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Association of lipid levels during gestation with hypertensive disorders of pregnancy and gestational diabetes mellitus: a prospective longitudinal cohort study

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Abstract

Objective: To study the association of lipid elevation levels during gestation with hypertensive disorders of pregnancy (HDP) and gestational diabetes mellitus (GDM).

Methods: This prospective cohort was conducted in a tertiary maternal hospital in Shanghai China through Feb 2014 to Nov 2014. Lipid profiles including triglycerides (TGs), total cholesterol, low-density lipoprotein and high-density lipoprotein of 1310 eligible women were assessed at the first trimester (10-13+wks), second trimester (22-28wks) and third trimester (30-35wks) consecutively. We planned to assess the association of lipid profiles and composite outcome of HDP/GDM as well as in HDP and GDM separately.

Results: Compared to normal women group, maternal TGs concentrations were higher in the HDP/ GDM group across the three trimesters (P<0.001) and TC and LDL-c concentration was only higher at first trimester in the HDP and GDM group (p<0.05). HDL-c levels were similar in the three groups. Compared to intermediate levels of TGs (25^{th}~ 75^{th} percentile), higher levels of TGs (>75^{th} percentile) were associated with an increased risk of HDP/GDM at each trimester with aORs (95%CI) of 2.04(1.41-2.95), 1.81(1.25-2.63) and 1.78(1.24-2.54) respectively. The greater TGs elevation from first to third trimester (>75^{th} percentile) was associated with an increased risk of HDP with an aOR of 2.09(1.16-3.78). The greater TGs elevation before 28wks was associated with an increased risk of GDM with an aOR of 1.67(1.10-2.54).TGs elevation was positively correlated with weight gain during gestation (R=0.089, P=0.005).

Conclusions: Controlling weight gain in pregnancy could decrease TGs elevation and then decrease the risk of HDP/GDM. TGs could be used as a follow-up index during pregnancy in complicated pregnancy, while other lipid levels are only meaningful in the first trimester.

Strengths and limitations of this study

- Our study is a large prospective longitudinal cohort study and most previous studies in lipid analysis are cross-sectional approach and retrospective nature, which could not avoid individual bias with respect to lipid increase during gestation.
- It's the first time to study the lipid elevation during pregnancy among same women.
- Maternal age, pre-pregnancy BMI, weight gain, fasting glucose levels, gestational age and fasting state are associated with lipid level and have been adjusted in our study.
- Diet controlling may affect the third trimester lipid interpretation in GDM group.
- No lipid profile before pregnancy may limit this study.

Introduction

Pregnancy has been considered as a 'stress test' for the body's metabolic and cardiovascular condition.[1 2] Hypertensive disorders of pregnancy(HDP) and gestational diabetes mellitus(GDM) are associated with an elevated risk for developing subsequent systemic hypertension and type II diabetes, affecting the cardiovascular system.[3] Hyperlipidemia, specifically hypertriglyceridemia is a well-known risk factor for these metabolic syndrome. It has been reported that triglyceride levels are significantly elevated in women with GDM/HDP compared to women without these metabolic syndromes, and these elevations are consistent in the first, second and third trimesters of pregnancy.[4 5] While the results of the levels of total cholesterol (TC), low-density lipoprotein cholesterol(LDL-c), high-density lipoprotein cholesterol (HDL-c) in these population with GDM/HDP are inconsistent in current studies.

Hyperlipidemia is common in the second half of pregnancy as a

physiologically required mechanism to maintain stable fuel supplementation to the fetus. It is also common in pregnancy with modest increases in lipids early in pregnancy and significant elevations of lipids later in pregnancy.[6] However, whether the elevation levels of the lipid concentrations during gestation are associated with the risk of GDM/HDP could not be clarified in previous cross-sectional approach and retrospective nature, thus it is difficult to ascertain which level of lipid elevation is physiological, or otherwise pathological. Furthermore, whether intra-pregnancy weight gain and dietary modification are correlated with the elevation of lipid levels have not been studied.

This prospective longitudinal cohort study was aimed to provide a description of lipids profile changes by gestational age during pregnancy. We sought to test the hypothesis that greater increase of triglycerides (TGs) levels during gestation is associated with pregnancy complications such as GDM and HDP. Also we tried to explore whether there is a correlation between weight gain and lipid profiles elevation during gestation.

Methods

Setting and participants

This prospective cohort study was conducted at International Peace Maternity & Child Healthcare Hospital (IPMCHH). IPMCHH is one of the largest obstetric care centers with annual delivery volume over fifteen thousands in Shanghai, China. Participants were recruited from Feb, 2014 to Nov 2014. Longitudinal lipid assessments were evaluated during three periods: 10-13^{+}GW (first prenatal visit), 22–28 GW (second trimester) and 30–35^{+}GW (third trimester). Women with pre-pregnancy cardiovascular disease, chronic hypertension, pre-pregnancy diabetes, or twin gestation were excluded. A total of 1376 women agreed to participate and signed consent. The study was approved by the ethics review board at IPMCHH.

Measurements

Blood samples were collected at the out-patient clinic by a trained professional nurse after a 10-12-hour fasting period. Serum TC, LDL-c, HDL-c and TGs concentrations and serum glucose concentration were measured on Hitachi type 7180 automatic biochemical analyzer (Japan, Hitachi High-Tech Science Systems Corporation) and monitored by a well-trained inspector. The intraassay and interassay coefficient of variation of this analysis was ≤ 5% and ≤10% respectively. Maternal body weight (kg) was obtained using an electronic scale during every follow-up visit and weight gain was recorded. Pre-pregnancy weight was recorded at the first obstetric clinic (self-reported). The pre-pregnancy body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters, and classified as low weight(<18.5 kg/m2), normal weight (18.5–24.9 kg/m2), overweight (25.0–29.9 kg/m2) or obesity (⩾30.0 kg/m2). Gestational age was based on the combination of last menstrual period and first-trimester ultrasound.

Other maternal characteristics including maternal age (years), education (years of schooling), gravidity, parity, medical history, reproductive and prenatal history, smoking status and alcohol use were obtained at first clinic visit. The participants were followed up to postpartum. Maternal weight, blood pressure and complications at every antenatal clinic were recorded. Labor and delivery summaries, postpartum and neonatal information were recorded according to criteria set forth on the standardized data collection form.

Operational Definitions

IPMCHH used International Association of Diabetes and Pregnancy Study Groups one-step criteria to diagnose GDM. The one step 75g, 2h OGTT was performed at 24-28 weeks of gestation, GDM was diagnosed when any one or more glucose values met or exceeded the following cut-offs: fasting ⩾5.1, 1-h

10.0 and 2-h 8.5 mmol/l. Women who were diagnosed as GDM received either nutritional counseling and/or dietary therapy, along with insulin if required. Hypertensive disorders of pregnancy included gestational hypertension and preeclampsia, blood pressure $\geqslant$ 140mmHg systolic or 90mmHg diastolic on at least two occasions 4-6 hours apart along with or without proteinuria (300mg of protein or more in a 24-hour urine collection or + on a urine dipstick).

Statistical analyses

Descriptive statistics include means and SDs for continuous variables, and numbers and percentages for categorical variables. The differences between variables in groups were assessed using chi-square tests or ANOVA as appropriate. Association between low HDL-c, high TGs, TC,LDL-c and GDM or HDP, the primary composite outcome was assessed by 2 multivariable logistic regressions if the factor had statistical significance in univariate factor analysis. Results for each lipid test (TC, LDL-c, HDL-c and TGs) during pregnancy in each trimester were divided into 3 groups: “low level” levels <25th percentile); “intermediate level” (between 25th and 75th percentiles); and “high level” (levels >75th percentile). The “intermediate level” (between 25th and 75th percentiles) was selected as reference. Variable selection in multivariable model was based on clinical and statistical significance. Confounding variables included maternal age, pre-pregnancy BMI, education years, fasting glucose levels, gestational age at blood drawn. Linear regression was used to analyze the correlation between TGs elevation and weight gain. All data were analyzed in SPSS, version 19.0 (IBM Corp, Chicago, USA). A P-value of <0.05 was considered statistically significant.

Results

From Feb, 2014 to Nov 2014, a total of 1376 eligible women consented to participate this study and had lipid concentrations tested at the first prenatal visit. During follow-up, 9 women experienced miscarriages or pregnancy

termination for fetal structure abnormalities before second trimester, 10 women were delivered before the third trimester blood measurement. After the third-trimester lipid assessment, 8 women were delivered less than 34 gestational weeks. 39 women chose other hospitals to deliver their babies and lost follow up. We were left with 1310 women (95.2%) for analysis. (Fig 1). Among these women, hypertensive disorders of pregnancy were diagnosed in 60 women (4.58%), 137 women (10.46%) were complicated with GDM. Composite endpoint of HDP/GDM occurred in 188 women (14.35%). All women were of Han ethnicity and no women smoked or consumed alcohol during pregnancy (data not shown).Other Maternal characteristics and neonatal outcomes of the study population are shown in Table 1. The women complicated with the composite of HDP/GDM were elder, heavier and with higher fasting glucose levels. Also the cesarean section rate was higher and babies were delivered earlier in the HDP/GDM group ($P<0.001$).There were no difference between groups with respect to gravidity, parity, the blood collection timing at each trimester, the neonatal gender, the average birthweight, the incidence of birthweight≥4000g and preterm birth rate.

The mean levels of lipid profile (TGs, TC, LDL-c and HDL-c) of three groups (HDP, GDM, NW) in different trimesters are presented at Fig 2. In normal women group, the TGs, TC and LDL-c concentration increased progressively throughout pregnancy while HDL-c concentration increased from first to second trimester then a slight decreased in the third trimester. Compared to normal women group, the levels of TGs concentration in HDP, GDM groups were higher in the three trimester assessment, while TC and LDL-c concentration was higher at first clinic visit and then lower compared to the normal group at second and third trimester. No statistical significance was observed with respect to HDL-c levels between three groups (Fig 2).

In the high levels of TGs group (>75th percentile), the rate of the composite endpoint (HDP/GDM) increased from 11.48% in the low levels of TGs (<25th percentile) to 23.15% at first time lipid assessment. Similar results existed in

second trimester TGs assessment (from 9.79% to 22.29%) and in third trimester (from 9.37% to 21.56%). (Fig 3)

The prevalence of HDP/GDM increased with levels of TCs from 11.29% in the group with low TCs to 18.31% (P=0.044) in the group with high TCs in first clinic visit. This trend was not found in second and third trimester assessment. Similar results existed in LDL-c assessment. The incidence of composite HDP/GDM increased with LDL-c levels in earlier assessment (from 10.97% to19.50%,P=0.006). This trend was not found in the second or third trimesters. (Fig 3)

Multivariate analysis

Compared to intermediate levels of TGs, high levels of TGs throughout pregnancy were associated with increased risks of the composite of HDP/GDM with aORs (95%CI) of 2.04(1.41-2.95), 1.81(1.25-2.63), 1.78(1.24-2.54) respectively in first, second and third trimester. High levels of TGs throughout pregnancy were also a risk factor of individual outcome of HDP with aORs (95%CI) of 1.94(1.05-3.59), 1.83(1.02-3.27), 2.89(1.72-4.84) respectively in first, second and third trimester (Table 2). We also found that high levels of TGs elevation from first to third trimester was associated with increased risks of the composite of HDP/GDM (aOR=1.58, 95%CI: 1.09-2.28, P=0.015) and individual outcome of HDP (aOR=2.09, 95%CI: 1.16~3.78,P=0.015). The high levels of TGs were associated with increased risks of GDM with aOR (95%CI) of 2.09 (1.37-3.17) and 1.93 (1.25-2.98) in first and second trimester respectively. However, High levels of TGs at third trimester was not a risk factor of GDM (aOR=1.51, 95%CI, 0.99~2.28, p=0.54). Meanwhile, high levels of TGs elevation from first to second trimester was associated with increased risks of GDM (aOR=1.67, 95%CI: 1.10-2.54, P=0.017).

