Accepted: 9 September 2022 | Published Online 17 October 2022

DOI: 10.1111/1471-0528.17297

BJOG An International Journal of Obstetrics and Gynaecology

RESEARCH ARTICLE

Basic Science

Maternal plasma diacylglycerols and triacylglycerols in the prediction of gestational diabetes mellitus

Guixue Hou[1] | Ya Gao[1,2] | Liona C. Poon[3] | Yan Ren[1,4] | Chunwei Zeng[1] | Bo Wen[1] | Argyro Syngelaki[5] | Liang Lin[1] | Jin Zi[1] | Fengxia Su[1] | Weiwei Xie[1] | Fang Chen[1] | Kypros H. Nicolaides[5]

[1]BGI-Shenzhen, Shenzhen, China

[2]Shenzhen Engineering Laboratory for Birth Defects Screening, Shenzhen, China

[3]Department of Obstetrics and Gynaecology, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong SAR, China

[4]Experiment Centre for Science and Technology, Shanghai University of Traditional Chinese Medicine, Shanghai, China

[5]Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK

Correspondence
Kypros H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK.
Email: kypros@fetalmedicine.com

Fang Chen, BGI-Shenzhen, BGI, Yantian, Shenzhen 518083, China.
Email: fangchen@genomics.cn

Funding information
The Fetal Medicine Foundation, Grant/Award Number: UK Charity No: 1037116

Abstract

Objective: To define the lipidomic profile in plasma across pregnancy, and identify lipid biomarkers for gestational diabetes mellitus (GDM) prediction in early pregnancy.

Design: Case–control study.

Setting: Tertiary referral maternity unit.

Population or Sample: Plasma samples from 100 GDM and 100 normal glucose tolerance (NGT) women, divided into a training set (GDM first trimester = 50, GDM second trimester = 40, NGT first trimester = 50, NGT second trimester = 50) and a validation set (GDM first trimester = 45, GDM second trimester = 34, NGT first trimester = 44, NGT second trimester = 40).

Methods: Plasma samples were collected in the first (11^{+0} to 13^{+6} weeks), second (19^{+0} to 24^{+6} weeks), and third trimesters (30^{+0} to 34^{+6} weeks), and tested by ultra-high-performance liquid chromatography coupled with electrospray ionisation-quadrupole-time of flight-mass spectrometry; The GDM prediction model was established by the machine-learning method of random forest.

Main outcome measures: Gestational diabetes mellitus.

Results: In both the GDM and NGT group, lyso-glycerophospholipids were down-regulated, whereas ceramides, sphingomyelins, cholesteryl ester, diacylglycerols (DGs) and triacylglycerols (TGs) and glucosylceramide were up-regulated across the three trimesters of pregnancy. In the training dataset, seven TGs and five DGs demonstrated good performance in the prediction of GDM in the first and second trimesters (area under the curve [AUC] = 0.96 with 95% confidence interval [CI] of 0.93–1 and AUC = 0.97 with 95% CI of 0.95–1, respectively), independent of maternal body mass index (BMI) and ethnicity. In the validation dataset, the predictive model achieved an AUC of 0.88 and 0.94 at the first and second trimesters, respectively.

Conclusions: Our results have proposed new lipid biomarkers for the first trimester prediction of GDM, independent of ethnicity and BMI.

KEYWORDS
diacylglycerols, gestational diabetes mellitus, lipidomics, triacylglycerols

Guixue Hou, Ya Gao, Liona C. Poon and Yan Ren contributed equally to the work.

1 | INTRODUCTION

Gestational diabetes mellitus (GDM) complicates 5%–25% of pregnancies, depending on the populations, methods of screening and glucose threshold values that define the disorder.[1,2] GDM is a major cause of perinatal adverse outcomes including fetal macrosomia, shoulder dystocia and neonatal hypoglycaemia.[3] GDM also increases the risks of maternal adverse outcomes including the need of caesarean delivery, birth trauma and long-term disorders of glucose metabolism.[4] Appropriate treatment of GDM has been shown to improve maternal, obstetric and perinatal outcomes but the risks for long-term cardiovascular and metabolic adverse outcomes remain high in women affected by GDM and their offspring.[4,5] For women with a history of prior GDM, it is recommended that early testing for GDM be undertaken at the first prenatal visit or before 24 weeks in future pregnancies.[6] However, in most circumstances, current guidelines recommend testing for GDM in the late second and early third trimesters of pregnancy, leaving a narrow window for interventions.[6,7] It would be desirable to predict GDM in early pregnancy, ideally during the first trimester when women attend for their 11- to 13-week scan for screening of fetal aneuploidies and preterm pre-eclampsia.[8,9] A number of first trimester biochemical predictors of GDM have been reported; however, the studies have mostly used a case–control design with small sample sizes.[10] The accuracy and reproducibility of these predictors have not been widely confirmed in independent cohorts, thus limiting the applicability in clinical practice.[11,12] To date, no predictor has been universally recommended for early GDM screening.

