre-term birth; f. Maternal lipid levels.

Abbreviation: Gestational diabetes mellitus(GDM), Positive screenes of Oral Glucose Tolerance test(OGTT+), Confidence interval(CI), Not documented(ND), Multiple logistic regression(MLOR), Analysis of covariance(ANCOVA), Standard deviation (SD), Interquartile range(IQR) and Appropriate for gestational age(AGA).

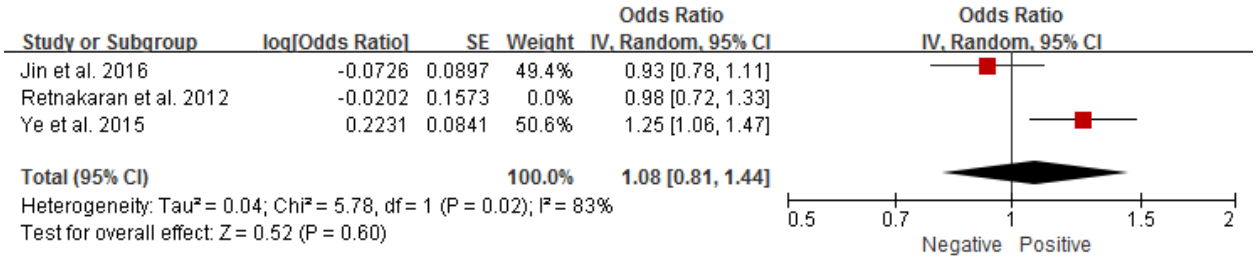
Meta-analysis

S8.4 Figure Meta-analysis of adjusted odds ratio for the association between maternal LDL-C levels and LGA in the third trimester



Sensitivity analysis

S8.5 Figure Sensitivity analysis _ Adjusted odds ratio _ The third trimester_ exclude studies adjust for other maternal lipid levels



Triglycerides (TG)

S8.4 Table Results summary of the association of maternal TG levels with LGA

Study ID	Countries	Population	Sample size	Trimesters	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	P	Statistical methods	Quality	The control of confounding factors					
												a	b	c	d	e	f
Jin et al.2016	China	non-GDM	934	1	ND	ND			ND	ND	7	ND	ND	ND	ND	×	ND
Vrijkotte et al.2012	Netherlands	non-GDM	4,008	1	Adjusted OR	**1.48**	**1.23**	**1.78**	ND	MLOR	8	√	√	×	×	×	×
Vrijkotte et al.2012	Netherlands	non-GDM	4,008	1	Crude OR	**1.44**	**1.20**	**1.71**	ND	Logistic regression	8	×	×	×	×	×	×
Lei et al.2016	China	General	5,535	2	Crude OR^	**1.60**	**1.42**	**2.01**	ND	Logistic regression	6	×	×	×	×	×	×
Di et al.2005	Italy	OGTT+	83	2	Crude OR^	5.60	0.93	33.77	ND	Chi-squared test	5	×	×	×	×	×	×
Jin et al.2016	China	non-GDM	934	2	ND	ND			ND	ND	7	ND	ND	ND	ND	√	ND
Retnakaran et al.2012	Canada	non-GDM	472	3	Crude OR	1.26	0.98	1.62	ND	Logistic regression	7	×	×	×	×	√	×
Ahmad et al. 2006	Malaysia	non-GDM	246	3	Crude OR^	**3.07**	**1.33**	**7.08**	ND	Chi-squared test	6	×	×	×	×	√	×
Kitajima et al.2001	Japan	OGTT +	146	3	Crude OR^	**14.80**	**1.59**	**137.28**	**0.012**	Chi-squared test	6	×	×	×	×	√	×
Mossayebi et al.2014	Iran	General	154	3	Adjusted OR	**1.04**	**1.02**	**1.05**	ND	MLOR	5	√	√	×	√	√	√
Ye et al.2015	China	non-GDM	1,204	3	Adjusted OR	**1.15**	**1.03**	**1.27**	ND	MLOR	8	√	√	√	√	√	×
Retnakaran et al.2012	Canada	non-GDM	472	3	Adjusted OR	0.98	0.70	1.38	ND	MLOR	7	√	√	√	√	√	√
Jin et al.2016	China	non-GDM	934	3	Adjusted OR	**1.13**	**1.02**	**1.26**	**0.025**	MLOR	7	√	√	√	×	√	×

Hou et al.2014	China	non-GDM	2,790	3	Adjusted OR¶	**3.30**	**1.18**	**9.27**	ND	MLOR	7	√	√	×	×	√	×
Ahmad et al. 2006	Malaysia	non-GDM	246	3	Adjusted OR^	**1.48**	**1.15**	**1.93**	ND	MLOR	6	×	√	×	√	√	×
Kitajima et al.2001	Japan	OGTT +	146	3	Adjusted OR^	**11.60**	**1.10**	**122.00**	**0.040**	MLOR	6	×	×	×	×	√	×
Son et al.2010	Korea	GDM	104	3	Adjusted OR^	**4.43**	**1.33**	**14.82**	ND	MLOR	5	√	√	√	×	√	×
Schaefer-Graf et al.2008	German	GDM	150	3	p	**ND**			**0.040**	MLOR	5	√	√	√	√	×	×
Laleh et al.2013	Iran	GDM	112	3	p	**+**			**0.040**	ANCOVA	7	√	√	×	×	×	×
					mmol/L	***Reference***		***LGA***									
Hou et al.2014	China	non-GDM	2,790	3	Median (IQR)	**3.02 (AGA) (2.48, 3.69)**		**3.19 (2.61, 3.97)**	**0.000**	Mann-Whitney U test	7	×	×	×	×	√	×
Slagjana et al.2014	Yugoslavia	non-GDM	200	3	$\bar{x}$±SD	**3.1±1.1**		**3.8±1.8**	**0.012**	Student t test	5	×	×	×	×	×	×

The bold font represents statistically significant results.

^ Results was calculated with self-defined cut-off point: Lei et al.2016, 3.49 mmol/L; Di et al.2005, 2.30mmol/L; Ahmad et al. 2006, 2.78mmol/L; Kitajima et al. 2001, 2.92 mmol/L; Son et al. 2010, 3.33mmol/L.

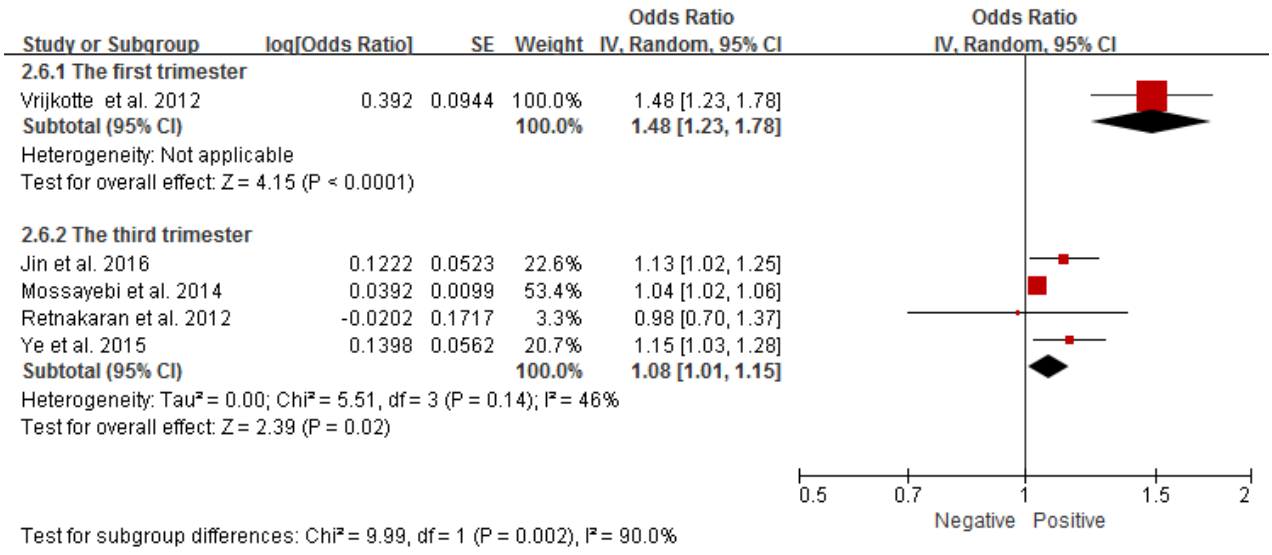
¶ Result was calculated by comparing the highest tertile with the lowest tertile maternal TG level

Confounding factors: a. Maternal age; b. Pre-pregnancy BMI; c. Gestational weight gain; d. Maternal glucose level; e. pre-term birth; f. Maternal lipid levels.