With respect to other lipid profiles, we found that high levels of LDL-c at first trimester was associated with an increased risk of the composite of HDP/GDM (aOR=1.46, 95%CI: 1.01-2.10, P=0.044). While high levels of TC at first

trimester was not a risk factor of the composite of HDP/GDM (aOR=1.38, 95%CI, 0.95~2.01, p=0.91). (Table 2)

Linear regression analysis showed TGs elevation was positively correlated with weight gain during gestation after adjustment with pre-pregnancy BMI. (R=0.089, P=0.005). The mean level of weight gain from first to third trimester in GDM group was significant lower than non-GDM group (7.80±3.22kg vs 9.32±3.00kg, P<0.001).

Discussion

Main findings:

This study yielded three main findings: (a) high levels of TGs during pregnancy were associated with an increased risk of HDP and gestational diabetes mellitus and high levels of TGs elevation throughout gestation also posed an increased risk of HDP/GDM; (b) high levels of LDL-c were associated with increased risks of the composite of HDP/GDM at first trimester. No significant difference was observed with respect to HDL-c levels among the three groups, (c) TGs elevation was positively correlated with weight gain during gestation.

Maternal fat depots occur during the first two-thirds of gestation associated with both hyperphagia and increased lipogenesis. Higher insulin levels or even enhanced insulin sensitivity during early pregnancy and increased activity of adipose tissue lipoprotein lipase (LPL) contribute to the lipogenesis and hyperlipidemia. In late pregnancy, there is an accelerated breakdown of fat depots to meet the maximal fetal growth and significant elevations of lipids later in pregnancy.[6] Decreased insulin sensitivity regulated by human placental lactogen, cortisol and sex steroids ,decreased adipose tissue LPL sensitivity , increased activity of hormone-sensitive lipase and increased amounts of free-fatty acids (FFAs) in the circulation are associated with the hyperlipidemia in late pregnancy.[7-9] Our findings that lipids level variations including TGs, TC and LDL increased gradually during gestation and peaked before the delivery are consistent with the results of other studies.[10 11] This elevation of lipid

profiles is the physiological requirement to maintain stable fuel supplementation to the fetus.

Both HDP and GDM are metabolic dysfunction disorders during pregnancy and they both have the characteristic of insulin resistance.[12 13] TGs, TC and LDL concentrations were higher in the HDP/GDM group in first trimester in our study. We speculate that lipids in early gestational age show more maternal basic lipid metabolic condition than the physiological requirement for fetal growth. We found that maternal TGs concentrations were higher in the HDP group across the three trimesters and increasing levels of TGs were associated with HDP. Our results are consistent with the recent study of Ray et al[4] that elevated serum levels of TGs are associated with the risk of developing pregnancy associated hypertension. The association between dyslipidemia and the risk of preeclampsia is biologically plausible and compatible with what is known about pathophysiology of preeclampsia. GDM are associated with an elevated risk for developing subsequent type II diabetes. Analysis of patients with gestational diabetes mellitus showed higher TGs during the gestation. Although debate regarding the state of insulin resistance in GDM is ongoing, the association between GDM and high TGs in present study and other studies support the insulin resistance theory.[5 14]

The association between the elevation levels of the lipid concentrations during gestation, specifically TGs, and the risk of GDM/HDP could not be clarified in previous cross-sectional approach and retrospective nature, thus it is difficult to ascertain which level of lipid elevation is physiological, or otherwise pathological.[15-17] We found that higher TGs elevation from the first trimester to the third trimester was associated with HDP, which could be explained as following. First, too much and too fast plasma lipid elevation may induce endothelial dysfunction secondary to oxidative stress.[18 19] The second possible mechanism is the pathologic process of preeclampsia via dysregulation of lipoprotein lipase resulting a dyslipidemic lipid profile.[20]

A third possible mechanism may be via the metabolic syndrome—metabolic characteristics of “insulin resistance syndrome” namely, hyperinsulinemia.[21] The result of the association between the elevation of TGs levels and GDM should be explained with caution in this study. Because the intervention including either nutritional counseling and/or dietary therapy, along with insulin if required has changed the nature process including insulin resistance in this subgroup, thus we only found that higher TGs elevation before these interventions was associated with an increased risk of the prevalence of GDM.

It is well known that the weight gain during gestation are associated with pregnancy outcomes. Thus IOM proposed certain range of weight gain for women with different pre-pregnancy BMI category.[22] However it is not yet known that whether weight gain is correlated with lipid changes during gestation. We found that TGs elevations were positively correlated with weight gain after adjustment with pre-pregnancy BMI. This finding has clinical implications. Through dietary modification and maternal weight control in pregnancy, the TGs levels elevate less and the prevalence of HDP could be lowered in the high level TGs group. Qiu et al. found high dietary fiber can attenuate TGs concentration and reduce preeclampsia risk.[23]

Strengths and limitations

Our study is a large prospective longitudinal cohort study, following the same women from early pregnancy to delivery. The lipid levels were assessed in first, second, third trimester and its elevation during gestation. Most previous studies in lipid analysis are cross-sectional approach and retrospective nature, which could not avoid individual bias with respect to lipid increase during gestation and it’s the first time to study the lipid elevation during pregnancy among same women. Maternal age, pre-pregnancy BMI, weight gain, fasting glucose levels, gestational age and fasting state are associated with lipid level and have been adjusted in our study. An important limitation in our study is all the GDM women accepted dietary guidance once the diagnosis was

established in our hospital. Diet controlling may affect the third trimester lipid interpretation in GDM group. Second, we did not get the lipid profile before pregnancy, thus we could not know whether the maternal weight control before pregnancy is associated with subsequent lipid levels and pregnancy outcomes. Third, the obesity rate is low in our population, it is impossible for us to analyze the association of lipid levels and HDP/GDM in this specific subgroup, which limits the generalization of our results to other population with much higher rate of obesity.

Conclusion

In summary, in a large prospective longitudinal cohort study, we found both hypertriglyceridemia and higher elevation of TG levels during gestation constitutes a risk of HDP/GDM. Maternal weight gain during pregnancy is positively correlated with TGs levels elevation. Controlling weight gain in pregnancy could decrease TGs elevation and then decrease the risk of HDP/GDM. TGs could be used as a follow-up index during pregnancy in complicated pregnancy, while other lipid levels are only meaningful in the first trimester.

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Contributors WC designed the study. WC, HS and XL performed the investigation and analyzed the data. YC and BH implemented the survey. All authors contributed to writing and interpretation.

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Competing interests None declared.

Ethics approval The study was approved by the ethics review board at International Peace Maternity & Child Healthcare Hospital.No:201424

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References

1. Sattar, N., & Greer, I. A. Pregnancy complications and maternal cardiovascular risk: opportunities for intervention and screening?. *Bmj.* 2002;325(7356):157-160.
2. Bartha, J. L., González-Bugatto, F., Fernández-Macías, R., González-González, N. L., Comino-Delgado, R., & Hervías-Vivancos, B. Metabolic syndrome in normal and complicated pregnancies. *European Journal of Obstetrics & Gynecology & Reproductive Biology.* 2008; 137(2):178-184.
3. Hermes, W., Franx, A., Pampus, M. G. V., Bloemenkamp, K. W. M., Bots, M. L., & Post, J. A. V. D., et al. Cardiovascular risk factors in women who had hypertensive disorders late in pregnancy: a cohort study. *Am J Obstet Gynecol.* 2013;208(6):474.e1-8.
4. Ray, J. G., Diamond, P., Singh, G., & Bell, C. M. Brief overview of maternal triglycerides as a risk factor for pre-eclampsia. *Bjog An International Journal of Obstetrics & Gynaecology.*2006;113(4): 379-86.
5. Ryckman, K. K., Spracklen, C. N., Smith, C. J., Robinson, J. G., & Saftlas, A. F. Maternal lipid levels during pregnancy and gestational diabetes: a systematic review and meta-analysis. *British Journal of Obstetrics & Gynaecology.*2015; 122(5): 643-51.
6. Herrera, E., & Ortega-Senovilla, H. Maternal lipid metabolism during normal pregnancy and its implications to fetal development. *Clinical Lipidology.*2010;5(6):899-911.
7. Kaaja, R. Lipid abnormalities in pre-eclampsia: implications for vascular health. Clinical Lipidology.2011;6(1):71-78.
8. Ryan, E. A., & Enns, L. Role of gestational hormones in the induction of insulin resistance.*Journal of Clinical Endocrinology & Metabolism.*1988; 67(2):341-7.
9. Williams, C., & Coltart, T. M. Adipose tissue metabolism in pregnancy: the

lipolytic effect of human placental lactogen. *British Journal of Obstetrics & Gynaecology.*1978;85(1):43-6.

10. Farias, D. R., Franco-Sena, A. B., Vilela, A., Lepsch, J., Mendes, R. H., & Kac, G. Lipid changes throughout pregnancy according to pre-pregnancy bmi: results from a prospective cohort. *BJOG.* 2016 Mar;123(4):570-8.
11. Vahratian, A., Misra, V. K., Trudeau, S., & Misra, D. P. Prepregnancy body mass index and gestational age-dependent changes in lipid levels during pregnancy. *Obstet Gynecol.* 2010 Jul;116(1):107-13
12. Kaaja, R., Tikkanen, M. J., Viinikka, L., & Ylikorkala, O. Serum lipoproteins, insulin, and urinary prostanoid metabolites in normal and hypertensive pregnant women. *Obstet Gynecol.* 1995 Mar;85(3):353-6.
13. Barquiel, B., Herranz, L., Hillman, N., Burgos, M. Á., & Pallardo, L. F. Prepregnancy body mass index and prenatal fasting glucose are effective predictors of early postpartum metabolic syndrome in spanish mothers with gestational diabetes. *Metab Syndr Relat Disord.* 2014 Nov;12(9):457-63.
14. Sánchez-Vera, I., Bonet, B., Viana, M., Quintanar, A., Martín, M. D., & Blanco, P., et al. Changes in plasma lipids and increased low-density lipoprotein susceptibility to oxidation in pregnancies complicated by gestational diabetes: consequences of obesity. *Metabolism.* 2007 Nov;56(11):1527-33.
15. Enquobahrie, D. A., Williams, M. A., Butler, C. L., Frederick, I. O., Miller, R. S., & Luthy, D. A. Maternal plasma lipid concentrations in early pregnancy and risk of preeclampsia. Am J Hypertens. 2004 Jul;17(7):574-81.
16. Lima, V. J. D., Ruschi, G. E., Marques, F. L., & Saas, N. P49 serum lipid levels in pregnancies complicated by preeclampsia. *Sao Paulo Medical Journal.*2011;129(2):73-6.
17. Kashinakunti, S. V. Lipid profile in preeclampsia – a case control study. *Journal of Clinical & Diagnostic Research.*2010(4): 2748-2751.
18. Sattar, N., Bendomir, A., Berry, C., Shepherd, J., Greer, I. A., & Packard, C. J. Lipoprotein subfraction concentrations in preeclampsia: pathogenic parallels to atherosclerosis. *Obstet Gynecol.* 1997 Mar;89(3):403-8
19. Fellow, A. G. B. H. F. Potential pathogenic roles of aberrant lipoprotein and fatty acid metabolism in pre-eclampsia. *Br J Obstet Gynaecol.* 1996 Jul;103(7):614-20
20. Zhang, C., Austin, M. A., Edwards, K. L., Farin, F. M., Li, N., & Hsu, L., et al. Functional variants of the lipoprotein lipase gene and the risk of preeclampsia among non-hispanic caucasian women. *Clin Genet.* 2006 Jan;69(1):33-9.
21. Kaaja, R., Laivuori, H., Laakso, M., Tikkanen, M. J., & Ylikorkala, O. Evidence of a state of increased insulin resistance in preeclampsia. *Metabolism.* 1999 Jul;48(7):892-6.
22. Obstetriciansgynecologists, A. C. O. Acog committee opinion no. 548: weight gain during pregnancy. *Obstet Gynecol.* 2013 Jan;121(1):210-2.
23. Qiu, C., Coughlin, K. B., Frederick, I. O., Sorensen, T. K., & Williams, M. A.