Metabolomics is a powerful technology for the discovery of early diagnostic biomarkers, owing to its capacity for detecting early dysregulations and disruptions in metabolism associated with metabolic diseases.[13] Previous studies have already used metabolomics approaches to investigate the changes of metabolites in GDM patients. However, most of these studies had a small sample size and focused on detecting polar metabolites such as amino acids, nitrogenous bases, hormones and their intermediate by-products.[14] In contrast, there are a limited number of lipidomics studies focusing on detecting non-polar metabolites. In addition, the findings of previous metabolomics studies that have attempted to identify biomarkers for diagnosing GDM have been inconsistent, and clinically useful biomarkers for GDM prediction with high accuracy are still waiting to be discovered.[15]

Lipids are a diverse group of non-polar chemicals, which have key biological functions of cell membrane composition, energy storage and cellular signal regulation and dysregulation of lipids has been observed in metabolic diseases.[16] Lipid homeostasis plays a crucial role in the pathophysiology of type 2 diabetes. Hyperlipidaemia is a recognised physiological finding in pregnancy. In women with GDM, the physiological changes in lipids are amplified and may indicate an underlying metabolic disturbance during pregnancy.[17] In this study, we used lipidomics technologies with a high coverage of lipid species to define the profile changes across the three trimesters in pregnant women with GDM and those with normal glucose tolerance (NGT). We aimed to identify a panel of lipid biomarkers for GDM prediction in early pregnancy.

2 | METHODS

2.1 | Recruitment of participants

The participants of this study were originally recruited to a large prospective observational study for early prediction of pregnancy complications in women attending for their routine first hospital visit in pregnancy at King's College Hospital, London, UK.[18] In the first visit, at 11^{+0}–13^{+6} weeks' gestation, maternal characteristics and medical history were recorded and combined screening for aneuploidies was performed. The second visit, at 19^{+0}–24^{+6} weeks' gestation, and third visit, at 30^{+0}– 34^{+6} weeks' gestation, included ultrasound examination of the fetal anatomy and estimation of fetal size from measurement of fetal head circumference, abdominal circumference and femur length.[19] At each visit, maternal blood was collected in ethylene diamine tetra-acetic acid (EDTA) blood collection tubes and maternal plasma was extracted and stored at −80°C for research purposes. Gestational age was determined by the measurement of fetal crown–rump length at 11–13 weeks' gestation.[20] Written informed consent was obtained from the women agreeing to participate in a study on adverse pregnancy outcome, which was approved by the hospital Ethics Committee. All women were screened for GDM at King's College Hospital, London, UK, based on a two-step approach. Random plasma glucose was measured at 24–28 weeks' gestation and if the concentration was >6.7 mmol/L an oral glucose tolerance test (OGTT) was carried out within the subsequent 2 weeks. The diagnosis of GDM is made if the fasting plasma glucose level is ≥6 mmol/L or the plasma glucose level 2 hours after the oral administration of 75 g glucose is ≥7.8 mmol/L.[21,22] In this study, we retrospectively retrieved the archived plasma samples of 100 singleton pregnancies with GDM and 100 singleton pregnancies with NGT for lipidomics profiling analysis based on matched sampling time (on the same day).

2.2 | Lipid extraction and untargeted lipidomic profiling

Lipid extraction was performed according to standardised methodology reported previously.[23,24] In short, the plasma samples (40 microlitres each) that were previously stored at −80°C were placed on ice and mixed with 3 volumes of precooled (−20°C) isopropanol. Samples were vortex-mixed for 1 minute. After 10 minute of incubation at room temperature, samples were stored overnight at −20°C to improve protein precipitation and then centrifuged at 14 000 *g* for 20 min. The supernatant was collected (100 microlitres) and diluted with 400 microlitres of IPA/

BJOG An International Journal of Obstetrics and Gynaecology

ACN/H2O (2:1:1 v:v:v) and stored at −80°C awaiting MS analysis.