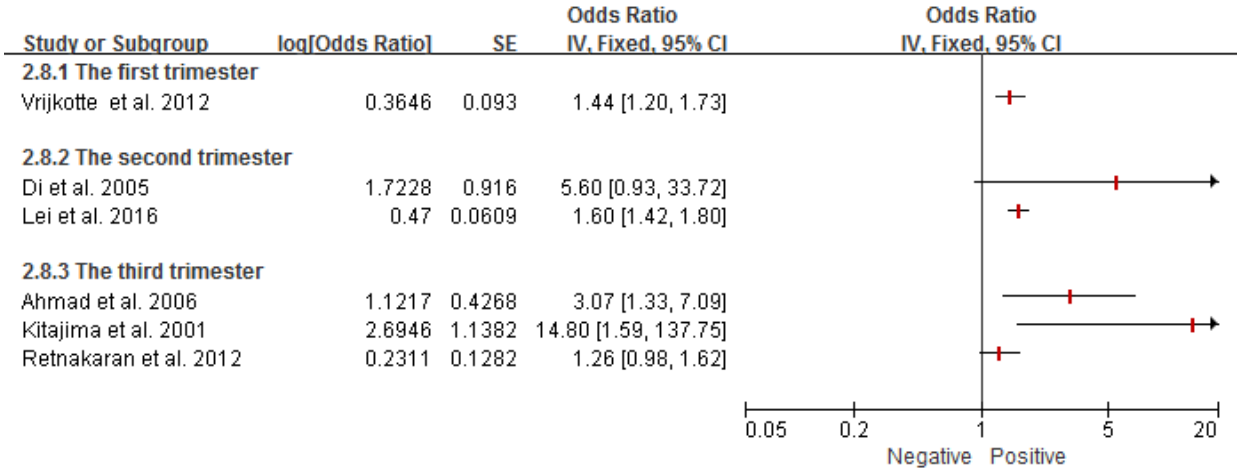
Abbreviation: Gestational diabetes mellitus(GDM), Positive screenes of Oral Glucose Tolerance test(OGTT+), Confidence interval(CI), Not documented(ND), Multiple logistic regression(MLOR), Analysis of covariance(ANCOVA), Standard deviation (SD), Interquartile range(IQR) and Appropriate for gestational age(AGA).

Meta-analysis

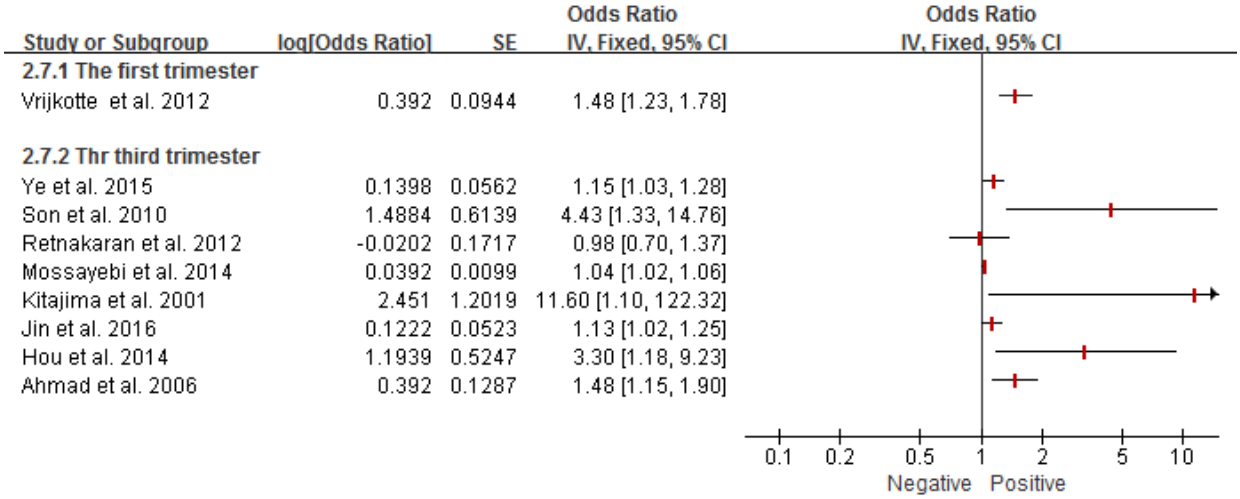
S8.6 Figure Meta-analysis of adjusted odds ratio for the association between maternal TG levels and LGA throughout pregnancy



S8.7 Figure Forest plots of crude odds ratio for the association between maternal TG levels and LGA throughout pregnancy

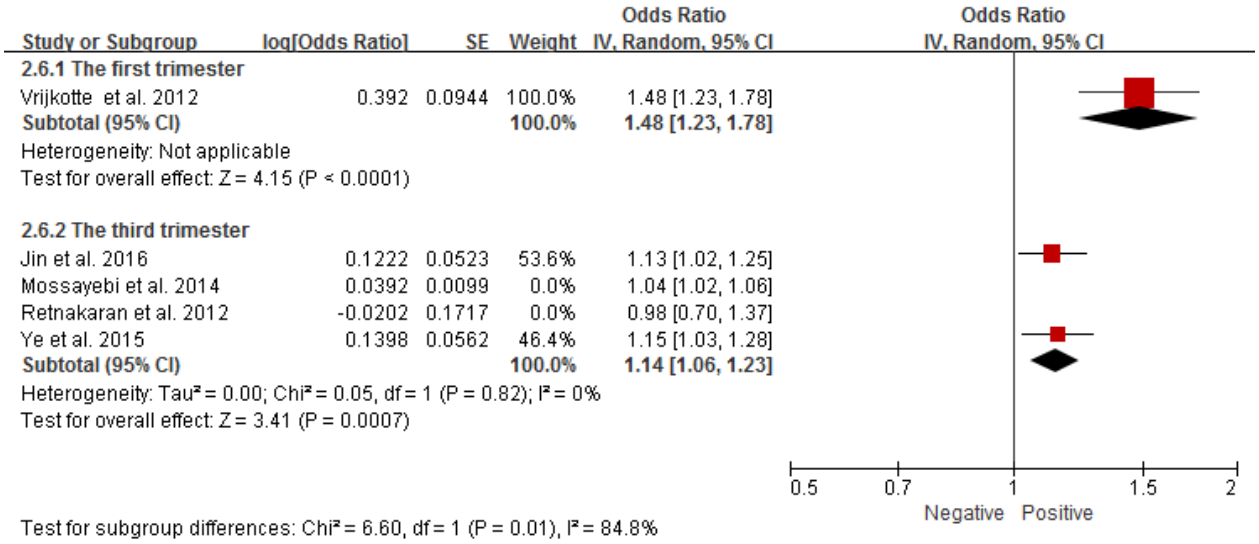


S8.8 Figure Forest plots of adjusted odds ratio for the association between maternal TG levels and LGA throughout pregnancy



Sensitivity analysis

S8.9 Figure Sensitivity analysis Exclude studies adjust for other maternal lipid levels



Free fatty acids (FFAs)

S8.5 Table Results summary of the association of maternal FFAs levels with LGA

Study ID	Countries	Population	Sample size	Trimesters	Reported measures	Effect size	p	Statistical methods	Quality	The control of confounding factors						Unit
										a	b	c	d	e	f	
Schaefer-Graf et al.2008	German	GDM	150	3	p	**ND**	**0.008**	MLOR	5	√	√	√	√	×	×	μmol/L
Kitajima et al.2001	Japan	OGTT +	146	3	ND	ND	ND	ND	6	×	×	×	×	√	×	ND

S7 Systematic review: Data analysis for Small for gestational age (SGA)

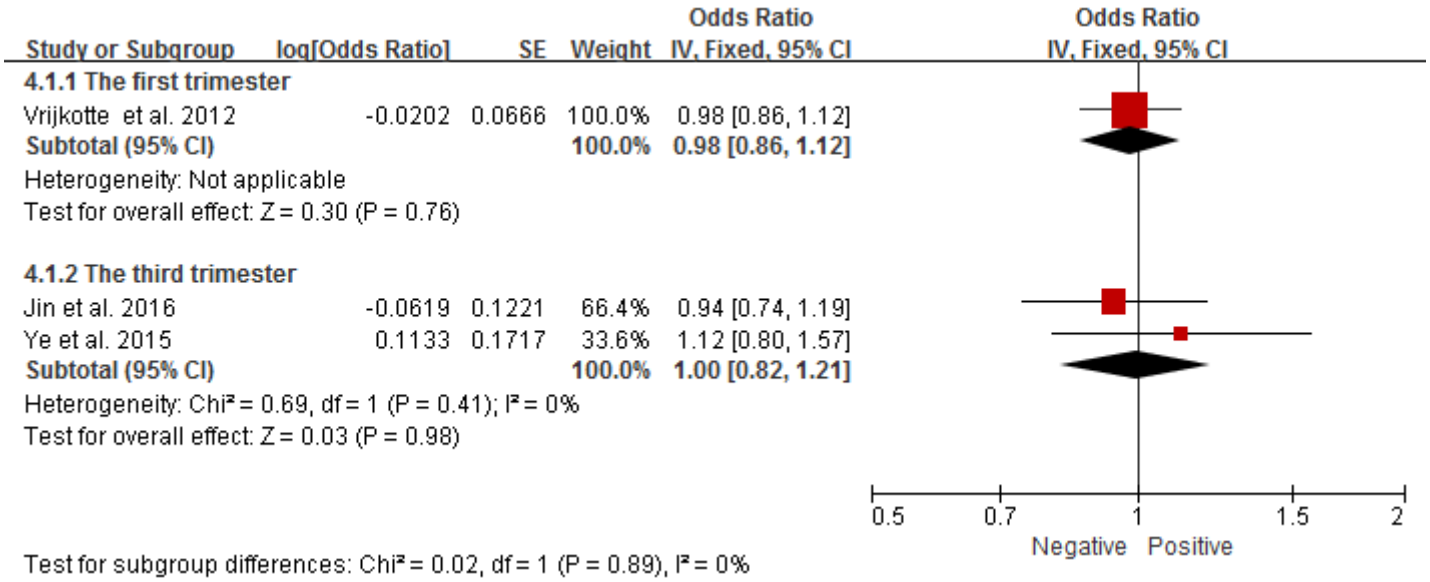
Total cholesterol (TC)

S9.1 Table Results summary of the association of maternal TC levels with SGA

Study ID	Countries	Population	Sample size	Trimesters	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors					
												a	b	c	d	e	f
Vrijkotte et al.2012	Netherlands	non-GDM	4,008	1	Crude OR	0.97	0.85	1.10	ND	Logistic regression	8	×	×	×	×	×	×
Vrijkotte et al.2012	Netherlands	non-GDM	4,008	1	Adjusted OR	0.98	0.86	1.12	ND	MLOR	8	√	√	×	×	×	×
Jin et al.2016	China	non-GDM	934	1	ND	ND			ND	ND	7	ND	ND	ND	ND	√	ND
Jin et al.2016	China	non-GDM	934	2	ND	ND			ND	ND	7	ND	ND	ND	ND	√	ND
Ye et al.2015	China	non-GDM	912	3	Adjusted OR	0.94	0.74	1.20	ND	MLOR	8	√	√	√	√	√	×
Jin et al.2016	China	non-GDM	934	3	Adjusted OR	1.12	0.80	1.56	0.520	MLOR	7	√	√	√	×	√	×
Slagjana et al.2014	Yugoslavia	non-GDM	200	3	p				>0.05	Student t test	5	×	×	×	×	×	×

Confounding factors: a. Maternal age; b. Pre-pregnancy BMI; c. Gestational weight gain; d. Maternal glucose level; e. pre-term birth; f. Maternal lipid levels.
Abbreviation: Gestational diabetes mellitus(GDM), Confidence interval(CI), Not documented(ND), Multiple logistic regression(MLOR).