Dietary fiber intake in early pregnancy and risk of subsequent preeclampsia. *Am J Hypertens.* 2008 Aug;21(8):903-9

Table1. Maternal characteristics and neonatal outcomes among complicated and control populations

	HDP/GDM(n=188)	NW(n=1122)	P value
Maternal age(years)	30.56±3.47	29.55±3.13	.000
Educational levels(years)	15.88±1.69	15.86±1.45	.851
Primiparous-n(%)	168(89.4%)	1001(89.2%)	.952
Pre-pregnancy BMI-n(%)	22.07±2.93	20.79±2.9	.000
<18.5(kg/m2)	12(6.4%)	123(11.0%)	
18.5–24.9(kg/m2)	149(79.3%)	938(83.9%)	
25.0–29.9(kg/m2)	24(12.8%)	56(5.0%)	
⩾30.0(kg/m2)	3(1.6%)	1(0.1%)	.000
Fasting glucose(mmol/L)	4.59±0.42	4.45±0.36	0.000
Blood drawn time(first clinic)(GW)	12.41±0.45	12.41±0.48	.986
Blood drawn time(second trimester)(GW)	24.93±0.88	24.97±0.94	.599
Blood drawn time(third trimester)(GW)	32.56±0.95	32.64±1	.297
Delivery gestation(GW)	39.12±0.99	39.5±1.17	.000
Birthweight(g)	3351.25±412.74	3350.03±412.74	.970
Placenta weight(g)	647.63±216.19	643.91±198.78	.815
Cesarean section-n(%)	84(44.7%)	381(34.0%)	.004
Sex(male) , n(%)	87(46.3%)	582(51.9%)	.156
Preterm delivery(34-37wks)	4(2.1%)	28(2.5%)	.762
Birthweight≥4000g- n(%)	13(6.9%)	75(6.7%)	.907

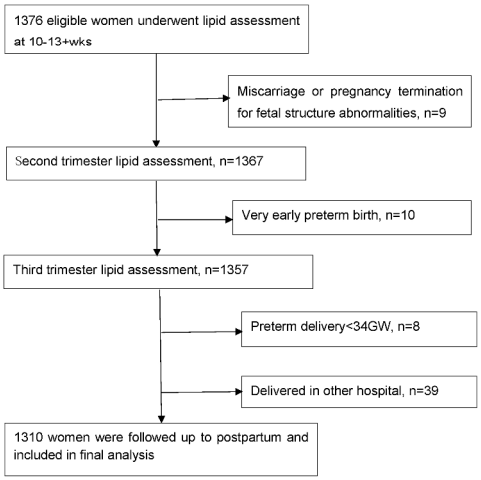
HDP/GDM, hypertensive disorders of pregnancy (HDP) /gestational diabetes mellitus (GDM); NW, normal women; BMI, Body Mass Index;

Table2. Multivariable logistic regression analysis predicting HDP,GDM, the composite of HDP/GDM

	HDP		GDM		Composite of HDP/GDM	
	OR(95%CI)	aOR(95%CI)	OR(95%CI)	aOR(95%CI)	OR(95%CI)	aOR(95%CI)
higher levels of TGs at 1st trimester	2.35(1.30-4.23)	1.94(1.05-3.59)*	2.21(1.48-3.29)	2.09(1.37-3.17)	2.28(1.60-3.25)	2.04(1.41-2.95)*
higher levels of TGs at 2nd trimester	1.97(1.12-3.45)*	1.83(1.02-3.27)*	2.05(1.38-3.04)*	1.93(1.25-2.98) *	1.97(1.39-2.79)*	1.81(1.25-2.63)*
higher levels of TGs at 3rd trimester	2.28(1.32-3.94)*	2.89(1.72-4.84)*	1.63(1.09-2.44)*	1.51(0.99-2.28)*	1.80(1.27-2.54)*	1.78(1.24-2.54)*
higher levels of TGs elevation	1.88(1.06-3.33)*	2.09(1.16-3.78)*	1.24(0.82-1.89)	1.26(0.83-1.94)	1.49(1.04-2.13)*	1.58(1.09-2.28)*
higher levels of TC at 1st trimester	1.77(0.97-3.26)	1.69(0.91-3.13)	1.10(0.72-1.68)	1.15(0.75-1.78)	1.37(0.95-1.97)	1.38(0.95-2.01)
higher levels of LDL at 1st trimester	1.72(0.95-3.10)	1.48(0.80-2.71)	1.43(0.96-2.14)	1.41(0.93-2.15)	1.56(1.09-2.12)*	1.46(1.01-2.10)*

HDP, hypertensive disorders of pregnancy; GDM, gestational diabetes mellitus; TGs, triglycerides; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol * P<0.05

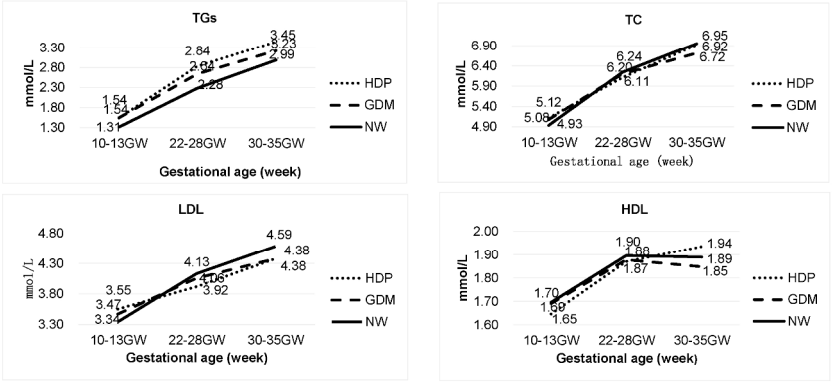
Figure 1, Flow chart of the study population



297x210mm (300 x 300 DPI)

Figure 2

The mean levels of lipid profile of individual group (HDP, GDM, NW) in different trimester

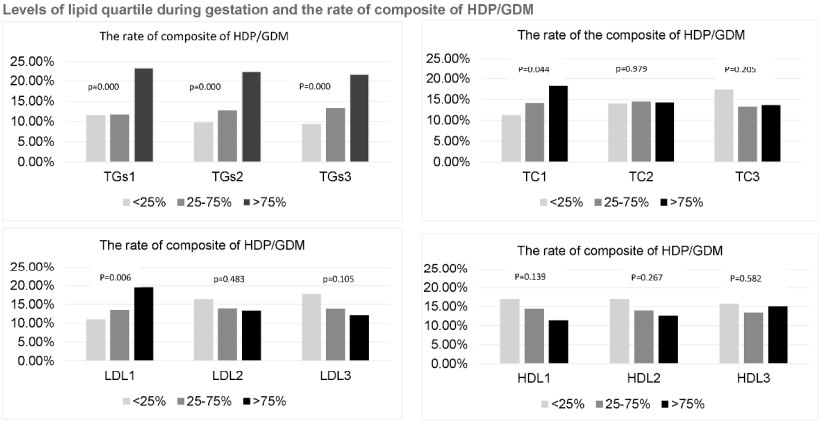


HDP, hypertensive disorders of pregnancy ; GDM, gestational diabetes mellitus; NW, normal women; TGs, triglycerides ;TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol

297x210mm (300 x 300 DPI)

Figure 3

Levels of lipid quartile during gestation and the rate of composite of HDP/GDM



HDP/GDM, hypertensive disorders of pregnancy (HDP) /gestational diabetes mellitus (GDM); TGs, triglycerides ;TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol

297x210mm (300 x 300 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5*
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	7
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	7

		Case-control study—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	6
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	7
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	7
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8 6
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Associations of lipid levels during gestation with hypertensive disorders of pregnancy and gestational diabetes mellitus: a prospective longitudinal cohort study

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Associations of lipid levels during gestation with hypertensive disorders of pregnancy and gestational diabetes mellitus: a prospective longitudinal cohort study

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Abstract

Objective: To assess associations of elevated lipid levels during gestation with hypertensive disorders of pregnancy (HDP) and gestational diabetes mellitus (GDM).

Methods: This prospective cohort study was conducted in a tertiary maternal hospital in Shanghai China, from February to November 2014. Lipid constituents, including triglycerides (TGs), total cholesterol, low-density lipoprotein, and high-density lipoprotein of 1310 eligible women were assessed in the first (10-13+ weeks), second (22-28 weeks) and third (30-35wks) trimesters, consecutively. Associations of lipid profiles with HDP and/or GDM outcomes were assessed.

Results: Compared with the normal group, maternal TG concentrations were higher in the HDP/GDM groups across the three trimesters (P<0.001); TC and LDL-c amounts were only higher in the first trimester for the HDP and GDM groups (p<0.05). HDL-c levels were similar in the three groups. Compared with intermediate TG levels (25^{th}~75^{th} percentile), higher TG amounts (>75^{th} percentile) were associated with increased risk of HDP/GDM in each trimester with aORs (95%CI) of 2.04 (1.41-2.95), 1.81 (1.25-2.63) and 1.78 (1.24-2.54), respectively. High TG elevation from first to third trimesters (>75^{th} percentile) was associated with increased risk of HDP, with an aOR of 2.09 (1.16-3.78). High TG elevation before 28 weeks was associated with increased risk of GDM, with an aOR of 1.67 (1.10-2.54). TG elevation was positively correlated with weight gain during gestation (R=0.089, P=0.005).

Conclusions: Controlling weight gain during pregnancy could decrease TG elevation and reduce the risk of HDP/GDM. TGs could be used as follow-up parameters during complicated pregnancy, while other lipids are meaningful only in the first trimester.

Strengths and limitations of this study

- This was a large prospective longitudinal cohort study, and previous reports assessing lipid levels have cross-sectional or retrospective designs, which could not avoid individual bias with respect to lipid level increase during gestation.
- Lipid elevation was assessed here for the first time throughout pregnancy (from first to third trimesters) among the same women in a large scale research.
- Maternal age, pre-pregnancy BMI, weight gain, fasting glucose levels, gestational age, and fasting state are associated with lipid levels, and were adjusted in this study.
- Diet control may affect lipid levels in the third trimester, which might result in biased interpretation in the GDM group.
- No lipid profile before pregnancy was obtained, constituting a study limitation.

Introduction

Pregnancy is considered a 'stress test' for metabolic and cardiovascular conditions of the body.[1 2] Hypertensive disorders of pregnancy (HDP) and gestational diabetes mellitus (GDM) are associated with an elevated risk of developing subsequent systemic hypertension and type II diabetes, affecting the cardiovascular system.[3] Hyperlipidemia, specifically hypertriglyceridemia, is a well-known risk factor for metabolic syndromes. Indeed, triglyceride levels are significantly elevated in women with GDM/HDP compared to those without these metabolic syndromes, and such elevations are consistent in the first, second and third trimesters of pregnancy.[4 5] However, associations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) levels in these populations with GDM/HDP are inconsistent in available reports.

Hyperlipidemia is common in the second half of pregnancy as a

physiologically required mechanism to maintain stable fuel supplementation to the fetus. It is also common to observe modest lipid level increases early in pregnancy, but significant elevations later in pregnancy.[6] However, whether elevated lipid concentrations during gestation are associated with the risk of GDM/HDP could not be clarified in previous cross-sectional and retrospective studies. Thus, it is difficult to ascertain which level of lipid elevation is physiological or pathological. In addition, whether intra-pregnancy weight gain and dietary modifications are correlated with elevated lipid levels remain unknown.

This prospective longitudinal cohort study aimed to provide a comprehensive description of lipid profile changes based on gestational age during pregnancy. We sought to test the hypothesis that higher increase of triglyceride (TG) levels during gestation is associated with pregnancy complications such as GDM and HDP. In addition, we explored a possible correlation between weight gain and lipid profile elevation during gestation.

Methods

Setting and participants

This prospective cohort study was conducted at International Peace Maternity & Child Healthcare Hospital (IPMCHH), one of the largest obstetric care centers in Shanghai, China, with an annual delivery volume over fifteen thousands. Participants were recruited from February to November 2014. Longitudinal lipid assessments were carried out during three periods: 10-13^{+} GW (first prenatal visit), 22–28 GW (second trimester) and 30–35^{+} GW (third trimester). Women with pre-pregnancy cardiovascular disease, chronic hypertension, pre-pregnancy diabetes, or twin pregnancy were excluded. A total of 1376 women agreed to participate in this study and provided signed informed consent. The study was approved by the ethics review board of IPMCHH.