Ultra-high-performance liquid chromatography coupled with electrospray ionisation-quadrupole-time of flight-mass spectrometry (G2-XS QTOF; Waters) was used for untargeted lipidomic profiling. The LC–MS parameter setting was similar to that previously reported.[24] Briefly, lipid molecules were separated with a CSH C18 column (2.1 × 100 mm, 1.7 micrometers; Waters) in a 10-minute LC gradient and detected with a XEVO-G2XS QTOF mass spectrometer in positive and negative mode, which was operated in MS^E mode from m/z 50–2000, with an acquisition time of 1 second per scan. Leucine encephalin was used as a lock mass for accurate mass measurements and 0.5-mM sodium formate solution was used for calibration.

Pooled plasma was used as quality control (QC) and injected in every ten injections for QC.[25,26] Identification was performed by PROGENESIS QI software searching the Human Metabolome Database (HMDB, http://www.hmdb.ca/)[27] and Lipidmaps[28] databases with accurate mass (<10 ppm) and MS/MS spectrum match. Data preprocessing and analysis were performed using the metaX package.[29] The QC-based robust locally estimated scatterplot smoothing signal correction[30,31] method was applied to correct batch variation. Features with a coefficient of variation of more than 30% in QC samples were excluded.[27]

TABLE 1 Characteristics of study population

	GDM ($n = 100$)	NGT ($n = 100$)	p-value
Maternal age in years, mean ± SD	33.2 ± 5.1	31.3 ± 6.0	0.016*
Body mass index in kg/m^2, mean ± SD	30.2 ± 7.3	25.3 ± 5.4	<0.0001*
Gestational age in weeks, mean ± SD	12.6 ± 0.5	12.6 ± 0.5	0.555
Crown-rump length in mm, mean ± SD	62.1 ± 7.3	62.7 ± 7.3	0.555
Racial origin			
White, *n* (%)	37 (37.0)	63 (63.0)	0.001*
Afro-Caribbean, *n* (%)	36 (36.0)	29 (29.0)	
South Asian, *n* (%)	17 (17.0)	2 (2.0)	
East Asian, *n* (%)	8 (8.0)	4 (4.0)	
Mixed, *n* (%)	2 (2.0)	2 (2.0)	
Gestational age at delivery in weeks, mean ± SD	38.3 ± 1.4	39.8 ± 0.8	<0.0001*
Birthweight in grams, mean ± SD	3159.5 ± 516.4	3349.5 ± 270.5	0.001*

Note: Comparisons using Student's *t*-test or chi-square test.

Abbreviations: GDM, Gestational diabetes mellitus; SD, standard deviation.

*$p < 0.05$.

2.3 | Statistical analysis

To reveal the hidden structure in lipidomics over time, soft clustering was provided based on fuzzy c-means algorithm implemented in the R package Mfuzz.[32] The optimised number of clusters was estimated by calculating minimum centroid distance. A machine-learning method, random forest (RF), was applied to identify features that could discriminate the GDM and NGT pregnant women, and the gestational change using the Caret package.[33] ROC curve analysis was used to assess the ability of a combination of identified lipid species in predicting GDM. Statistical analyses were performed by R language using packages including ggplot2,[34] and Two-way analysis of variance (ANOVA2) was processed by the two-factor analysis module in MetaboAnalyst.[35] Each lipid class was examined for the association with lipid changes in GDM using linear models adjusted for body mass index (BMI), ethnicity and maternal age. Associations with lipid change in BMI were adjusted for GDM, ethnicity and maternal age. The *p*-values were corrected for multiple comparisons using the Benjamini–Hochberg procedure.

2.4 | GDM-related lipid identification using MS/MS fragmentation

The RF-selected features were further identified by targeted fragmentation and the MS/MS spectrum was manually checked under the guidelines of the Lipidomics Standards Initiative (LSI; https://lipidomics-standards-initiative.org/). All representative matched MS/MS spectra are shown in Figure S2.