S9.1 Figure Meta-analysis of adjusted odds ratio for the association between maternal TC levels and SGA throughout pregnancy



High-density lipoprotein cholesterol (HDL-C)

S9.2 Table Results summary of the association of maternal HDL-C levels with SGA

Study ID	Countries	Population	Sample size	Trimesters	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors					
												a	b	c	d	e	f
Jin et al.2016	China	non-GDM	934	1	Adjusted OR	1.41	0.32	5.38	ND	MLOR	7	√	√	√	×	√	×
Lei et al.2016	China	General	5,535	2	Crude OR^	1.13	0.80	1.61	ND	Logistic regression	6	×	×	×	×	×	×
Jin et al.2016	China	non-GDM	934	2	Adjusted OR	1.88	0.47	7.59	ND	MLOR	7	√	√	√	×	√	×
Ye et al.2015	China	non-GDM	912	3	Adjusted OR	1.57	0.87	2.83	ND	MLOR	8	√	√	√	√	√	×
Jin et al.2016	China	non-GDM	934	3	Adjusted OR	**3.15**	**1.15**	**8.65**	**0.026**	MLOR	7	√	√	√	×	√	×
Slagjana et al.2014	Yugoslavia	non-GDM	200	3	p				>0.05	Student t test	5	×	×	×	×	×	×

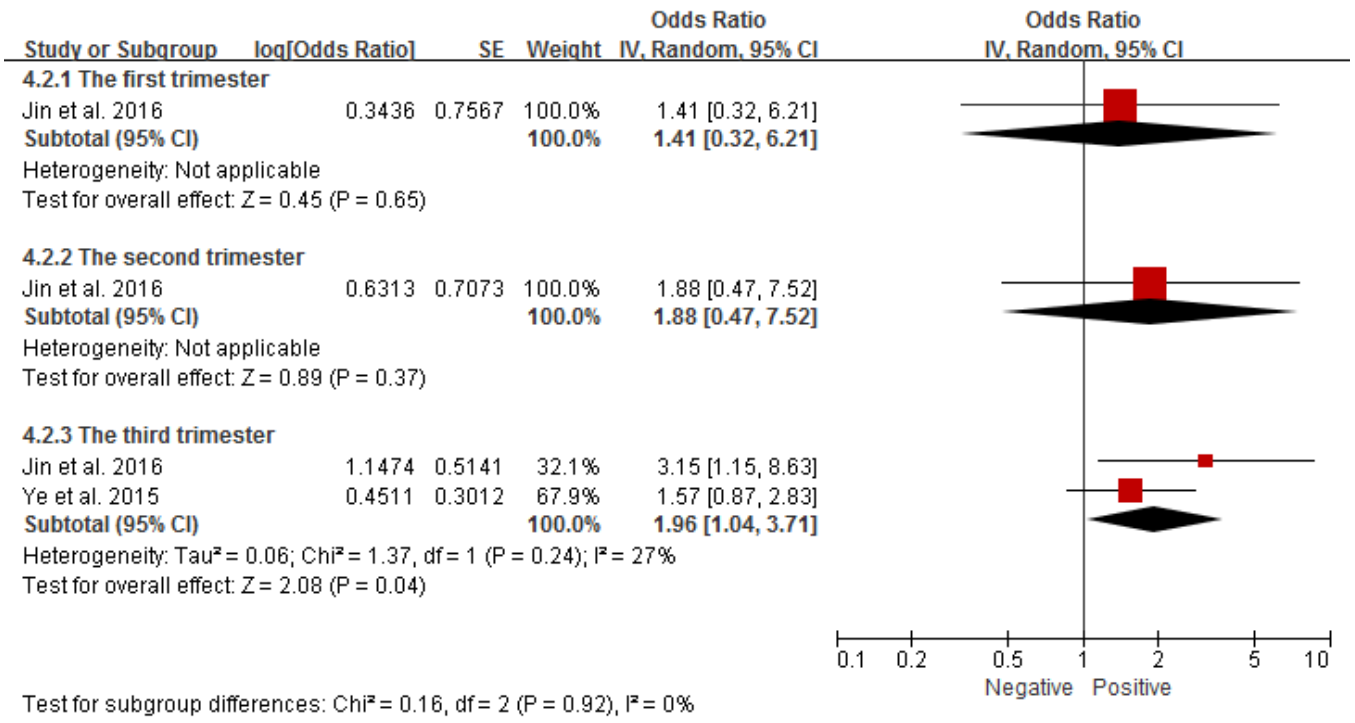
The bold font represents statistically significant results.

^ Results was calculated with self-defined cut-off point: 1.3 mmol/L

Confounding factors: a. Maternal age; b. Pre-pregnancy BMI; c. Gestational weight gain; d. Maternal glucose level; e. pre-term birth; f. Maternal lipid levels.

Abbreviation: Gestational diabetes mellitus(GDM), Confidence interval(CI), Not documented(ND), Multiple logistic regression(MLOR).

S9.2 Figure Meta-analysis of adjusted odds ratio for the association between maternal HDL-C levels and SGA throughout pregnancy



Low-density lipoprotein cholesterol (LDL-C)

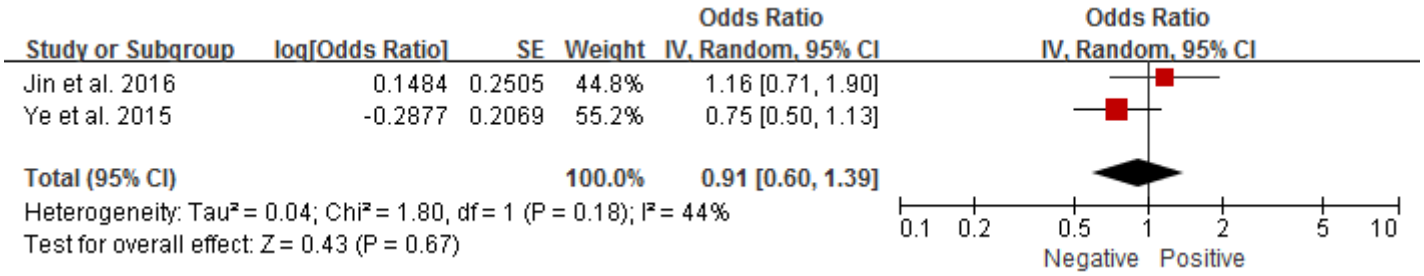
S9.3 Table Results summary of the association of maternal LDL-C levels with SGA

Study ID	Countries	Population	Sample size	Trimesters	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors					
												a	b	c	d	e	f
Jin et al.2016	China	non-GDM	934	1	ND	ND			ND	ND	7	ND	ND	ND	ND	√	ND
Jin et al.2016	China	non-GDM	934	2	ND	ND			ND	ND	7	ND	ND	ND	ND	√	ND
Ye et al.2015	China	non-GDM	912	3	Adjusted OR	0.75	0.50	1.14	ND	MLOR	8	√	√	√	√	√	×
Jin et al.2016	China	non-GDM	934	3	Adjusted OR	1.16	0.71	1.89	0.565	MLOR	7	√	√	√	×	√	×
Slagjana et al.2014	Yugoslavia	non-GDM	200	3	p				>0.05	Student t test	5	×	×	×	×	×	×

Confounding factors: a. Maternal age; b. Pre-pregnancy BMI; c. Gestational weight gain; d. Maternal glucose level; e. pre-term birth; f. Maternal lipid levels.

Abbreviation: Gestational diabetes mellitus(GDM), Confidence interval(CI), Not documented(ND), Multiple logistic regression(MLOR).