Measurements

Blood samples were collected at the out-patient clinic by a trained nurse after a 10-12-hour fasting period. Serum TC, LDL-c, HDL-c, TG, and glucose concentrations were measured on Hitachi type 7180 automatic biochemical analyzer (Japan, Hitachi High-Tech Science Systems Corporation) and monitored by a well-trained inspector. Intra- and inter-assay coefficients of variation in this analysis were ≤ 5% and ≤10%, respectively. Maternal body weight (kg) was obtained on an electronic scale at every follow-up visit, with weight gain recorded. Pre-pregnancy weights were recorded at the first obstetric clinic (self-reported). Pre-pregnancy body-mass index (BMI) was derived as the weight (kilograms) divided by the square of the height (meters), and the patients were classified as low weight (<18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2) or obesity (⩾30.0 kg/m^2). Gestational age was based on the combination of last menstrual period and first-trimester ultrasound.

Other maternal characteristics, including maternal age (years), education (years of schooling), gravidity, parity, medical history, reproductive and prenatal history, smoking status, and alcohol use, were obtained at the first clinic visit. All participants were followed up to the postpartum period. Maternal weight, blood pressure, and complications at each antenatal clinical visit were recorded. Labor and delivery records, as well as postpartum and neonatal information were recorded according to criteria included in the standardized data collection form.

Operational definitions

IPMCHH used International Association of Diabetes and Pregnancy Study Groups one-step criteria for GDM diagnosis. The one step approach with 75g, 2h OGTT was performed at 24-28 weeks of gestation. GDM was diagnosed

when 1 or more glucose indexes met or exceeded the following cut-offs: fasting, ⩾5.1 mmol/l; 1h, 10.0 mmol/l; 2h, 8.5 mmol/l. Women diagnosed with GDM received nutritional counseling and/or dietary therapy, along with insulin if required. Hypertensive disorders of pregnancy included gestational hypertension and preeclampsia, blood pressure ⩾140 mmHg systolic or 90 mmHg diastolic on at least two occasions 4-6 hours apart, with or without proteinuria (300 mg protein or more in 24-hour urine sample or + on a urine dipstick).

Statistical analyses

Descriptive statistics included means and standard deviations (SDs) for continuous variables, and numbers and percentages for categorical variables. Group comparisons were performed by chi-square test or ANOVA as appropriate. Associations of low HDL-c, high TGs, TC, and LDL-c with GDM or HDP, as well as primary composite outcome were assessed by multivariable logistic regression for factors with statistical significance in univariate analysis. Data for each lipid test (TC, LDL-c, HDL-c and TG levels) in each pregnancy trimester were divided into 3 groups: “low level” (<25th percentile); “intermediate level” (between 25th and 75th percentiles), and “high level” (>75th percentile). “Intermediate level” was selected as reference. Variable selection in the multivariable model was based on clinical and statistical significance. Confounding variables included maternal age, pre-pregnancy BMI, education years, fasting glucose levels, and gestational age at blood collection. Linear regression was used to assess the correlation between TG elevation and weight gain. SPSS version 19.0 (SPSS, USA) was used for all analyses. P<0.05 was considered statistically significant.

Results

From February to November 2014, a total of 1376 eligible women consented to participate in this study, and their lipid concentrations were tested at the first

prenatal visit. During follow-up, 9 women had miscarriage or pregnancy termination for fetal structure abnormalities before the second trimester; 10 others delivered before third trimester blood collection. After third-trimester lipid assessment, 8 women delivered at less than 34 gestational weeks. Meanwhile, 39 women chose other hospitals for delivery, and were lost to follow up. Therefore, 1310 women (95.2%) were included in the final analysis. (Fig 1).

Hypertensive disorders of pregnancy were diagnosed in 60 of the 1310 women (4.58%) with 23 preeclampsia and 37 gestational hypertension. Most cases were detected after 34 weeks of gestation. A total of 6 HDP cases were diagnosed before 34 gestational weeks, and good blood pressure control and term delivery were achieved. There was no significant difference in lipid levels between the preeclampsia and gestational hypertension groups (data not shown). Meanwhile, 137 women (10.46%) had GDM complications, including five cases who received insulin therapy. The HDP/GDM composite endpoint occurred in 188 women (14.35%). All women were of Han ethnicity, and none smoked or consumed alcohol during pregnancy (data not shown). Other maternal characteristics and neonatal outcomes of the study population are shown in Table 1. Women complicated with the HDP/GDM composite outcome were elder (30.56±3.47 vs 29.55±3.13, P<0.001) and heavier (Pre-pregnancy BMI 22.07±2.93 vs 20.79±2.9, P<0.001), with higher fasting glucose levels at first trimester (4.59±0.42vs 4.45±0.36, P<0.001). In addition, the cesarean section rate (44.7% vs 34.0%, P=0.004) was higher and babies were delivered earlier in the HDP/GDM group (39.12±0.99 39.5±1.17, P<0.001).There were no differences between groups in gravidity, parity, blood collection timing in each trimester, neonatal gender, average birthweight, incidence of birthweight ≥4000 g and preterm birth rate.

Lipid (TG, TC, LDL-c and HDL-c) profiles in the three groups (HDP, GDM and normal women [NW]) in different trimesters are depicted in Fig 2. In the NW group, TG, TC and LDL-c concentrations increased progressively

throughout pregnancy; meanwhile, HDL-c amounts increased from the first to second trimester with a slight decrease in the third trimester. Compared with the normal women group, the HDP and GDM groups showed higher TG concentrations throughout pregnancy, while TC and LDL-c concentrations were higher at the first clinical visit, but lower in the second and third trimesters. No statistically significant difference was observed in HDL-c levels among the three groups (Fig 2).

In the 4th quartile TG level group (>75th percentile), the rate of composite endpoint (HDP/GDM) increased to 23.15%, from the 11.48% in the1st quartile TG group (<25th percentile) at first lipid assessment. Similar results were obtained in the second (9.79% vs. 22.29%) and third (9.37% vs. 21.56%) trimesters (Fig 3).

HDP/GDM prevalence increased with TC levels, from 11.29% in the 1st quartile TC group to 18.31% (P=0.044) in the 4th quartile TC group at first clinical visit. Such trend was not found in the second and third trimesters. Similar results were found for LDL-c. Incidence of composite HDP/GDM increased with LDL-c levels in early pregnancy, from 10.97% in the 1st quartile level group to 19.50% in the 4th quartile level group (P=0.006). Such a trend was not found in the second and third trimesters (Fig 3).

Associations of lipid profile with HDP and GDM

Compared with intermediate TG levels, the 4th quartile TG levels throughout pregnancy were associated with increased risks of combined HDP and GDM with aORs (95%CI) of 2.04 (1.41-2.95), 1.81 (1.25-2.63), and 1.78 (1.24-2.54), respectively, in the first, second and third trimesters. The 4th quartile levels of TGs throughout pregnancy were also a risk factor for the individual outcome of HDP with aORs (95%CI) of 1.94 (1.05-3.59), 1.83 (1.02-3.27), and 2.89 (1.72-4.84), respectively, in the first, second and third trimesters (Table 2). Interestingly, TG elevation from the first to third trimesters was associated with increased risks of combined HDP/GDM (aOR=1.58, 95%CI 1.09-2.28, P=0.015)

as well as HDP (aOR=2.09, 95%CI 1.16~3.78, P=0.015). The 4th quartile levels of TGs were associated with increased risks of GDM with aORs (95%CI) of 2.09 (1.37-3.17) and 1.93 (1.25-2.98) in the first and second trimesters, respectively. However, elevated TG level in the third trimester was not a risk factor for GDM (aOR=1.51, 95%CI 0.99~2.28, p=0.54). Meanwhile, TG elevation from the first to second trimester was associated with increased risk of GDM (aOR=1.67, 95%CI: 1.10-2.54, P=0.017).

With respect to other lipid profiles, The 4th quartile levels of LDL-c in the first trimester were associated with increased risk of combined HDP and GDM (aOR=1.46, 95%CI 1.01-2.10, P=0.044). Meanwhile, elevated TC in the first trimester was not a risk factor for the composite HDP/GDM outcome (aOR=1.38, 95%CI 0.95~2.01, p=0.91) (Table 2).

Linear regression analysis showed that TG elevation was positively correlated with weight gain during gestation after adjusting for pre-pregnancy BMI (R=0.089, P=0.005). Weight gain from the first to third trimesters in the GDM group was significant lower than that of the non-GDM group (7.80±3.22kg vs 9.32±3.00kg, P<0.001).

Discussion

Main findings:

This study yielded three main findings: (a) First, high levels of TGs during pregnancy were associated with increased risk of HDP and gestational diabetes mellitus; in addition, TG elevation throughout gestation also conferred increased risk of combined HDP and GDM. (b) Then, high LDL-c amounts were associated with increased risk of composite HDP/GDM in the first trimester; no significant difference was observed in HDL-c levels among the three groups. (c) Finally, TG elevation was positively correlated with weight gain during gestation.

Maternal fat depots occurring during the first two trimesters of gestation are associated with both hyperphagia and increased lipogenesis. Elevated insulin

levels or even enhanced insulin sensitivity in early pregnancy and increased activity of adipose tissue lipoprotein lipase (LPL) contribute to lipogenesis and hyperlipidemia. In late pregnancy, there is an accelerated breakdown of fat depots to meet maximal fetal growth requirements, with significant elevation of lipids later in pregnancy.[6] Decreased insulin sensitivity (regulated by human placental lactogen, cortisol, and sex steroids), reduced adipose tissue LPL sensitivity, increased activity of hormone-sensitive lipase, and enhanced amounts of free-fatty acids (FFAs) in circulation are associated with hyperlipidemia in late pregnancy.[7-9] Our findings that the levels of lipids, including TGs, TC and LDL, increased gradually during gestation and peaked before delivery are consistent with other studies.[10 11] This elevation of lipid amounts is a physiological requirement for maintaining stable fuel supplementation to the fetus.

Both HDP and GDM are metabolic dysfunction disorders during pregnancy, and have the characteristics of insulin resistance.[12 13] TG, TC and LDL concentrations were higher in the HDP/GDM group in the first trimester as shown above. These findings suggested that lipids in early gestational age show a more maternal metabolic condition than the physiological requirement for fetal growth. We found that maternal TG concentrations were higher in the HDP group across the three pregnancy trimesters, with elevated TG levels associated with HDP. These findings are consistent with a recent study by Ray et al[4] demonstrating that elevated serum levels of TGs are associated with the risk of developing pregnancy associated hypertension. The association between dyslipidemia and the risk of preeclampsia is biologically plausible and compatible with the current knowledge of preeclampsia pathophysiology. GDM is associated with an elevated risk of developing subsequent type II diabetes. Patients with gestational diabetes mellitus showed higher TG amounts during pregnancy. Despite ongoing debate regarding insulin resistance status in GDM, the association found between GDM and high TG levels in the present and other studies support the insulin resistance theory.[5 14]

The associations of elevated concentrations of lipids (specifically TGs) during gestation with the risk of GDM/HDP could not be clarified in cross-sectional and retrospective studies, making it difficult to ascertain which level of lipid elevation is physiological or pathological.[15-17] As shown above, high TG elevation from the first to third trimesters was associated with HDP, which could be explained as follows. First, too much and too fast plasma lipid elevation may induce endothelial dysfunction secondary to oxidative stress.[18 19] A second possible mechanism is the pathologic process of preeclampsia via dysregulation of lipoprotein lipase, resulting a dyslipidemic lipid profile.[20] A third possible mechanism may be via the metabolic characteristics of the "insulin resistance syndrome" namely, hyperinsulinemia.[21] The association of elevated TG levels with GDM should be interpreted with caution in this study. Because interventions, including nutritional counseling and/or dietary therapy alongside insulin if required, could change the natural process of insulin resistance in this subgroup, we only found that stark TG elevation before intervention was associated with increased risk of GDM prevalence.