3 | RESULTS

3.1 | Study design and data QC

Maternal characteristics of the study population are shown in Table 1. In the GDM group, compared with the NGT group, the mean maternal age and BMI were higher and there was a higher proportion of non-white women. Additionally, the GDM group, compared with the NGT group, delivered earlier and their neonates had a lower birthweight, which is consistent with previous reports.[36,37]

All plasma samples from GDM and NGT subjects in the three trimesters of pregnancy were quantified using lipid profiling technologies to evaluate the systematic lipid variation related with pregnancy, GDM and racial origin (Figure 1A). After removing sample outliers, there were 268 GDM subjects (corresponding to 95, 74 and 99 GDM samples in the first, second and third trimesters, respectively) and 280 NGT subjects (corresponding to 94, 90 and 96 NGT samples in the first, second and third trimesters, respectively) whose samples could be used for further analysis. For GDM biomarker analysis, samples were divided into training dataset and validation dataset (Figure 1B). Potential biomarkers discovered in the training dataset were quantified

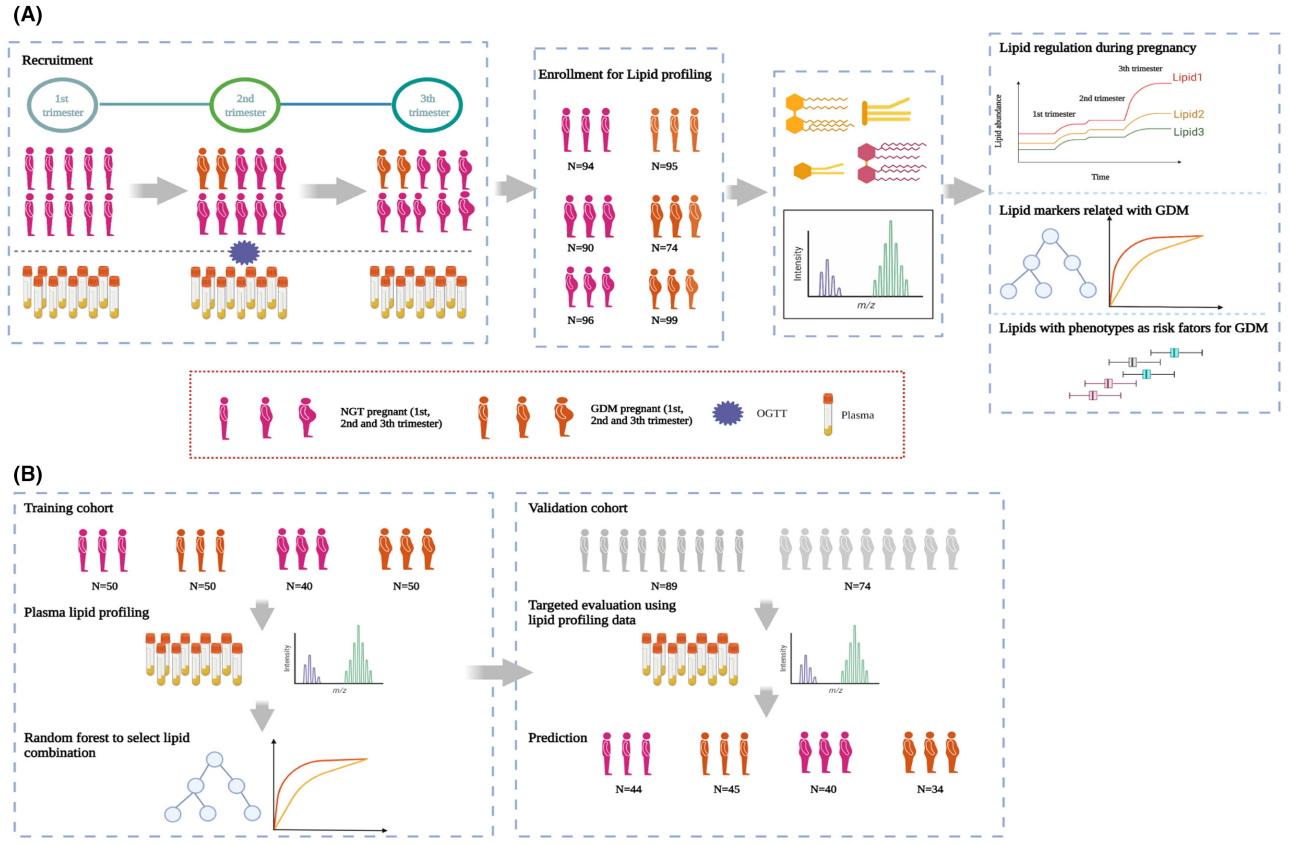

FIGURE 1 Study design using lipid profiling of plasma in gestational diabetes mellitus (GDM) and normal glucose tolerance (NGT) subjects. (A) Study design of GDM related lipid profiling. (B) Selection and validation of lipid signatures in the training and validation datasets.

and subsequently evaluated for GDM prediction in the validation dataset. The principal component analysis showed that all QC samples that spiked at certain intervals clustered together, verifying an acceptable reproducibility and stability of the results (Figure S1).