S9.3 Figure Meta-analysis of adjusted odds ratio for the association between maternal LDL-C levels and SGA in the third trimester



Triglycerides (TG)

S9.4 Table Results summary of the association of maternal TG levels with SGA

Study ID	Countries	Population	Sample size	Trimesters	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors					
												a	b	c	d	e	f
Jin et al.2016	China	non-GDM	934	1	ND	ND			ND	ND	7	ND	ND	ND	ND	√	ND
Vrijkotte et al.2012	Netherlands	non-GDM	4,008	1	Crude OR	1.06	0.87	1.29	ND	Logistic regression	8	×	×	×	×	×	×
Vrijkotte et al.2012	Netherlands	non-GDM	4,008	1	Adjusted OR	0.97	0.79	1.19	ND	MLOR	8	√	√	×	×	×	×
Jin et al.2016	China	non-GDM	934	2	ND	ND			ND	ND	7	ND	ND	ND	ND	√	ND
Lei et al.2016	China	General	5,535	2	Crude OR^	**1.51**	**1.08**	**2.12**	ND	Logistic regression	6	×	×	×	×	×	×
Ye et al.2015	China	non-GDM	912	3	Adjusted OR	0.69	0.47	1.03	ND	MLOR	8	√	√	√	√	√	×
Jin et al.2016	China	non-GDM	934	3	Adjusted OR	**0.63**	**0.40**	**0.99**	**0.046**	MLOR	7	√	√	√	×	√	×
Slagjana et al.2014	Yugoslavia	non-GDM	200	3	p				**0.012**	Student t test	5	×	×	×	×	×	×

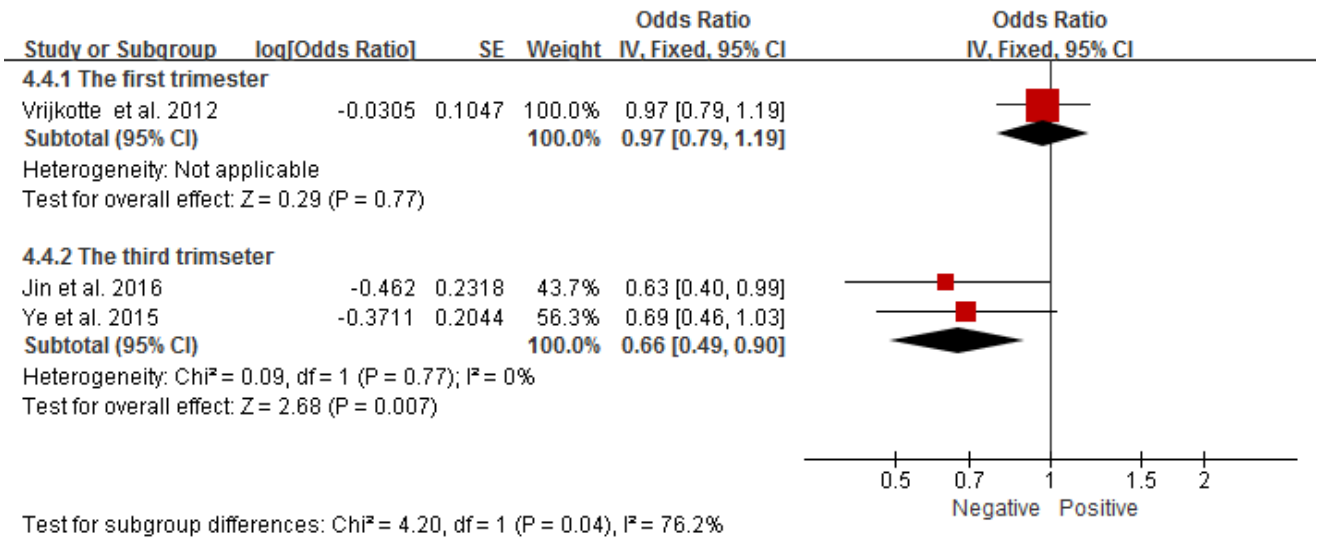
The bold font represents statistically significant results.

^ Results was calculated with self-defined cut-off point: 3.49 mmol/L

Confounding factors: a. Maternal age; b. Pre-pregnancy BMI; c. Gestational weight gain; d. Maternal glucose level; e. pre-term birth; f. Maternal lipid levels.

Abbreviation: Gestational diabetes mellitus(GDM), Confidence interval(CI), Not documented(ND), Multiple logistic regression(MLOR).

S9.4 Figure Meta-analysis of adjusted odds ratio for the association between maternal TG levels and SGA throughout pregnancy



S8 Systematic review: Data analysis for Macrosomia

Total cholesterol (TC)

S10.1 Table Results summary of the association of maternal TC levels with macrosomia

Study ID	Countries	Population	Sample size	Tri.	Reported measures	Effect size	Lower r 95% CI	Upper r 95% CI	p	Statistical methods	Quality	The control of confounding factors							
												a	b	c	d	e	f	g	h
Jin et al.2016	China	non-GDM	934	1	ND	ND			ND	ND	7	ND	ND	ND	ND	ND	ND	×	ND
Clausen et al.2005	Norway	General	1,037	2	Crude OR*	1.10	0.60	2.00	ND	Logistic regression	8	×	×	×	×	×	×	√	×
Clausen et al.2005	Norway	General	1,037	2	Adjusted OR*	1.10	0.60	2.00	ND	MLOR	8	×	×	√	×	×	×	√	×
Zhou et al.2012	China	General	1,000	2	P				>0.05	Non-parametric Mann-Whitney Test	5	×	×	×	×	×	×	×	×
Jin et al.2016	China	non-GDM	934	2	ND	ND			ND	ND	7	ND	ND	ND	ND	ND	ND	√	ND
Jin et al.2016	China	non-GDM	934	3	Adjusted OR	0.99	0.81	1.21	0.903	MLOR	7	×	√	√	√	√	×	√	×

Laleh et al.2013	Iran	GDM	112	3	P	ND	>0.05	Bonferroni multiple comparison test	7	×	×	√	√	×	×	×	×
Mossayebi et al.2014	Iran	General	154	3	ND	ND	ND	ND	5	ND	ND	ND	ND	ND	ND	√	ND

* Result was calculated by comparing the highest quartile with the lowest quartile maternal TC level

Confounding factors: a. Maternal age; b. Pre-pregnancy BMI; c. Gestational weight gain; d. Maternal glucose level; e. pre-term birth; f. Maternal lipid levels.

Abbreviation: Gestational diabetes mellitus(GDM), Positive screenes of Oral Glucose Tolerance test(OGTT+), Confidence interval(CI), No documented(ND), Multiple logistic regression(MLOR), Analysis of covariance(ANCOVA), Standard deviation (SD), Interquartile range(IQR) and Appropriate for gestational age(AGA).

High-density lipoprotein cholesterol (HDL-C)

S10.2 Table Results summary of the association of maternal HDL-C levels with macrosomia

Study ID	Countries	Population	Sample size	Tri.	Reported measures	Effect size	Lower r 95% CI	Upper r 95% CI	p	Statistical methods	Quality	The control of confounding factors							
												a	b	c	d	e	f	g	h
Jin et al.2016	China	non-GDM	934	1	Adjusted OR	0.51	0.19	1.36	0.178	MLOR	7	×	√	√	√	√	×	√	×
Zawiejska et al. 2008	Poland	GDM	357	2	Crude RR	0.59	0.32	1.02	ND	Chi-squared test	5	×	×	×	×	×	×	×	×
Clausen et al.2005	Norway	General	1,025	2	Crude OR*	**0.30**	**0.20**	**0.60**	ND	Logistic regression	8	×	×	×	×	×	×	√	×
Clausen et al.2005	Norway	General	1,025	2	Adjusted OR*	**0.30**	**0.20**	**0.60**	ND	MLOR	8	×	×	√	×	×	×	√	×
Zhou et al.2012	China	General	1,000	2	Adjusted OR^	**0.61**	**0.38**	**0.98**	ND	MLOR	5	×	×	√	√	√	×	×	×
Jin et al.2016	China	non-GDM	934	2	Adjusted OR	**0.25**	**0.09**	**0.73**	0.011	MLOR	7	×	√	√	√	√	×	√	×
Jin et al.2016	China	non-GDM	934	3	Adjusted OR	**0.46**	**0.22**	**0.94**	0.034	MLOR	7	×	√	√	√	√	×	√	×

Laleh et al.2013	Iran	GDM	112	3	p	ND	>0.05	Bonferroni multiple comparison test	7	×	×	√	√	×	×	×	×
Mossayebi et al.2014	Iran	General	154	3	ND	ND	ND	ND	5	ND	ND	ND	ND	ND	ND	√	ND

The bold font represents statistically significant results.

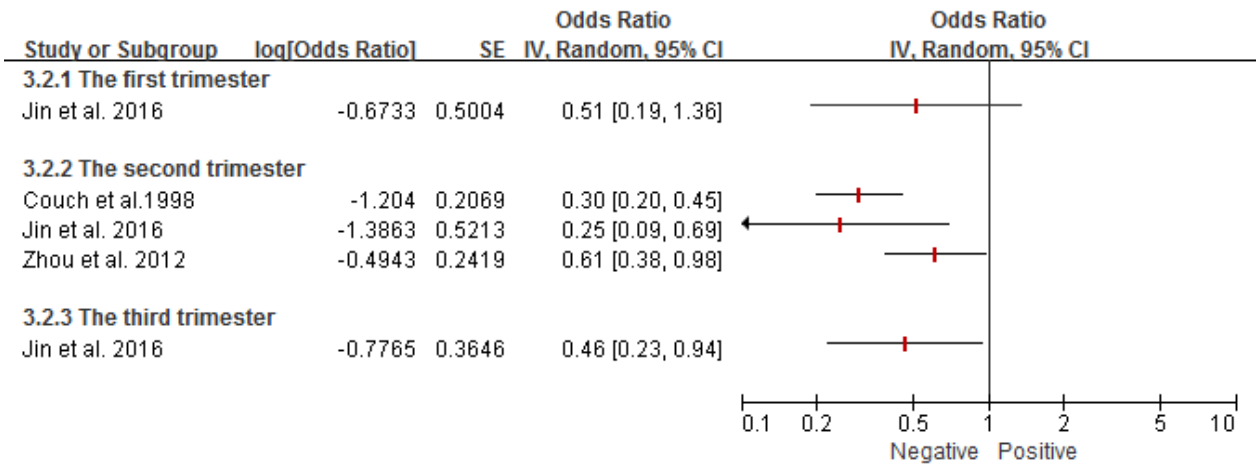
^ Results was calculated with self-defined cut-off point: 2.205mmol/L

* Result was calculated by comparing the highest quartile with the lowest quartile maternal HDL-C level

Confounding factors: a. Maternal age; b. Pre-pregnancy BMI; c. Gestational weight gain; d. Maternal glucose level; e. pre-term birth; f. Maternal lipid levels.