It is well known that weight gain during gestation is associated with pregnancy outcomes. Thus, IOM proposed a certain range of weight gain for women with different pre-pregnancy BMI category.[22] However, it remains unknown whether weight gain is correlated with lipid level changes during gestation. As shown above, TG elevation was positively correlated with weight gain after adjusting for pre-pregnancy BMI. This finding has clinical implications. Through dietary modifications and maternal weight control during pregnancy, TG level elevation could be reduced and HDP prevalence could be lowered in the high level TG group. Qiu et al. found high dietary fibers can decrease TG concentration and reduce preeclampsia risk.[23]

Strengths and limitations

This was a large prospective longitudinal cohort study, with the same women assessed from early pregnancy to delivery. Lipid levels were assessed in the

first, second, third trimesters, as well as elevations during gestation. Although several meta-analyses have been published in this field, few studies examined lipids at multiple points during pregnancy[5]. This study allows understanding of the relationship between lipid levels during pregnancy and the development of hypertension and GDM. Maternal age, pre-pregnancy BMI, weight gain, fasting glucose levels, gestational age and fasting state are associated with lipid levels, and were adjusted in this study. An important limitation of this study is that all women with GDM received dietary guidance once diagnosis was established. Diet control may affect the third trimester lipid levels in the GDM group. In addition, we did not assess lipid profiles before pregnancy; thus, whether maternal weight control before pregnancy is associated with subsequent lipid levels and pregnancy outcomes remains unclear. Finally, the obesity rate was low in the study population, making it impossible to analyze the associations of lipid levels with HDP/GDM in this specific subgroup; this limits the generalization of our findings to other populations with much higher rates of obesity.

Conclusion

Overall, in a large prospective longitudinal cohort study, we found that both hypertriglyceridemia and highly elevated TG levels during gestation constitute risk factors for HDP/GDM. Maternal weight gain during pregnancy was positively correlated with TG level elevation. Controlling weight gain in pregnancy could decrease TG elevation and reduce the risk of HDP/GDM. TGs could be used as a follow-up index in complicated pregnancy, while the levels of other lipids are meaningful only in the first trimester.

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Contributors WC designed the study. WC, HS and XL performed the experiments and analyzed the data. YC and BH implemented the survey. All authors contributed to data interpretation and manuscript writing.

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Competing interests None Declared.

Ethics approval The study was approved by the ethics review board of International Peace Maternity & Child Healthcare Hospital (No:201424).

Data sharing statement No additional data are available.

References

1. Sattar, N., & Greer, I. A. Pregnancy complications and maternal cardiovascular risk: opportunities for intervention and screening?. *Bmj.* 2002;325(7356):157-160.
2. Bartha, J. L., González-Bugatto, F., Fernández-Macías, R., González-González, N. L., Comino-Delgado, R., & Hervías-Vivancos, B. Metabolic syndrome in normal and complicated pregnancies. *European Journal of Obstetrics & Gynecology & Reproductive Biology.* 2008; 137(2):178-184.
3. Hermes, W., Franx, A., Pampus, M. G. V., Bloemenkamp, K. W. M., Bots, M. L., & Post, J. A. V. D., et al. Cardiovascular risk factors in women who had hypertensive disorders late in pregnancy: a cohort study. *Am J Obstet Gynecol.* 2013;208(6):474.e1-8.
4. Ray, J. G., Diamond, P., Singh, G., & Bell, C. M. Brief overview of maternal triglycerides as a risk factor for pre-eclampsia. *Bjog An International Journal of Obstetrics & Gynaecology.*2006;113(4): 379-86.
5. Ryckman, K. K., Spracklen, C. N., Smith, C. J., Robinson, J. G., & Saftlas, A. F. Maternal lipid levels during pregnancy and gestational diabetes: a systematic review and meta-analysis. *British Journal of Obstetrics & Gynaecology.*2015; 122(5): 643-51.
6. Herrera, E., & Ortega-Senovilla, H. Maternal lipid metabolism during

normal pregnancy and its implications to fetal development. *Clinical Lipidology.*2010;5(6):899-911.
7. Kaaja, R. Lipid abnormalities in pre-eclampsia: implications for vascular health. Clinical Lipidology.2011;6(1):71-78.
8. Ryan, E. A., & Enns, L. Role of gestational hormones in the induction of insulin resistance.*Journal of Clinical Endocrinology & Metabolism.*1988; 67(2):341-7.
9. Williams, C., & Coltart, T. M. Adipose tissue metabolism in pregnancy: the lipolytic effect of human placental lactogen. *British Journal of Obstetrics & Gynaecology.*1978;85(1):43-6.
10. Farias, D. R., Franco-Sena, A. B., Vilela, A., Lepsch, J., Mendes, R. H., & Kac, G. Lipid changes throughout pregnancy according to pre-pregnancy bmi: results from a prospective cohort. *BJOG.* 2016 Mar;123(4):570-8.
11. Vahratian, A., Misra, V. K., Trudeau, S., & Misra, D. P. Prepregnancy body mass index and gestational age-dependent changes in lipid levels during pregnancy. *Obstet Gynecol.* 2010 Jul;116(1):107-13
12. Kaaja, R., Tikkanen, M. J., Viinikka, L., & Ylikorkala, O. Serum lipoproteins, insulin, and urinary prostanoid metabolites in normal and hypertensive pregnant women. *Obstet Gynecol.* 1995 Mar;85(3):353-6.
13. Barquiel, B., Herranz, L., Hillman, N., Burgos, M. Á., & Pallardo, L. F. Prepregnancy body mass index and prenatal fasting glucose are effective predictors of early postpartum metabolic syndrome in spanish mothers with gestational diabetes. *Metab Syndr Relat Disord.* 2014 Nov;12(9):457-63.
14. Sánchez-Vera, I., Bonet, B., Viana, M., Quintanar, A., Martín, M. D., & Blanco, P., et al. Changes in plasma lipids and increased low-density lipoprotein susceptibility to oxidation in pregnancies complicated by gestational diabetes: consequences of obesity. *Metabolism.* 2007 Nov;56(11):1527-33.
15. Enquobahrie, D. A., Williams, M. A., Butler, C. L., Frederick, I. O., Miller, R. S., & Luthy, D. A. Maternal plasma lipid concentrations in early pregnancy and risk of preeclampsia. Am J Hypertens. 2004 Jul;17(7):574-81.
16. Lima, V. J. D., Ruschi, G. E., Marques, F. L., & Saas, N. P49 serum lipid levels in pregnancies complicated by preeclampsia. *Sao Paulo Medical Journal.*2011;129(2):73-6.
17. Kashinakunti, S. V. Lipid profile in preeclampsia – a case control study. *Journal of Clinical & Diagnostic Research.*2010(4): 2748-2751.
18. Sattar, N., Bendomir, A., Berry, C., Shepherd, J., Greer, I. A., & Packard, C. J. Lipoprotein subfraction concentrations in preeclampsia: pathogenic parallels to atherosclerosis. *Obstet Gynecol.* 1997 Mar;89(3):403-8
19. Fellow, A. G. B. H. F. Potential pathogenic roles of aberrant lipoprotein and fatty acid metabolism in pre-eclampsia. *Br J Obstet Gynaecol.* 1996 Jul;103(7):614-20
20. Zhang, C., Austin, M. A., Edwards, K. L., Farin, F. M., Li, N., & Hsu, L., et al. Functional variants of the lipoprotein lipase gene and the risk of

preeclampsia among non-hispanic caucasian women. *Clin Genet.* 2006 Jan;69(1):33-9.

21. Kaaja, R., Laivuori, H., Laakso, M., Tikkanen, M. J., & Ylikorkala, O. Evidence of a state of increased insulin resistance in preeclampsia. *Metabolism.* 1999 Jul;48(7):892-6.
22. Obstetriciansgynecologists, A. C. O. Acog committee opinion no. 548: weight gain during pregnancy. *Obstet Gynecol.* 2013 Jan;121(1):210-2.
23. Qiu, C., Coughlin, K. B., Frederick, I. O., Sorensen, T. K., & Williams, M. A. Dietary fiber intake in early pregnancy and risk of subsequent preeclampsia. *Am J Hypertens.* 2008 Aug;21(8):903-9

Table1. Maternal characteristics and neonatal outcomes among patients with complications and control populations

	HDP/GDM(n=188)	NW(n=1122)	P value
Maternal age(years)	30.56±3.47	29.55±3.13	<0.001
Educational levels(years)	15.88±1.69	15.86±1.45	.851
Primiparous-n(%)	168(89.4%)	1001(89.2%)	.952
Pre-pregnancy BMI-n(%)	22.07±2.93	20.79±2.9	<0.001
<18.5(kg/m2)	12(6.4%)	123(11.0%)	
18.5–24.9(kg/m2)	149(79.3%)	938(83.9%)	
25.0–29.9(kg/m2)	24(12.8%)	56(5.0%)	
⩾30.0(kg/m2)	3(1.6%)	1(0.1%)	<0.001
Fasting glucose at blood test 1(mmol/L)	4.59±0.42	4.45±0.36	<0.001
GW at blood test 1	12.41±0.45	12.41±0.48	.986
GW at blood test 2	24.93±0.88	24.97±0.94	.599
GW at blood test 3	32.56±0.95	32.64±1	.297
Delivery gestation(GW)	39.12±0.99	39.5±1.17	<0.001
Birthweight(g)	3351.25±412.74	3350.03±412.74	.970
Placenta weight(g)	647.63±216.19	643.91±198.78	.815
Cesarean section-n(%)	84(44.7%)	381(34.0%)	.004
Sex(male) , n(%)	87(46.3%)	582(51.9%)	.156
Preterm delivery(34-37wks)	4(2.1%)	28(2.5%)	.762
Birthweight≥4000g- n(%)	13(6.9%)	75(6.7%)	.907

HDP/GDM, hypertensive disorders of pregnancy (HDP) /gestational diabetes mellitus (GDM); NW, normal women; BMI, body mass index; GW, gestational week.

Table2. Multivariable logistic regression analysis predicting HDP and/or GDM

	HDP		GDM		Composite of HDP/GDM	
	OR(95%CI)	aOR(95%CI)	OR(95%CI)	aOR(95%CI)	OR(95%CI)	aOR(95%CI)
4th quartile TGs at 1st trimester	2.35(1.30-4.23)	1.94(1.05-3.59)*	2.21(1.48-3.29)	2.09(1.37-3.17)	2.28(1.60-3.25)	2.04(1.41-2.95)*
4th quartile TGs at 2nd trimester	1.97(1.12-3.45)*	1.83(1.02-3.27)*	2.05(1.38-3.04)*	1.93(1.25-2.98) *	1.97(1.39-2.79)*	1.81(1.25-2.63)*
4th quartile TGs at 3rd trimester	2.28(1.32-3.94)*	2.89(1.72-4.84)*	1.63(1.09-2.44)*	1.51(0.99-2.28)*	1.80(1.27-2.54)*	1.78(1.24-2.54)*
4th quartile TGs elevation	1.88(1.06-3.33)*	2.09(1.16-3.78)*	1.24(0.82-1.89)	1.26(0.83-1.94)	1.49(1.04-2.13)*	1.58(1.09-2.28)*
4th quartile TC at 1st trimester	1.77(0.97-3.26)	1.69(0.91-3.13)	1.10(0.72-1.68)	1.15(0.75-1.78)	1.37(0.95-1.97)	1.38(0.95-2.01)
4th quartile LDL at 1st trimester	1.72(0.95-3.10)	1.48(0.80-2.71)	1.43(0.96-2.14)	1.41(0.93-2.15)	1.56(1.09-2.12)*	1.46(1.01-2.10)*

Adjusted confounding variables included maternal age, pre-pregnancy BMI, education years, fasting glucose levels, and gestational age at blood collection

HDP, hypertensive disorders of pregnancy; GDM, gestational diabetes mellitus; TGs, triglycerides; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol * P<0.05

Figure legends:

Figure 1, Flow chart of the study population

Figure 2

The mean levels of lipid profile of individual group (HDP, GDM, NW) in different trimester