3.2 | Lipid changes across the first, second and third trimesters

For plasma collected from the three trimesters, untargeted lipidomics profiling quantified 963 lipids by MS1 level identification, including 12 cholesteryl ester (CEs), 32 ceramides (Cers), 72 diacylglycerols (DGs), 13 glucosylceramide (GlcCers), 11 lactosylceramide (LacCers), 20 lyso-phosphatidylcholines (Lyso-PCs), 17 lyso-phosphatidylethanolamines (Lyso-PEs), 13 phosphatidic acids (PAs), 233 phosphatidylcholines (PCs), 153 phosphatidylethanolamines (PEs), 67 phosphatidylglycerols (PGs), 43 phosphoinositols (PIs), 32 phosphoserines (PSs), 25 sphingomyelins (SMs) and 220 triacylglycerols (TGs).

In NGT pregnant women, time-clustering analysis identified six expression clusters for all profiled lipids. Lipids in clusters 1 and 3 were up-regulated throughout pregnancy, whereas lipids in clusters 5 and 6 were down-regulated throughout pregnancy. Lipids in cluster 4 had the maximum expression in the second trimester, whereas lipids in cluster 2 had the minimum expression in the second trimester (Figure 2A). As deciphered by the heatmap of the percentages for each lipid species in Figure 2B, the abundance of lyso-glycerophospholipids, including Lyso-PCs, Lyso-PEs and LacCers were downregulated, whereas Cers, SMs, CEs, DGs and TGs and GlcCers were enriched in the up-regulating clusters across the three trimesters of pregnancy.

3.3 | Potential lipid biomarkers to differentiate GDM from NGT groups

Fold-change comparison of lipid abundance between the GDM and NGT groups throughout pregnancy demonstrated significant differences with an adjusted p-value of <0.05 (Figure 3A). In particular, at the first, second and third trimesters of pregnancy, 54.7, 15.1 and 20.8% of DG species had o average a 1.32- (1.14–1.68), 1.31- (1.15–1.57) and 1.13-fold (0.7–1.43) higher expression in the GDM group than in the NGT group, respectively. On the other hand, 25.1% and 12.0% of TG species that had on average a 1.21- (0.66–1.88) and 1.13-fold (0.7–1.44) higher expression in the first and second trimester in the GDM group than in the NGT group, respectively. The medium expression level of DGs, FAs and PEs in the GDM group was constantly higher than that in the NGT group. In contrast, the medium expression level