Abbreviation: Gestational diabetes mellitus(GDM), Positive screenes of Oral Glucose Tolerance test(OGTT+), Confidence interval(CI), Not documented(ND), Multiple logistic regression(MLOR), Analysis of covariance(ANCOVA), Standard deviation (SD), Interquartile range(IQR) and Appropriate for gestational age(AGA).

S10.1 Figure Forest plots of adjusted odds ratio for the association between maternal HDL-C levels and macrosomia throughout pregnancy



Low-density lipoprotein cholesterol (LDL-C)

S10.3 Table Results summary of the association of maternal LDL-C levels with macrosomia

Study ID	Countries	Population	Sample size	Tri.	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors							
												a	b	c	d	e	f	g	h
Jin et al.2016	China	non-GDM	934	1	ND	ND			ND	ND	7	ND	ND	ND	ND	ND	ND	×	ND
Clausen et al.2005	Norway	General	1,018	2	Crude OR*	**2.20**	**1.20**	**4.00**	ND	Logistic regression	8	×	×	×	×	×	×	√	×
Clausen et al.2005	Norway	General	1,018	2	Adjusted OR*	**2.10**	**1.20**	**3.90**	ND	MLOR	8	×	×	√	×	×	×	√	×
Zhou et al.2012	China	General	1,000	2	p				>0.05	Non-parametric Mann-Whitney Test	5	×	×	×	×	×	×	×	×
Jin et al.2016	China	non-GDM	934	2	ND	ND			ND	ND	7	ND	ND	ND	ND	ND	ND	√	ND
Jin et al.2016	China	non-GDM	934	3	Adjusted OR	0.93	0.69	1.25	0.621	MLOR	7	×	√	√	√	√	×	√	×
Laleh et al.2013	Iran	GDM	112	3	p	ND			>0.05	Bonferroni multiple comparison test	7	×	×	√	√	×	×	×	×
Mossayebi et al.2014	Iran	General	154	3	ND	ND			ND	ND	5	ND	ND	ND	ND	ND	ND	√	ND

The bold font represents statistically significant results.

* Result was calculated by comparing the highest quartile with the lowest quartile maternal LDL-C level

Confounding factors: a. Maternal age; b. Pre-pregnancy BMI; c. Gestational weight gain; d. Maternal glucose level; e. pre-term birth; f. Maternal lipid levels.

Abbreviation: Gestational diabetes mellitus(GDM), Positive screenes of Oral Glucose Tolerance test(OGTT+), Confidence interval(CI), Not documented(ND), Multiple logistic regression(MLOR), Analysis of covariance(ANCOVA), Standard deviation (SD), Interquartile range(IQR) and Appropriate for gestational age(AGA).

Triglycerides (TG)

S10.4 Table Results summary of the association of maternal TG levels with macrosomia

Study ID	Countries	Population	Sample size	Tri.	Reported measures	Effect size	Lower 95%CI	Upper 95%CI	p	Statistical methods	Quality	The control of confounding factors							
												a	b	c	d	e	f	g	h
Jin et al.2016	China	non-GDM	934	1	ND	ND			ND	ND	7	ND	ND	ND	ND	ND	ND	×	ND
Clausen et al.2005	Norway	General	988	2	Crude OR*	**2.90**	**1.40**	**5.90**	ND	Logistic regression	8	×	×	×	×	×	×	√	×
Clausen et al.2005	Norway	General	988	2	Adjusted OR*	**2.90**	**1.40**	**5.90**	ND	MLOR	8	×	×	√	×	×	×	√	×
Zhou et al.2012	China	General	1,000	2	p				>0.05	Non-parametric Mann-Whitney Test	5	×	×	×	×	×	×	×	×
Jin et al.2016	China	non-GDM	934	2	ND	ND			ND	ND	7	ND	ND	ND	ND	ND	ND	√	ND
Mossayebi et al.2014	Iran	General	154	3	Adjusted OR	**1.04**	**1.02**	**1.07**	ND	MLOR	5	×	×	√	√	×	√	√	√
Jin et al.2016	China	non-GDM	934	3	Adjusted OR	**1.19**	**1.02**	**1.39**	**0.024**	MLOR	7	×	√	√	√	√	×	√	×
Lin et al.2013	China	General	ND	ND	OR^	**2.20**	**1.54**	**3.14**	ND	ND	NA	ND	ND	ND	ND	ND	ND	ND	ND
Laleh et al.2013	Iran	GDM	112	3	p	+			**0.001**	Bonferroni multiple comparison test	7	×	×	√	√	×	×	×	×

The bold font represents statistically significant results.

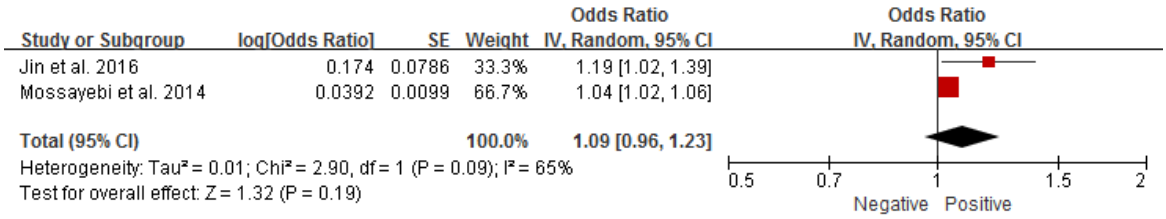
^ Results was calculated with self-defined cut-off point: 2.27 mmol/L

* Result was calculated by comparing the highest quartile with the lowest quartile maternal TG level

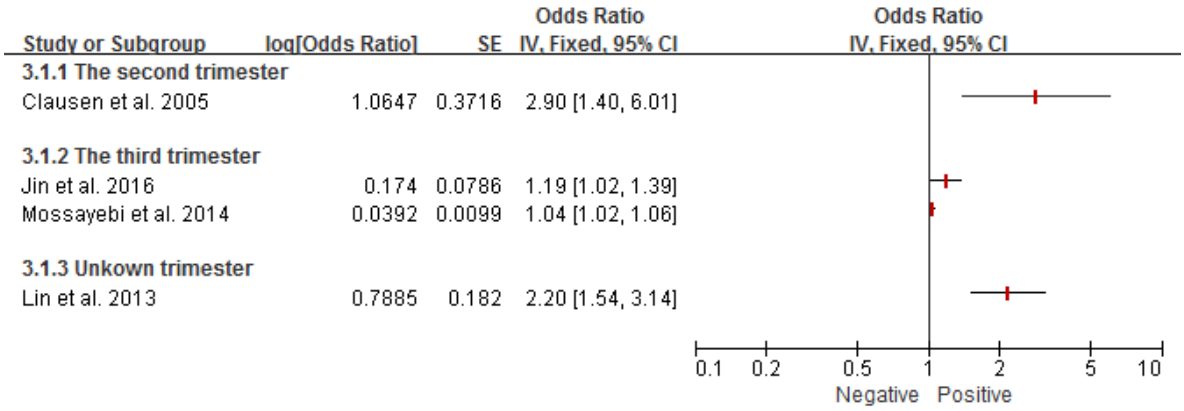
Confounding factors: a. Maternal age; b. Pre-pregnancy BMI; c. Gestational weight gain; d. Maternal glucose level; e. pre-term birth; f. Maternal lipid levels.

Abbreviation: Gestational diabetes mellitus(GDM), Positive screenes of Oral Glucose Tolerance test(OGTT+), Confidence interval(CI), No documented(ND), Not applicable(NA), Multiple logistic regression(MLOR), Analysis of covariance(ANCOVA), Standard deviation (SD), Interquartile range(IQR) and Appropriate for gestational age(AGA).

S10.2 Figure Meta-analysis of adjusted odds ratio for the association between maternal TG levels and macrosomia



S10.3 Figure Forest plots of adjusted odds ratio for the association between maternal TG levels and macrosomia



S9 Bayesian study: Sample size calculation

We powered the study for the potentially least associated maternal metabolic risk factor (triglycerides) for birthweight. Knopp et al. reported a correlation between maternal triglycerides and birthweight of r = 0.09 ($p < 0.05$) in non-GDM women (361)(1). We, conservatively assumed an effect size of 0.08. STATA 14.0 is used to calculate the sample size. After using 'Fisher's z tests comparing one correlation to a reference value' tool, a sample of 1225 will give 80% power to detect a correlation of 0.08 at the 5% significance level (two-sided). We conservatively assumed a 20% attrition rate due to missing data and loss to follow up, the giving a sample size of 1,531.

S10 Bayesian study: Testing for degradation for cord blood insulin

No prior literature reported the impact of long-term -80 °C storage on plasma insulin. We therefore fit a regression model to detect the potential degradation for cord blood insulin. The median storage duration of cord blood sample is 488 (IQR 394 to 707) days. Cord blood insulin was found to be slightly degraded over time (r = -0.07, $p = 0.01$). In the multivariate regression model, we included sample storage time as a covariate. In the additive Bayesian Network analysis, adjustments were made to account for any degradation by correcting the initial value using linear regression methods (adjusted cord blood insulin = initial value of cord blood insulin + (mean value of sample storage time – sample storage time) * β, β = -0.0044446).