Figure 3

Levels of lipid quartile during gestation and the rate of composite of HDP/GDM

Figure 1, Flow chart of the study population

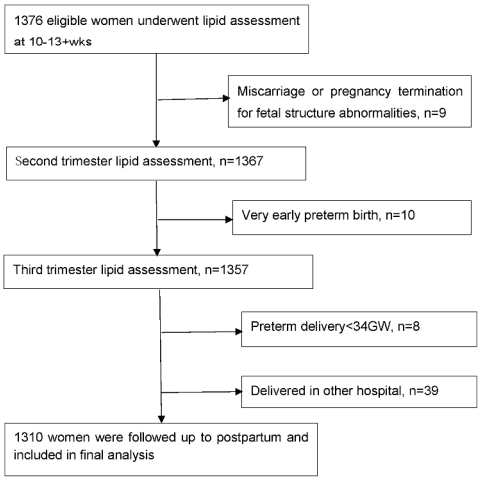

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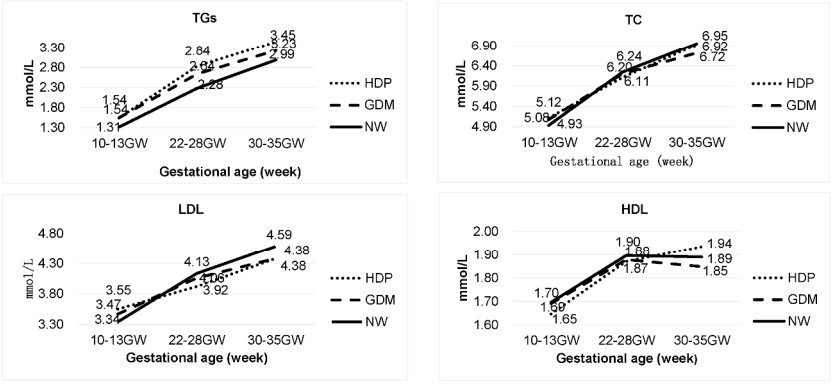
Figure 2

The mean levels of lipid profile of individual group (HDP, GDM, NW) in different trimester



HDP, hypertensive disorders of pregnancy ; GDM, gestational diabetes mellitus; NW, normal women; TGs, triglycerides ;TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol

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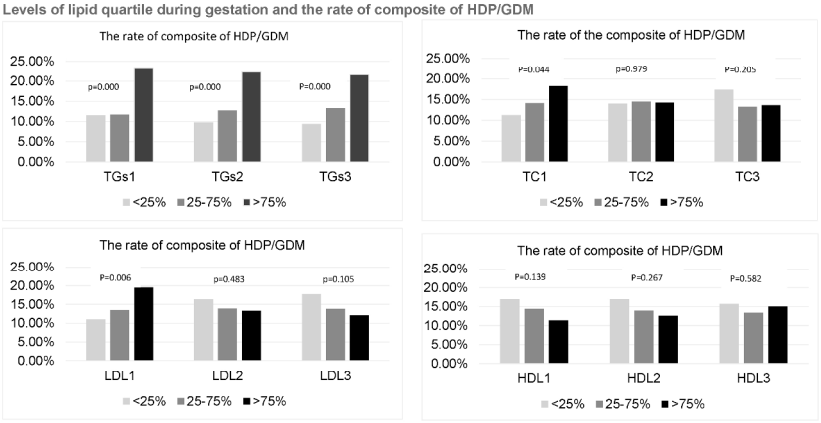
Figure 3

Levels of lipid quartile during gestation and the rate of composite of HDP/GDM



HDP/GDM, hypertensive disorders of pregnancy (HDP) /gestational diabetes mellitus (GDM); TGs, triglycerides ;TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol

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STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5*
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	7
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	7

		Case-control study—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	6
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	7
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	7
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8 6
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Associations of lipid levels during gestation with hypertensive disorders of pregnancy and gestational diabetes mellitus: a prospective longitudinal cohort study

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Associations of lipid levels during gestation with hypertensive disorders of pregnancy and gestational diabetes mellitus: a prospective longitudinal cohort study

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Abstract

Objective: To assess associations of elevated lipid levels during gestation with hypertensive disorders of pregnancy (HDP) and gestational diabetes mellitus (GDM).

Methods: This prospective cohort study was conducted in a tertiary maternal hospital in Shanghai China, from February to November 2014. Lipid constituents, including triglycerides (TGs), total cholesterol, low-density lipoprotein, and high-density lipoprotein of 1310 eligible women were assessed in the first (10-13+ weeks), second (22-28 weeks) and third (30-35wks) trimesters, consecutively. Associations of lipid profiles with HDP and/or GDM outcomes were assessed.

Results: Compared with the normal group, maternal TG concentrations were higher in the HDP/GDM groups across the three trimesters (P<0.001); TC and LDL-c amounts were only higher in the first trimester for the HDP and GDM groups (p<0.05). HDL-c levels were similar in the three groups. Compared with intermediate TG levels (25^{th}~75^{th} percentile), higher TG amounts (>75^{th} percentile) were associated with increased risk of HDP/GDM in each trimester with aORs (95%CI) of 2.04 (1.41-2.95), 1.81 (1.25-2.63) and 1.78 (1.24-2.54), respectively. High TG elevation from first to third trimesters (>75^{th} percentile) was associated with increased risk of HDP, with an aOR of 2.09 (1.16-3.78). High TG elevation before 28 weeks was associated with increased risk of GDM, with an aOR of 1.67 (1.10-2.54). TG elevation was positively correlated with weight gain during gestation (R=0.089, P=0.005).

Conclusions: Controlling weight gain during pregnancy could decrease TG elevation and reduce the risk of HDP/GDM. TGs could be used as follow-up parameters during complicated pregnancy, while other lipids are meaningful only in the first trimester.

Strengths and limitations of this study

- This was a large prospective longitudinal cohort study, and previous reports assessing lipid levels have cross-sectional or retrospective designs, which could not avoid individual bias with respect to lipid level increase during gestation.
- Lipid elevation was assessed here for the first time throughout pregnancy (from first to third trimesters) among the same women in a large scale research.
- Maternal age, pre-pregnancy BMI, weight gain, fasting glucose levels, gestational age, and fasting state are associated with lipid levels, and were adjusted in this study.
- Diet control may affect lipid levels in the third trimester, which might result in biased interpretation in the GDM group.
- No lipid profile before pregnancy was obtained, constituting a study limitation.

Introduction

Pregnancy is considered a 'stress test' for metabolic and cardiovascular conditions of the body.[1 2] Hypertensive disorders of pregnancy (HDP) and gestational diabetes mellitus (GDM) are associated with an elevated risk of developing subsequent systemic hypertension and type II diabetes, affecting the cardiovascular system.[3] Hyperlipidemia, specifically hypertriglyceridemia, is a well-known risk factor for metabolic syndromes. Indeed, triglyceride levels are significantly elevated in women with GDM/HDP compared to those without these metabolic syndromes, and such elevations are consistent in the first, second and third trimesters of pregnancy.[4 5] However, associations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) levels in these populations with GDM/HDP are inconsistent in available reports.

Hyperlipidemia is common in the second half of pregnancy as a

physiologically required mechanism to maintain stable fuel supplementation to the fetus. It is also common to observe modest lipid level increases early in pregnancy, but significant elevations later in pregnancy.[6] However, whether elevated lipid concentrations during gestation are associated with the risk of GDM/HDP could not be clarified in previous cross-sectional and retrospective studies. Thus, it is difficult to ascertain which level of lipid elevation is physiological or pathological. In addition, whether intra-pregnancy weight gain and dietary modifications are correlated with elevated lipid levels remain unknown.

This prospective longitudinal cohort study aimed to provide a comprehensive description of lipid profile changes based on gestational age during pregnancy. We sought to test the hypothesis that higher increase of triglyceride (TG) levels during gestation is associated with pregnancy complications such as GDM and HDP. In addition, we explored a possible correlation between weight gain and lipid profile elevation during gestation.

Methods

Setting and participants

This prospective cohort study was conducted at International Peace Maternity & Child Healthcare Hospital (IPMCHH), one of the largest obstetric care centers in Shanghai, China, with an annual delivery volume over fifteen thousands. Participants were recruited from February to November 2014. Longitudinal lipid assessments were carried out during three periods: 10-13^{+} GW (first prenatal visit), 22–28 GW (second trimester) and 30–35^{+} GW (third trimester). Women with pre-pregnancy cardiovascular disease, chronic hypertension, pre-pregnancy diabetes, or twin pregnancy were excluded. A total of 1376 women agreed to participate in this study and provided signed informed consent. The study was approved by the ethics review board of IPMCHH.

Measurements

Blood samples were collected at the out-patient clinic by a trained nurse after a 10-12-hour fasting period. Serum TC, LDL-c, HDL-c, TG, and glucose concentrations were measured on Hitachi type 7180 automatic biochemical analyzer (Japan, Hitachi High-Tech Science Systems Corporation) and monitored by a well-trained inspector. Intra- and inter-assay coefficients of variation in this analysis were ≤ 5% and ≤10%, respectively. Maternal body weight (kg) was obtained on an electronic scale at every follow-up visit, with weight gain recorded. Pre-pregnancy weights were recorded at the first obstetric clinic (self-reported). Pre-pregnancy body-mass index (BMI) was derived as the weight (kilograms) divided by the square of the height (meters), and the patients were classified as low weight (<18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2) or obesity (⩾30.0 kg/m^2). Gestational age was based on the combination of last menstrual period and first-trimester ultrasound.

Other maternal characteristics, including maternal age (years), education (years of schooling), gravidity, parity, medical history, reproductive and prenatal history, smoking status, and alcohol use, were obtained at the first clinic visit. All participants were followed up to the postpartum period. Maternal weight, blood pressure, and complications at each antenatal clinical visit were recorded. Labor and delivery records, as well as postpartum and neonatal information were recorded according to criteria included in the standardized data collection form.

Operational definitions

IPMCHH used International Association of Diabetes and Pregnancy Study Groups one-step criteria for GDM diagnosis. The one step approach with 75g, 2h OGTT was performed at 24-28 weeks of gestation. GDM was diagnosed

when 1 or more glucose indexes met or exceeded the following cut-offs: fasting, ⩾5.1 mmol/l; 1h, 10.0 mmol/l; 2h, 8.5 mmol/l. Women diagnosed with GDM received nutritional counseling and/or dietary therapy, along with insulin if required. Hypertensive disorders of pregnancy included gestational hypertension and preeclampsia, blood pressure ⩾140 mmHg systolic or 90 mmHg diastolic on at least two occasions 4-6 hours apart, with or without proteinuria (300 mg protein or more in 24-hour urine sample or + on a urine dipstick).

Statistical analyses

Descriptive statistics included means and standard deviations (SDs) for continuous variables, and numbers and percentages for categorical variables. Group comparisons were performed by chi-square test or ANOVA as appropriate. Associations of low HDL-c, high TGs, TC, and LDL-c with GDM or HDP, as well as primary composite outcome were assessed by multivariable logistic regression for factors with statistical significance in univariate analysis. Data for each lipid test (TC, LDL-c, HDL-c and TG levels) in each pregnancy trimester were divided into 3 groups: “low level” (<25th percentile); “intermediate level” (between 25th and 75th percentiles), and “high level” (>75th percentile). “Intermediate level” was selected as reference. Variable selection in the multivariable model was based on clinical and statistical significance. Confounding variables included maternal age, pre-pregnancy BMI, education years, fasting glucose levels, and gestational age at blood collection. Linear regression was used to assess the correlation between TG elevation and weight gain. SPSS version 19.0 (SPSS, USA) was used for all analyses. P<0.05 was considered statistically significant.

Results

From February to November 2014, a total of 1376 eligible women consented to participate in this study, and their lipid concentrations were tested at the first

prenatal visit. During follow-up, 9 women had miscarriage or pregnancy termination for fetal structure abnormalities before the second trimester; 10 others delivered before third trimester blood collection. After third-trimester lipid assessment, 8 women delivered at less than 34 gestational weeks. Meanwhile, 39 women chose other hospitals for delivery, and were lost to follow up. Therefore, 1310 women (95.2%) were included in the final analysis. (Fig 1).

Hypertensive disorders of pregnancy were diagnosed in 60 of the 1310 women (4.58%) with 23 preeclampsia and 37 gestational hypertension. Most cases were detected after 34 weeks of gestation. A total of 6 HDP cases were diagnosed before 34 gestational weeks, and good blood pressure control and term delivery were achieved. There was no significant difference in lipid levels between the preeclampsia and gestational hypertension groups (data not shown). Meanwhile, 137 women (10.46%) had GDM complications, including five cases who received insulin therapy. The HDP/GDM composite endpoint occurred in 188 women (14.35%). All women were of Han ethnicity, and none smoked or consumed alcohol during pregnancy (data not shown). Other maternal characteristics and neonatal outcomes of the study population are shown in Table 1. Women complicated with the HDP/GDM composite outcome were elder (30.56±3.47 vs 29.55±3.13, P<0.001) and heavier (Pre-pregnancy BMI 22.07±2.93 vs 20.79±2.9, P<0.001), with higher fasting glucose levels at first trimester (4.59±0.42vs 4.45±0.36, P<0.001). In addition, the cesarean section rate (44.7% vs 34.0%, P=0.004) was higher and babies were delivered earlier in the HDP/GDM group (39.12±0.99 39.5±1.17, P<0.001).There were no differences between groups in gravidity, parity, blood collection timing in each trimester, neonatal gender, average birthweight, incidence of birthweight ≥4000 g and preterm birth rate.

Lipid (TG, TC, LDL-c and HDL-c) profiles in the three groups (HDP, GDM and normal women [NW]) in different trimesters are depicted in Fig 2. In the NW group, TG, TC and LDL-c concentrations increased progressively

throughout pregnancy; meanwhile, HDL-c amounts increased from the first to second trimester with a slight decrease in the third trimester. Compared with the normal women group, the HDP and GDM groups showed higher TG concentrations throughout pregnancy, while TC and LDL-c concentrations were higher at the first clinical visit, but lower in the second and third trimesters. No statistically significant difference was observed in HDL-c levels among the three groups (Fig 2).

In the 4th quartile TG level group (>75th percentile), the rate of composite endpoint (HDP/GDM) increased to 23.15%, from the 11.48% in the1st quartile TG group (<25th percentile) at first lipid assessment. Similar results were obtained in the second (9.79% vs. 22.29%) and third (9.37% vs. 21.56%) trimesters (Fig 3).

HDP/GDM prevalence increased with TC levels, from 11.29% in the 1st quartile TC group to 18.31% (P=0.044) in the 4th quartile TC group at first clinical visit. Such trend was not found in the second and third trimesters. Similar results were found for LDL-c. Incidence of composite HDP/GDM increased with LDL-c levels in early pregnancy, from 10.97% in the 1st quartile level group to 19.50% in the 4th quartile level group (P=0.006). Such a trend was not found in the second and third trimesters (Fig 3).

Associations of lipid profile with HDP and GDM

Compared with intermediate TG levels, the 4th quartile TG levels throughout pregnancy were associated with increased risks of combined HDP and GDM with aORs (95%CI) of 2.04 (1.41-2.95), 1.81 (1.25-2.63), and 1.78 (1.24-2.54), respectively, in the first, second and third trimesters. The 4th quartile levels of TGs throughout pregnancy were also a risk factor for the individual outcome of HDP with aORs (95%CI) of 1.94 (1.05-3.59), 1.83 (1.02-3.27), and 2.89 (1.72-4.84), respectively, in the first, second and third trimesters (Table 2). Interestingly, TG elevation from the first to third trimesters was associated with increased risks of combined HDP/GDM (aOR=1.58, 95%CI 1.09-2.28, P=0.015)

as well as HDP (aOR=2.09, 95%CI 1.16~3.78, P=0.015). The 4th quartile levels of TGs were associated with increased risks of GDM with aORs (95%CI) of 2.09 (1.37-3.17) and 1.93 (1.25-2.98) in the first and second trimesters, respectively. However, elevated TG level in the third trimester was not a risk factor for GDM (aOR=1.51, 95%CI 0.99~2.28, p=0.54). Meanwhile, TG elevation from the first to second trimester was associated with increased risk of GDM (aOR=1.67, 95%CI: 1.10-2.54, P=0.017).

With respect to other lipid profiles, The 4th quartile levels of LDL-c in the first trimester were associated with increased risk of combined HDP and GDM (aOR=1.46, 95%CI 1.01-2.10, P=0.044). Meanwhile, elevated TC in the first trimester was not a risk factor for the composite HDP/GDM outcome (aOR=1.38, 95%CI 0.95~2.01, p=0.91) (Table 2).

Linear regression analysis showed that TG elevation was positively correlated with weight gain during gestation after adjusting for pre-pregnancy BMI (R=0.089, P=0.005). Weight gain from the first to third trimesters in the GDM group was significant lower than that of the non-GDM group (7.80±3.22kg vs 9.32±3.00kg, P<0.001).

Discussion

Main findings:

This study yielded three main findings: (a) First, high levels of TGs during pregnancy were associated with increased risk of HDP and gestational diabetes mellitus; in addition, TG elevation throughout gestation also conferred increased risk of combined HDP and GDM. (b) Then, high LDL-c amounts were associated with increased risk of composite HDP/GDM in the first trimester; no significant difference was observed in HDL-c levels among the three groups. (c) Finally, TG elevation was positively correlated with weight gain during gestation.

Maternal fat depots occurring during the first two trimesters of gestation are associated with both hyperphagia and increased lipogenesis. Elevated insulin

levels or even enhanced insulin sensitivity in early pregnancy and increased activity of adipose tissue lipoprotein lipase (LPL) contribute to lipogenesis and hyperlipidemia. In late pregnancy, there is an accelerated breakdown of fat depots to meet maximal fetal growth requirements, with significant elevation of lipids later in pregnancy.[6] Decreased insulin sensitivity (regulated by human placental lactogen, cortisol, and sex steroids), reduced adipose tissue LPL sensitivity, increased activity of hormone-sensitive lipase, and enhanced amounts of free-fatty acids (FFAs) in circulation are associated with hyperlipidemia in late pregnancy.[7-9] Our findings that the levels of lipids, including TGs, TC and LDL, increased gradually during gestation and peaked before delivery are consistent with other studies.[10 11] This elevation of lipid amounts is a physiological requirement for maintaining stable fuel supplementation to the fetus.

Both HDP and GDM are metabolic dysfunction disorders during pregnancy, and have the characteristics of insulin resistance.[12 13] TG, TC and LDL concentrations were higher in the HDP/GDM group in the first trimester as shown above. These findings suggested that lipids in early gestational age show a more maternal metabolic condition than the physiological requirement for fetal growth. We found that maternal TG concentrations were higher in the HDP group across the three pregnancy trimesters, with elevated TG levels associated with HDP. These findings are consistent with a recent study by Ray et al[4] demonstrating that elevated serum levels of TGs are associated with the risk of developing pregnancy associated hypertension. The association between dyslipidemia and the risk of preeclampsia is biologically plausible and compatible with the current knowledge of preeclampsia pathophysiology. GDM is associated with an elevated risk of developing subsequent type II diabetes. Patients with gestational diabetes mellitus showed higher TG amounts during pregnancy. Despite ongoing debate regarding insulin resistance status in GDM, the association found between GDM and high TG levels in the present and other studies support the insulin resistance theory.[5 14]

The associations of elevated concentrations of lipids (specifically TGs) during gestation with the risk of GDM/HDP could not be clarified in cross-sectional and retrospective studies, making it difficult to ascertain which level of lipid elevation is physiological or pathological.[15-17] As shown above, high TG elevation from the first to third trimesters was associated with HDP, which could be explained as follows. First, too much and too fast plasma lipid elevation may induce endothelial dysfunction secondary to oxidative stress.[18 19] A second possible mechanism is the pathologic process of preeclampsia via dysregulation of lipoprotein lipase, resulting a dyslipidemic lipid profile.[20] A third possible mechanism may be via the metabolic characteristics of the "insulin resistance syndrome" namely, hyperinsulinemia.[21] The association of elevated TG levels with GDM should be interpreted with caution in this study. Because interventions, including nutritional counseling and/or dietary therapy alongside insulin if required, could change the natural process of insulin resistance in this subgroup, we only found that stark TG elevation before intervention was associated with increased risk of GDM prevalence.

It is well known that weight gain during gestation is associated with pregnancy outcomes. Thus, IOM proposed a certain range of weight gain for women with different pre-pregnancy BMI category.[22] However, it remains unknown whether weight gain is correlated with lipid level changes during gestation. As shown above, TG elevation was positively correlated with weight gain after adjusting for pre-pregnancy BMI. This finding has clinical implications. Through dietary modifications and maternal weight control during pregnancy, TG level elevation could be reduced and HDP prevalence could be lowered in the high level TG group. Qiu et al. found high dietary fibers can decrease TG concentration and reduce preeclampsia risk.[23]

Strengths and limitations

This was a large prospective longitudinal cohort study, with the same women assessed from early pregnancy to delivery. Lipid levels were assessed in the

first, second, third trimesters, as well as elevations during gestation. Although several meta-analyses have been published in this field, few studies examined lipids at multiple points during pregnancy[5]. This study allows understanding of the relationship between lipid levels during pregnancy and the development of hypertension and GDM. Maternal age, pre-pregnancy BMI, weight gain, fasting glucose levels, gestational age and fasting state are associated with lipid levels, and were adjusted in this study. An important limitation of this study is that all women with GDM received dietary guidance once diagnosis was established. Diet control may affect the third trimester lipid levels in the GDM group. In addition, we did not assess lipid profiles before pregnancy; thus, whether maternal weight control before pregnancy is associated with subsequent lipid levels and pregnancy outcomes remains unclear. Finally, the obesity rate was low in the study population, making it impossible to analyze the associations of lipid levels with HDP/GDM in this specific subgroup; this limits the generalization of our findings to other populations with much higher rates of obesity.

Conclusion

Overall, in a large prospective longitudinal cohort study, we found that both hypertriglyceridemia and highly elevated TG levels during gestation constitute risk factors for HDP/GDM. Maternal weight gain during pregnancy was positively correlated with TG level elevation. Controlling weight gain in pregnancy could decrease TG elevation and reduce the risk of HDP/GDM. TGs could be used as a follow-up index in complicated pregnancy, while the levels of other lipids are meaningful only in the first trimester.

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Contributors WC designed the study. WC, HS and XL performed the experiments and analyzed the data. YC and BH implemented the survey. All authors contributed to data interpretation and manuscript writing.

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Competing interests None Declared.

Ethics approval The study was approved by the ethics review board of International Peace Maternity & Child Healthcare Hospital (No:201424).

Data sharing statement No additional data are available.