S11 Bayesian study: Adjusting gestational age at sampling for maternal lipid profile

The average sampling time for maternal overnight fasting blood sample at the second trimester is 20.46 gestation weeks. The table below represented the estimate of associations between maternal plasma lipid levels and blood sampling gestational age.

lipids	Regression coefficient (β)
Total cholesterol	0.098
HDL-C	0.015
LDL-C	0.075
Triglycerides	0.059

Linear regression model

Adjustment Equation: Adjusted lipids = initial value + (20.46 – sampling time) * β

S12 Bayesian study: Baseline characteristics of participants with and without missing mid-pregnancy weight records.

Characteristics	Non-missing (n=1,449)	Missing (n=73)	p
Maternal age at enrolment (years)*	29.53 (3.30)	28.99 (3.11)	0.18
Primiparous$	289 (19.94)	10 (13.70)	0.19
Early pregnancy cigarette exposure$	415 (28.68)	22 (28.77)	0.99
GDM$	168(11.59)	13 (17.81)	0.11
Pre-pregnancy BMI (kg/m^2)*	20.47 (2.69)	20.37 (2.58)	0.76

*Mean (Standard deviation), T test used
$Number (proportion), Chi-squared test used

S13 Bayesian study: Sensitivity analysis results

By GDM and non-GDM

β(95%CI)	Pre-pregnancy BMI Z-Score	GWG Z-Score	Glucose Z-Score	HDL-C Z-Score	TG Z-Score
Birthweight Z-Score					
Model 1 (All)	**0.20(0.15, 0.24)**	**0.18(0.13, 0.23)**	**0.08(0.04, 0.12)**	**-0.05(-0.09, -0.00)**	**0.12(0.08, 0.16)**
non-GDM	**0.20(0.15, 0.25)**	**0.18(0.12, 0.24)**	**0.06(0.01, 0.11)**	-0.04(-0.09, 0.00)	**0.13(0.08, 0.17)**
GDM	**0.19(0.09, 0.29)**	0.11(-0.03, 0.25)	**0.16(0.07, 0.24)**	-0.08(-0.19, 0.03)	0.04(-0.09, 0.17)
p	0.84	0.62	0.06	0.55	0.22
Cord blood insulin Z-Score					
Model 2 (All)	**0.10(0.05, 0.15)**	0.06(-0.01, 0.12)	**0.13(0.08, 0.18)**	-0.04(-0.09, 0.01)	**0.06(0.01, 0.11)**
non-GDM	**0.03(0.01, 0.05)**	0.05(-0.01, 0.12)	**0.10(0.04, 0.16)**	-0.03(-0.08, 0.03)	**0.06(0.01, 0.12)**
GDM	**0.14(0.03, 0.25)**	0.09(-0.07,0,26)	**0.19(0.09, 0.29)**	-0.12(-0.27, 0.03)	0.01(-0.12, 0.15)
p	0.38	0.65	0.13	0.23	0.50

Model 1: Adjusted for maternal age, ethic group, parity, early pregnancy cigarette exposures, and delivery mode. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.
Model 2: Adjusted for maternal age, ethic group, parity, early pregnancy cigarette exposures, gestational age, neonatal gender, delivery mode, and sample storage duration. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.
BMI, body mass index; GWG, gestational weight gain; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; GDM, gestational diabetes mellitus.

By lean and overweight group

β(95%CI)	Pre-pregnancy BMI Z-Score	GWG Z-Score	Glucose Z-Score	HDL-C Z-Score	TG Z-Score
Birthweight Z-Score					
Model 1 (All)	**0.20(0.15, 0.24)**	**0.18(0.13, 0.23)**	**0.08(0.04, 0.12)**	**-0.05(-0.09, -0.00)**	**0.12(0.08, 0.16)**
lean	**0.23(0.16, 0.29)**	**0.15(0.09, 0.21)**	**0.08(0.03, 0.12)**	-0.02(-0.07, 0.02)	**0.11(0.06, 0.15)**
overweight	**0.20(0.03, 0.36)**	**0.13(0.01, 0.25)**	0.05(-0.07, 0.17)	-0.09(-0.22, 0.05)	0.13(-0.01, 0.27)
p	0.73	0.79	0.67	0.37	0.75
Cord blood insulin Z-Score					
Model 2 (All)	**0.10(0.05, 0.15)**	0.06(-0.01, 0.12)	**0.13(0.08, 0.18)**	-0.04(-0.09, 0.01)	**0.06(0.01, 0.11)**
lean	**0.15(0.07, 0.23)**	0.05(-0.01, 0.12)	**0.13(0.08, 0.19)**	-0.03(-0.08, 0.02)	**0.07(0.02, 0.12)**
overweight	0.07(-0.09, 0.24)	0.10(-0.04, 0.25)	**0.11(0.00, 0.22)**	-0.11(-0.26, 0.03)	-0.00(-0.13, 0.13)
p	0.41	0.54	0.72	0.25	0.34

Model 1: Adjusted for maternal age, ethic group, parity, early pregnancy cigarette exposures, and delivery mode. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.
Model 2: Adjusted for maternal age, ethic group, parity, early pregnancy cigarette exposures, gestational age, neonatal gender, delivery mode, and sample storage duration. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.
BMI, body mass index; GWG, gestational weight gain; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; GDM, gestational diabetes mellitus.

By fasting maternal blood and non-fasting maternal blood

β(95%CI)	Pre-pregnancy BMI Z-Score	GWG Z-Score	Glucose Z-Score	HDL-C Z-Score	TG Z-Score
Birthweight Z-Score					
Model 1 (All)	-	-	-	**-0.05(-0.09, -0.00)**	**0.12(0.08, 0.16)**
fasting	-	-	-	**-0.05(-0.10, -0.01)**	**0.12(0.08, 0.16)**
non-fasting	-	-	-	0.01(-0.12, 0.14)	0.07(-0.07, 0.21)
p	-	-	-	0.39	0.50
Cord blood insulin Z-Score					
Model 2 (All)	-	-	-	-0.04(-0.09, 0.01)	**0.06(0.01, 0.11)**
fasting	-	-	-	-0.04(-0.09, 0.01)	**0.06(0.01, 0.11)**
non-fasting	-	-	-	-0.01(-0.17, 0.14)	0.11(-0.04, 0.25)
p	-	-	-	0.73	0.56

Model 1: Adjusted for maternal age, ethic group, parity, early pregnancy cigarette exposures, and delivery mode. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.
Model 2: Adjusted for maternal age, ethic group, parity, early pregnancy cigarette exposures, gestational age, neonatal gender, delivery mode, and sample storage duration. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.
BMI, body mass index; GWG, gestational weight gain; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; GDM, gestational diabetes mellitus.

By primiparous women and non-primiparous women

β(95%CI)	Pre-pregnancy BMI Z-Score	GWG Z-Score	Glucose Z-Score	HDL-C Z-Score	TG Z-Score
Birthweight Z-Score					
Model 1 (All)	**0.20(0.15, 0.24)**	**0.18(0.13, 0.23)**	**0.08(0.04, 0.12)**	-0.05(-0.09, 0.00)	**0.12(0.08, 0.16)**
primiparous	**0.18(0.13, 0.22)**	**0.15(0.09, 0.21)**	**0.08(0.03, 0.12)**	-0.05(-0.09, 0.00)	**0.11(0.07, 0.16)**
non-primiparous	**0.28(0.18, 0.37)**	0.12(-0.01, 0.25)	**0.11(0.01, 0.21)**	-0.04(-0.13, 0.05)	**0.14(0.06, 0.23)**
p	0.06	0.74	0.52	0.87	0.51
Cord blood insulin Z-Score					
Model 2 (All)	**0.10(0.05, 0.15)**	0.06(-0.01, 0.13)	**0.13(0.08, 0.18)**	-0.04(-0.09, 0.01)	**0.06(0.01, 0.11)**
primiparous	**0.10(0.04, 0.15)**	0.06(-0.01, 0.13)	**0.14(0.09, 0.19)**	-0.03(-0.08, 0.04)	**0.07(0.01, 0.12)**
non-primiparous	**0.10(0.00, 0.20)**	-0.00(-0.12, 0.13)	0.09(-0.01,0.19)	**-0.10(-0.19, -0.00)**	0.05(-0.04, 0.14)
p	0.89	0.39	0.42	0.24	0.75

Model 1: Adjusted for maternal age, ethic group, parity, early pregnancy cigarette exposures, and delivery mode. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.
Model 2: Adjusted for maternal age, ethic group, parity, early pregnancy cigarette exposures, gestational age, neonatal gender, delivery mode, and sample storage duration. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.
BMI, body mass index; GWG, gestational weight gain; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; GDM, gestational diabetes mellitus.

By before and after multiple imputation

β(95%CI)	Pre-pregnancy BMI Z-Score	GWG Z-Score	Glucose Z-Score	HDL-C Z-Score	TG Z-Score
Birthweight Z-Score					
before	**0.20(0.15, 0.24)**	**0.18(0.12, 0.23)**	**0.08(0.04, 0.12)**	**-0.04(-0.09, -0.00)**	**0.12(0.08, 0.16)**
after	**0.20(0.15, 0.24)**	**0.18(0.13, 0.23)**	**0.08(0.04, 0.12)**	**-0.05(-0.09, -0.00)**	**0.12(0.08, 0.16)**
Cord blood insulin Z-Score					
before	**0.11(0.06, 0.16)**	**0.07(0.00, 0.13)**	**0.14(0.09, 0.19)**	-0.04(-0.09, 0.01)	**0.07(0.02, 0.12)**
after	**0.10(0.05, 0.15)**	0.06(-0.01, 0.12)	**0.13(0.08, 0.18)**	-0.04(-0.09, 0.01)	**0.06(0.01, 0.11)**

Model for birthweight Z-Score: Adjusted for maternal age, ethic group, parity, and early pregnancy cigarette exposures. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.
Model for cord blood insulin Z-Score: Adjusted for maternal age, ethic group, parity, early pregnancy cigarette exposures, gestational age, neonatal gender, delivery mode, and sample storage duration. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.
BMI, body mass index; GWG, gestational weight gain; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; GDM, gestational diabetes mellitus.