References

1. Sattar, N., & Greer, I. A. Pregnancy complications and maternal cardiovascular risk: opportunities for intervention and screening?. *Bmj.* 2002;325(7356):157-160.
2. Bartha, J. L., González-Bugatto, F., Fernández-Macías, R., González-González, N. L., Comino-Delgado, R., & Hervías-Vivancos, B. Metabolic syndrome in normal and complicated pregnancies. *European Journal of Obstetrics & Gynecology & Reproductive Biology.* 2008; 137(2):178-184.
3. Hermes, W., Franx, A., Pampus, M. G. V., Bloemenkamp, K. W. M., Bots, M. L., & Post, J. A. V. D., et al. Cardiovascular risk factors in women who had hypertensive disorders late in pregnancy: a cohort study. *Am J Obstet Gynecol.* 2013;208(6):474.e1-8.
4. Ray, J. G., Diamond, P., Singh, G., & Bell, C. M. Brief overview of maternal triglycerides as a risk factor for pre-eclampsia. *Bjog An International Journal of Obstetrics & Gynaecology.*2006;113(4): 379-86.
5. Ryckman, K. K., Spracklen, C. N., Smith, C. J., Robinson, J. G., & Saftlas, A. F. Maternal lipid levels during pregnancy and gestational diabetes: a systematic review and meta-analysis. *British Journal of Obstetrics & Gynaecology.*2015; 122(5): 643-51.
6. Herrera, E., & Ortega-Senovilla, H. Maternal lipid metabolism during

normal pregnancy and its implications to fetal development. *Clinical Lipidology.*2010;5(6):899-911.
7. Kaaja, R. Lipid abnormalities in pre-eclampsia: implications for vascular health. Clinical Lipidology.2011;6(1):71-78.
8. Ryan, E. A., & Enns, L. Role of gestational hormones in the induction of insulin resistance.*Journal of Clinical Endocrinology & Metabolism.*1988; 67(2):341-7.
9. Williams, C., & Coltart, T. M. Adipose tissue metabolism in pregnancy: the lipolytic effect of human placental lactogen. *British Journal of Obstetrics & Gynaecology.*1978;85(1):43-6.
10. Farias, D. R., Franco-Sena, A. B., Vilela, A., Lepsch, J., Mendes, R. H., & Kac, G. Lipid changes throughout pregnancy according to pre-pregnancy bmi: results from a prospective cohort. *BJOG.* 2016 Mar;123(4):570-8.
11. Vahratian, A., Misra, V. K., Trudeau, S., & Misra, D. P. Prepregnancy body mass index and gestational age-dependent changes in lipid levels during pregnancy. *Obstet Gynecol.* 2010 Jul;116(1):107-13
12. Kaaja, R., Tikkanen, M. J., Viinikka, L., & Ylikorkala, O. Serum lipoproteins, insulin, and urinary prostanoid metabolites in normal and hypertensive pregnant women. *Obstet Gynecol.* 1995 Mar;85(3):353-6.
13. Barquiel, B., Herranz, L., Hillman, N., Burgos, M. Á., & Pallardo, L. F. Prepregnancy body mass index and prenatal fasting glucose are effective predictors of early postpartum metabolic syndrome in spanish mothers with gestational diabetes. *Metab Syndr Relat Disord.* 2014 Nov;12(9):457-63.
14. Sánchez-Vera, I., Bonet, B., Viana, M., Quintanar, A., Martín, M. D., & Blanco, P., et al. Changes in plasma lipids and increased low-density lipoprotein susceptibility to oxidation in pregnancies complicated by gestational diabetes: consequences of obesity. *Metabolism.* 2007 Nov;56(11):1527-33.
15. Enquobahrie, D. A., Williams, M. A., Butler, C. L., Frederick, I. O., Miller, R. S., & Luthy, D. A. Maternal plasma lipid concentrations in early pregnancy and risk of preeclampsia. Am J Hypertens. 2004 Jul;17(7):574-81.
16. Lima, V. J. D., Ruschi, G. E., Marques, F. L., & Saas, N. P49 serum lipid levels in pregnancies complicated by preeclampsia. *Sao Paulo Medical Journal.*2011;129(2):73-6.
17. Kashinakunti, S. V. Lipid profile in preeclampsia – a case control study. *Journal of Clinical & Diagnostic Research.*2010(4): 2748-2751.
18. Sattar, N., Bendomir, A., Berry, C., Shepherd, J., Greer, I. A., & Packard, C. J. Lipoprotein subfraction concentrations in preeclampsia: pathogenic parallels to atherosclerosis. *Obstet Gynecol.* 1997 Mar;89(3):403-8
19. Fellow, A. G. B. H. F. Potential pathogenic roles of aberrant lipoprotein and fatty acid metabolism in pre-eclampsia. *Br J Obstet Gynaecol.* 1996 Jul;103(7):614-20
20. Zhang, C., Austin, M. A., Edwards, K. L., Farin, F. M., Li, N., & Hsu, L., et al. Functional variants of the lipoprotein lipase gene and the risk of

preeclampsia among non-hispanic caucasian women. *Clin Genet.* 2006 Jan;69(1):33-9.

21. Kaaja, R., Laivuori, H., Laakso, M., Tikkanen, M. J., & Ylikorkala, O. Evidence of a state of increased insulin resistance in preeclampsia. *Metabolism.* 1999 Jul;48(7):892-6.
22. Obstetriciansgynecologists, A. C. O. Acog committee opinion no. 548: weight gain during pregnancy. *Obstet Gynecol.* 2013 Jan;121(1):210-2.
23. Qiu, C., Coughlin, K. B., Frederick, I. O., Sorensen, T. K., & Williams, M. A. Dietary fiber intake in early pregnancy and risk of subsequent preeclampsia. *Am J Hypertens.* 2008 Aug;21(8):903-9

Table1. Maternal characteristics and neonatal outcomes among patients with complications and control populations

	HDP/GDM(n=188)	NW(n=1122)	P value
Maternal age(years)	30.56±3.47	29.55±3.13	<0.001
Educational levels(years)	15.88±1.69	15.86±1.45	.851
Primiparous-n(%)	168(89.4%)	1001(89.2%)	.952
Pre-pregnancy BMI-n(%)	22.07±2.93	20.79±2.9	<0.001
<18.5(kg/m2)	12(6.4%)	123(11.0%)	
18.5–24.9(kg/m2)	149(79.3%)	938(83.9%)	
25.0–29.9(kg/m2)	24(12.8%)	56(5.0%)	
⩾30.0(kg/m2)	3(1.6%)	1(0.1%)	<0.001
Fasting glucose at blood test 1(mmol/L)	4.59±0.42	4.45±0.36	<0.001
GW at blood test 1	12.41±0.45	12.41±0.48	.986
GW at blood test 2	24.93±0.88	24.97±0.94	.599
GW at blood test 3	32.56±0.95	32.64±1	.297
Delivery gestation(GW)	39.12±0.99	39.5±1.17	<0.001
Birthweight(g)	3351.25±412.74	3350.03±412.74	.970
Placenta weight(g)	647.63±216.19	643.91±198.78	.815
Cesarean section-n(%)	84(44.7%)	381(34.0%)	.004
Sex(male) , n(%)	87(46.3%)	582(51.9%)	.156
Preterm delivery(34-37wks)	4(2.1%)	28(2.5%)	.762
Birthweight≥4000g- n(%)	13(6.9%)	75(6.7%)	.907

HDP/GDM, hypertensive disorders of pregnancy (HDP) /gestational diabetes mellitus (GDM); NW, normal women; BMI, body mass index; GW, gestational week.

Table2. Multivariable logistic regression analysis predicting HDP and/or GDM

	HDP		GDM		Composite of HDP/GDM	
	OR(95%CI)	aOR(95%CI)	OR(95%CI)	aOR(95%CI)	OR(95%CI)	aOR(95%CI)
4th quartile TGs at 1st trimester	2.35(1.30-4.23)	1.94(1.05-3.59)*	2.21(1.48-3.29)	2.09(1.37-3.17)	2.28(1.60-3.25)	2.04(1.41-2.95)*
4th quartile TGs at 2nd trimester	1.97(1.12-3.45)*	1.83(1.02-3.27)*	2.05(1.38-3.04)*	1.93(1.25-2.98) *	1.97(1.39-2.79)*	1.81(1.25-2.63)*
4th quartile TGs at 3rd trimester	2.28(1.32-3.94)*	2.89(1.72-4.84)*	1.63(1.09-2.44)*	1.51(0.99-2.28)*	1.80(1.27-2.54)*	1.78(1.24-2.54)*
Higher (4th quartile) TGs elevation**	1.88(1.06-3.33)*	2.09(1.16-3.78)*	1.24(0.82-1.89)	1.26(0.83-1.94)	1.49(1.04-2.13)*	1.58(1.09-2.28)*
4th quartile TC at 1st trimester	1.77(0.97-3.26)	1.69(0.91-3.13)	1.10(0.72-1.68)	1.15(0.75-1.78)	1.37(0.95-1.97)	1.38(0.95-2.01)
4th quartile LDL at 1st trimester	1.72(0.95-3.10)	1.48(0.80-2.71)	1.43(0.96-2.14)	1.41(0.93-2.15)	1.56(1.09-2.12)*	1.46(1.01-2.10)*

Adjusted confounding variables included maternal age, pre-pregnancy BMI, education years, fasting glucose levels, and gestational age at blood collection

**Adjusted before mentioned variables plus weight gain during gestation

HDP, hypertensive disorders of pregnancy; GDM, gestational diabetes mellitus; TGs, triglycerides; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol * P<0.05

Figure legends:

Figure 1, Flow chart of the study population

Figure 2

The mean levels of lipid profile of individual group (HDP, GDM, NW) in different trimester

Figure 3

Levels of lipid quartile during gestation and the rate of composite of HDP/GDM

Figure 1, Flow chart of the study population

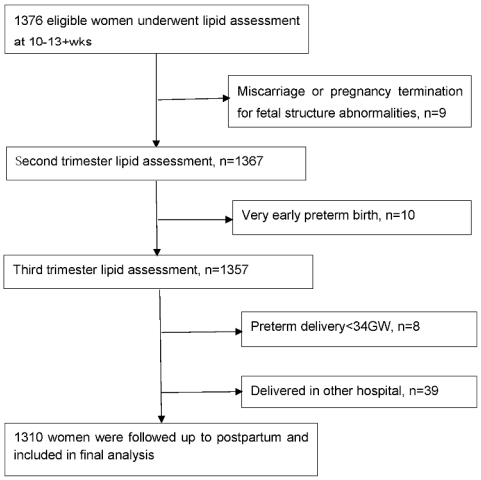

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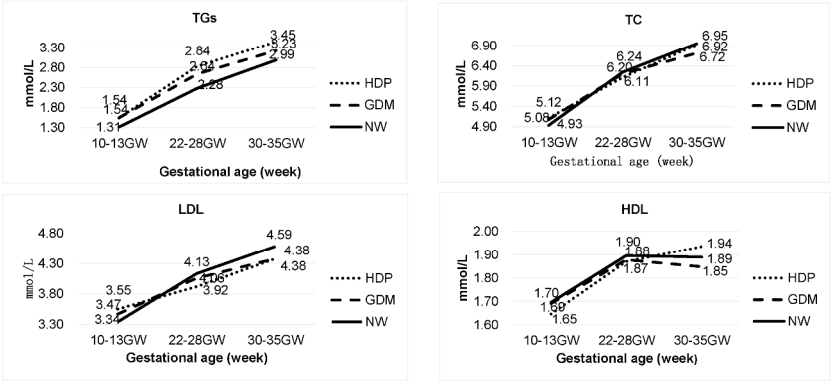
Figure 2

The mean levels of lipid profile of individual group (HDP, GDM, NW) in different trimester



HDP, hypertensive disorders of pregnancy ; GDM, gestational diabetes mellitus; NW, normal women; TGs, triglycerides ;TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol

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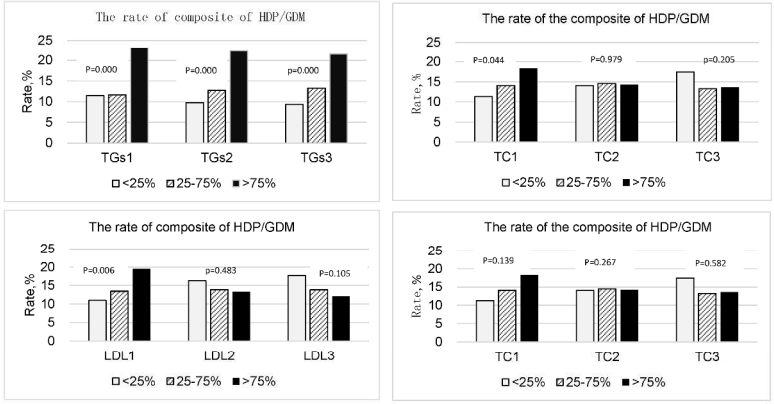
Figure 3

Levels of lipid quartile during gestation and the rate of composite of HDP/GDM



HDP/GDM, hypertensive disorders of pregnancy (HDP) /gestational diabetes mellitus (GDM); TGs, triglycerides;TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol

Figure 3 Levels of lipid quartile during gestation and the rate of composite of HDP/GDM

297x210mm (300 x 300 DPI)

STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5*
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	7
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	7

		Case-control study—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	6
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	7
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	7
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	7
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8 6
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.