S14 Bayesian study: Additive Bayesian Network methodologies

Introduction to Additive Bayesian Network analysis

A Bayesian network is a probabilistic graphical model that represents a set of variables and their conditional dependencies via a directed acyclic graphs (DAGs).(320) It is a well-established unsupervised machine learning methodology that is typically referred to as structure discovery model for dealing with multidimensional data.(321) Unlike other widely used multivariate approaches, such as principal component analysis, propensity score matching analysis and multivariable regression model, graphical modelling does not involve any dimension reduction. Most graphical models, including path analysis and structural equation modelling, rely on a pre-specified structure, whereas Bayesian network is entirely data-driven.

Unlike the contingency table parameterization in standard Bayesian network models, Additive Bayesian networks (ABN) allow us to obtain interpretable DAGs where each node in graph comprises a generalized linear model (GLM) or a generalized linear mixed model (GLMM, if binary variable involved).(323, 399) There are two mutually dependent parts in ABN model: a network structure (i.e. the DAG) and a set of parameters. Each node (corresponding to the variables in the dataset) in the DAG is the equivalent of a potential dependent variable in a Bayesian GLM or GLMM regression model. While other DAG nodes where relevant as identified by the unsupervised learning act as covariates, having a role of corresponding parameters. Therefore, an ABN model is ideally suited to analyzing highly complex epidemiological data comprising many inter-dependent variables.

The technical process of ABN

After an initial data preparation phase we used a three-step procedure to determine an optimal DAGs for our data.

Step 0 Data pre-processing

Ten variables were chosen for ABN based on our knowledge gained from prior literature and findings of the classical statistical analyses. These included maternal age, maternal pre-pregnancy BMI, maternal fasting glucose concentration in OGTT, early gestational weight gain (GWG, adjusted for gestational age at weight measurement), maternal fasting plasma high-density lipoprotein cholesterol (HDL-C, adjusted for gestational age at blood sampling) in 2nd trimester, maternal fasting plasma triglycerides in 2nd trimester (adjusted for gestational age at blood sampling), birthweight Z-Score (adjusted for gestational age at delivery and neonatal gender), cord blood insulin (CBI, adjusted for sample storage duration) concentration, gestational age at delivery, and neonatal gender. All continuous variables were standardized to Z-Scores to eliminate the influence of different measurement units (maternal triglycerides and cord blood insulin were log-transformed before standardization). Participants with data missing for at least one of these ten variables (6% of participants) were excluded from the analysis. The number of mother-child pairs that was finally included in ABN analysis is 1,429.

Step 1 Identification of the optimal model

The identification of the single optimal model is referred to as structure discovery. The purpose of this step is to combine all individual GLMs into a single, probabilistically cohesive model describing all the inter-dependent relationships via a DAG. We blocked all directions of arcs between variables that are biologically impossible to occur. This was done using the adjacency matrix in figure S1.

```
ban <- matrix( c(
  # 01 02 03 04 05 06 07 08 09 10
     0, 1, 1, 1, 1, 1, 1, 1, 1, 1, # 01 mage
     0, 0, 1, 1, 1, 1, 1, 1, 1, 1, # 02 prebmi
     0, 0, 0, 0, 0, 0, 1, 1, 1, 1, # 03 gwg
     0, 0, 0, 0, 0, 0, 1, 1, 1, 1, # 04 glu
     0, 0, 0, 0, 0, 0, 1, 1, 1, 1, # 05 hdl
     0, 0, 0, 0, 0, 0, 1, 1, 1, 1, # 06 tg
     0, 0, 0, 0, 0, 0, 0, 0, 0, 0, # 07 bwz
     0, 0, 0, 0, 0, 0, 0, 0, 0, 0, # 08 ins
     1, 1, 1, 1, 1, 1, 1, 1, 0, 1, # 09 sex
     0, 0, 0, 0, 0, 0, 0, 0, 0, 0 # 10 gaw
), byrow=TRUE, ncol=10)
```

Variable labels explanation: 01 mage, maternal age; 02 prebmi, maternal pre-pregnancy BMI; 03 gwg, gestational weight gain; 04 glu, maternal fasting glucose level; 05 hdl, maternal plasma high-density lipoprotein cholesterol level; 06 tg, maternal plasma triglyceride level; 07 bwz, birthweight Z-Score; 08 ins, cord blood insulin; 09 sex, neonatal gender; 10 gaw, gestational age at delivery. Same labels also apply to the numbers across the top of the matrix.
DAG definition. Rows are children nodes, columns are parent nodes. 1 represents block from parent node (column) towards child node (row), 0 represents unblock.

Figure S1 ABN block matrix definition

To find the DAG with the best goodness of fit (network score - log marginal likelihood), exact searches were conducted across the parent limits (the limit number of arcs from parent nodes to child node), starting from a minimum of 1 and reaching a maximum of 9. As shown in Figure S2, we found that the goodness of fit (maximum marginal likelihood=-19153.30) does not improve when the number of parent limit is greater than 4.

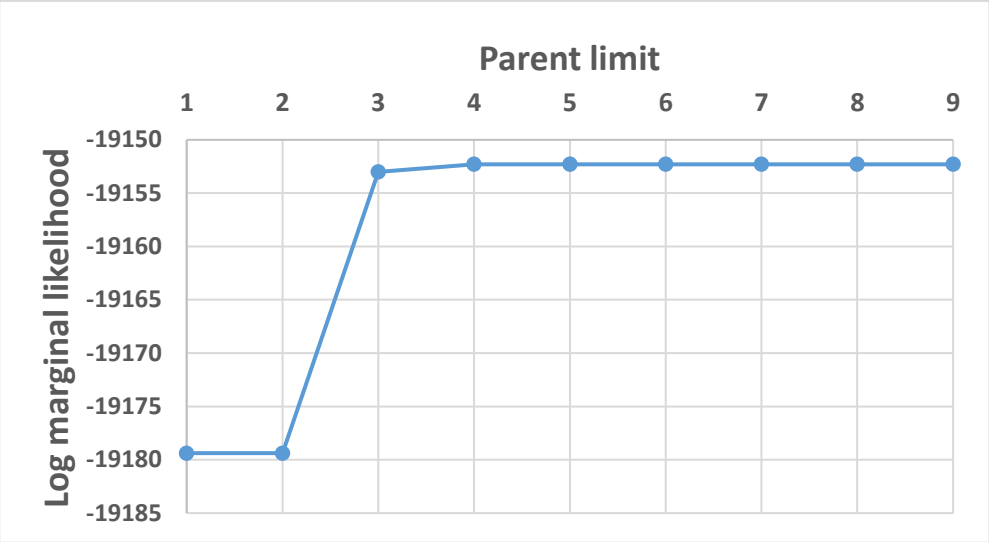
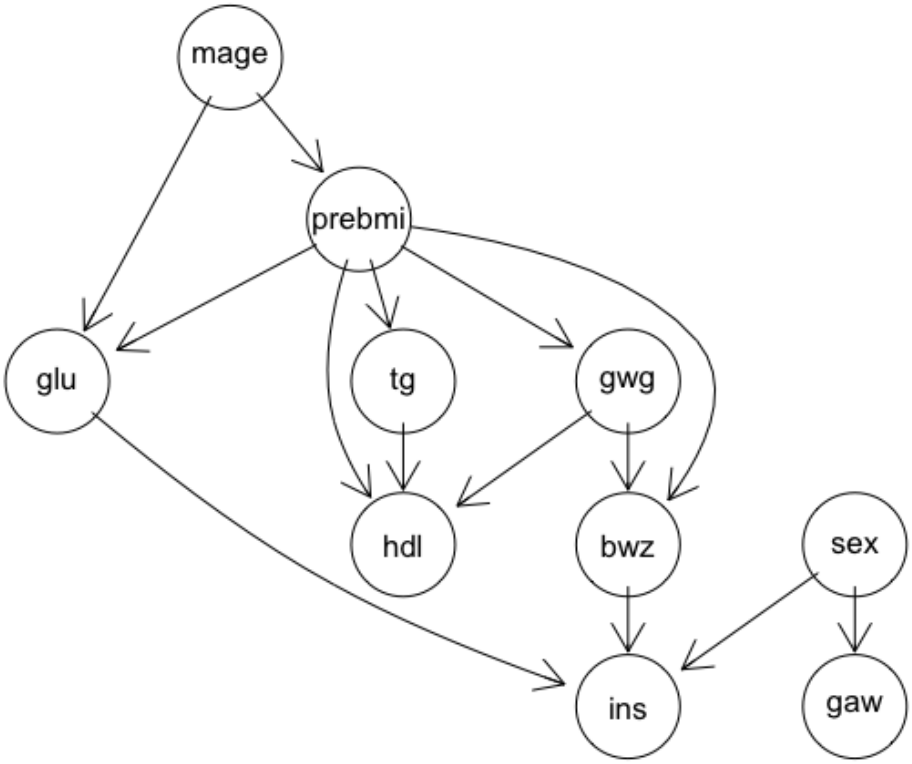


Figure S2 Comparison of goodness of fits for different parent limits



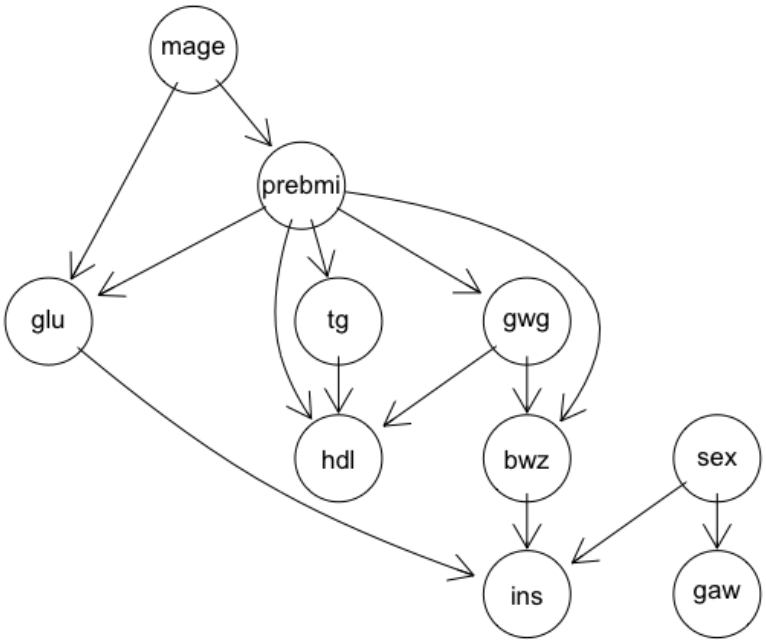
Variables explanation: mage, maternal age; prebmi, maternal pre-pregnancy BMI; gwg, gestational weight gain; glu, maternal fasting glucose level; hdl, maternal plasma high-density lipoprotein cholesterol level; tg, maternal plasma triglyceride level; bwz, birthweight Z-Score; ins, cord blood insulin; sex, neonatal gender; gaw, gestational age at delivery.

Figure S3 The identified optimal DAG from the initial search

Step 2 Adjustment for overfitting: parametric bootstrapping

We have identified the optimal DAG, but there is a risk of overfitting because of the combinatoric nature of Bayesian hypotheses. To address this, 12,800 independent parametric bootstrapping analyses were performed. This involves simulating data sets of the same size as the original dataset, and see how often the different structural features are recovered. Arcs present in less than 50% frequencies of the globally optimal DAGs estimated from the bootstrap data were considered not to be robust and need to be trimmed (removed) from the DAG generated in the first step.

The resulting optimal summary network was inferred from data with a total of 14 high-confidence arcs across 10 variables (Figure S3). The DAGs presented using pruning at 50% was constructed from 12,800 searches with a parent limit of four parents per node. Collating results across these 12,800 searches, all 14 arcs were recovered for at least 12,742 times, as resulting from the frequencies matrix at Figure S4.



Variables explanation: mage, maternal age; prebmi, maternal pre-pregnancy BMI; gwg, gestational weight gain; glu, maternal fasting glucose level; hdl, maternal plasma high-density lipoprotein cholesterol level; tg, maternal plasma triglyceride level; bwz, birthweight Z-Score; ins, cord blood insulin; sex, neonatal gender; gaw, gestational age at delivery.

Figure S4 Optimal final DAG (Containing 14 arcs after removal of arcs supported at less than 50% in bootstrapping)

```
> total.dag
        mage prebmi   gwg   glu hdl    tg   bwz ins   sex gaw
mage       0      0     0     0   0     0     0   0     0   0
prebmi 12800      0     0     0   0     0     0   0     0   0
gwg        0  12800     0     0   0     0     0   0     0   0
glu    12800  12800     0     0   0     0     0   0     0   0
hdl        0  12800 12742     0   0 12799     0   0     0   0
tg         0  12800     0     0   0     0     0   0     0   0
bwz        0  12800 12800     0   0     0     0   0     0   0
ins        0      0     0 12800   0     0 12800   0 12800   0
sex        0      0     0     0   0     0     0   0     0   0
gaw        0      0     0     0   0     0     0   0 12800   0
```

Variables explanation: mage, maternal age; prebmi, maternal pre-pregnancy BMI; gwg, gestational weight gain; glu, maternal fasting glucose level; hdl, maternal plasma high-density lipoprotein cholesterol level; tg, maternal plasma triglyceride level; bwz, birthweight Z-Score; ins, cord blood insulin; sex, neonatal gender; gaw, gestational age at delivery.
Rows are children nodes, columns are parent nodes. The number in each cell represents the frequencies at which each arc (from parent node towards child node) was recovered during 12,800 times of bootstrapping.

Figure S5 Frequencies at which each arc in the original DAG was recovered during bootstrapping

Step 3 Estimating marginal from the final DAG

Once the optimal DAG has been identified, we need to examine the strength of the various arcs in our analysis. This process is very similar to when estimating the marginal for the bootstrapping. All of the effect parameters of this analysis has been provided in Table S1 with 95% confidence intervals.

Arcs	Effect estimate (β, 95%CI)	95% CI
Mage → prebmi	0.19	(0.14, 0.24)
Prebmi → gwg	-0.12	(-0.17, -0.06)
mage → glu	0.11	(0.06, 0.16)
Prebmi → glu	0.14	(0.09, 0.19)
Prebmi → hdl	-0.12	(-0.17, -0.07)
Gwg → hdl	0.09	(0.05, 0.14)
Tg → hdl	-0.33	(-0.38, -0.28)
Prebmi → tg	0.23	(0.18, 0.28)
Prebmi → bwz	0.27	(0.22, 0.32)
Gwg → bwz	0.17	(0.12, 0.22)
Glu → ins	0.12	(0.07, 0.17)
Bwz → ins	0.24	(0.19, 0.29)
Sex → ins	0.19	(0.09, 0.29)
Sex → gaw	0.20	(0.10, 0.31)

Variables explanation: mage, maternal age; prebmi, maternal pre-pregnancy BMI; gwg, gestational weight gain; glu, maternal fasting glucose level; hdl, maternal plasma high-density lipoprotein cholesterol level; tg, maternal plasma triglyceride level; bwz, birthweight Z-Score; ins, cord blood insulin; sex, neonatal gender; gaw, gestational age at delivery.

Table S1

S15 Bayesian study: Results summary of the association between maternal TC/LDL-C and birthweight, cord blood insulin, and the risk of LGA/SGA

β (95%CI)	TC (mmol/L)	LDL-C (mmol/L)
Regression Coefficients (95%CI)		
Birthweight(g)[Δ]	-0.78(-20.32, 18.76)	0.66(-23.23, 21.91)
Cord blood insulin[¶] **(μU/mL)**	-0.14(-0.77, 0.49)	-0.14(-0.87, 0.58)
Odds Ratio (95%CI)		
LGA[§]	0.99(0.79, 1.24)	1.00(0.77, 1.30)
SGA[§]	0.91(0.73, 1.15)	0.98(0.75, 1.27)

Δ Adjusted for maternal age, ethnic group, parity, gestational age, neonatal gender, and early pregnancy cigarette exposures. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.

¶ Adjusted for maternal age, ethnic group, parity, gestational age, neonatal gender, early pregnancy cigarette exposures, and delivery mode. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.

§ Adjusted for maternal age, ethnic group, parity, and early pregnancy cigarette exposures. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.

TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; LGA, large-for-gestational age; SGA, small-for-gestational age.

S16 The association between cord blood metabolic parameter concentrations and storage duration.

Cord blood glucose concentration in EDTA tube at -80℃was not related to storage duration (r = 0.03, p = 0.27), while cord blood insulin was found to be slightly degraded over time (r = -0.07, p = 0.01). Therefore, cord blood insulin was adjusted for storage duration using regression model, the equation of which is as follows: log (adjusted insulin) = log (raw insulin) + slope (mean of storage duration -storage duration). This equation yield a downward adjusted of raw insulin values where storage duration < 536 days and an upward adjustment where storage duration > 536 days. The slope value was -0.0003 in the linear regression model.

S17 Regression analysis for the association between birthweight Z-Score and cord blood glucose Z-Score

```
. reg dlglu_z mage multipara mode preterm sex passsmoke mnationality ib1.bwt_c gdm
```

Source	SS	df	MS		
				Number of obs	= 1,519
				F(10, 1508)	= 72.48
Model	492.994268	10	49.2994268	Prob > F	= 0.0000
Residual	1025.73777	1,508	.680197458	R-squared	= 0.3246
				Adj R-squared	= 0.3201
Total	1518.73203	1,518	1.00048224	Root MSE	= .82474

dlglu_z	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
mage	.0020768	.0072899	0.28	0.776	-.0122226	.0163762
multipara	-.3927499	.059565	-6.59	0.000	-.5095889	-.2759109
mode	-1.360137	.0557865	-24.38	0.000	-1.469565	-1.25071
preterm	-.0520068	.1042104	-0.50	0.618	-.2564196	.1524059
sex	-.03393	.0427075	-0.79	0.427	-.1177024	.0498423
passsmoke	-.0829623	.0470173	-1.76	0.078	-.1751884	.0092638
mnationality	-.3450131	.1416707	-2.44	0.015	-.6229057	-.0671205
bwt_c						
SGA	-.0916044	.0840019	-1.09	0.276	-.2563773	.0731684
LGA	-.0798336	.0893415	-0.89	0.372	-.2550803	.0954132
gdm	.0488811	.0662326	0.74	0.461	-.0810367	.178799
_cons	2.030061	.2567603	7.91	0.000	1.526415	2.533706