BJOG An International Journal of Obstetrics and Gynaecology

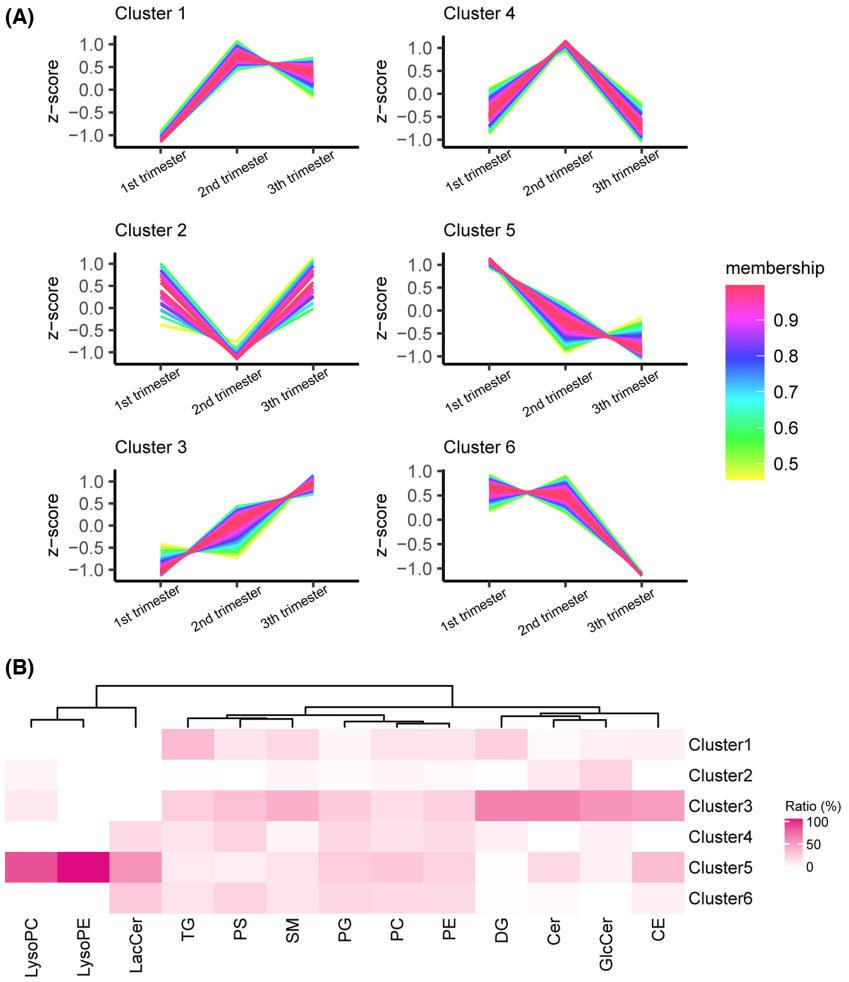

FIGURE 2 Temporal clustering of lipids in normal glucose tolerance (NGT) pregnant women across three trimesters. (A) Temporal clustering of lipids quantified in NGT pregnant women across the three trimesters (First: 11–13 weeks; second: 20–24 weeks; third: 30–34 weeks). (B) Heatmap of percentage for lipid classes enriched in the six clusters.

of PCs, PGs, PIs and PSs in the GDM group was constantly lower than that in the NGT group.

To explore potential lipids related to GDM in the first trimester of pregnancy, all lipid profiling data were divided into two datasets (Figure 1B), In the training dataset (first trimester GDM = 50, second trimester GDM = 40, first trimester NGT = 50, and second trimester NGT = 50), all differential lipids (p-value <0.05) were used for biomarker selection using RF analysis. There were 12 lipid signatures with significant expression differences across each trimester of pregnancy between the NGT and GDM groups, including five DGs and seven TGs. Targeted MS/MS fragmentation was used for further identification (Figure S2). Seven lipid signatures (DG[16:0_18:1], TG[16:0_16:1_18:1], DG[18:0_16:1], TG[16:0_16:1_20:1], DG[32:0], TG[16:0_16:0_18:1] and DG[O-34:1]) showed increased expression across each trimester of pregnancy, and were more highly expressed in the GDM than in the NGT group (Figure 3B). Four lipid signatures (DG[34:0], TG[16:0_18:0_18:1], TG[18:1_18:1_22:6] and TG[16:1_20:2_22:1]) showed a higher expression in the GDM than in the NGT group, although their expression decreased followed the increase in gestational age. One lipid signature (TG[18:2_18:2_22:6]) showed decreased expression across each trimester and had lower expression in the GDM than in the NGT group. For the prediction of GDM, receiver operating characteristic (ROC) of the combination of these 12 lipid signatures yielded area under the ROC curves (AUCs) of 0.96 (95% CI 0.93–1) and 0.97 (95% CI 0.95–1) in the first and second trimesters of pregnancy, respectively, which were significantly higher than other respective values (Figure 3C). Linear regression analysis was performed to clarify the correlation of the 12 identified lipids with maternal BMI and age (Figure S3); the level of correlation coefficient was very low ($R^2 < 0.2$). Thus, the prominent expression differences of the 12 lipids in GDM women were not confounded by maternal BMI or age. To validate the predictive performance of these 12 lipid signatures in plasma selected as potential indicators to predict GDM, their abundances in the GDM (first trimester: 45 and second trimester: 34) and NGT (first trimester: 44 and second trimester: 40) subjects from the validation dataset were explored (Figure 1B). The AUC could reach 0.88 with 95% CI (0.80–0.95) and 0.94 with 95% CI (0.89–0.99) using these 12 molecules in the first and second trimesters (Figure 3C).

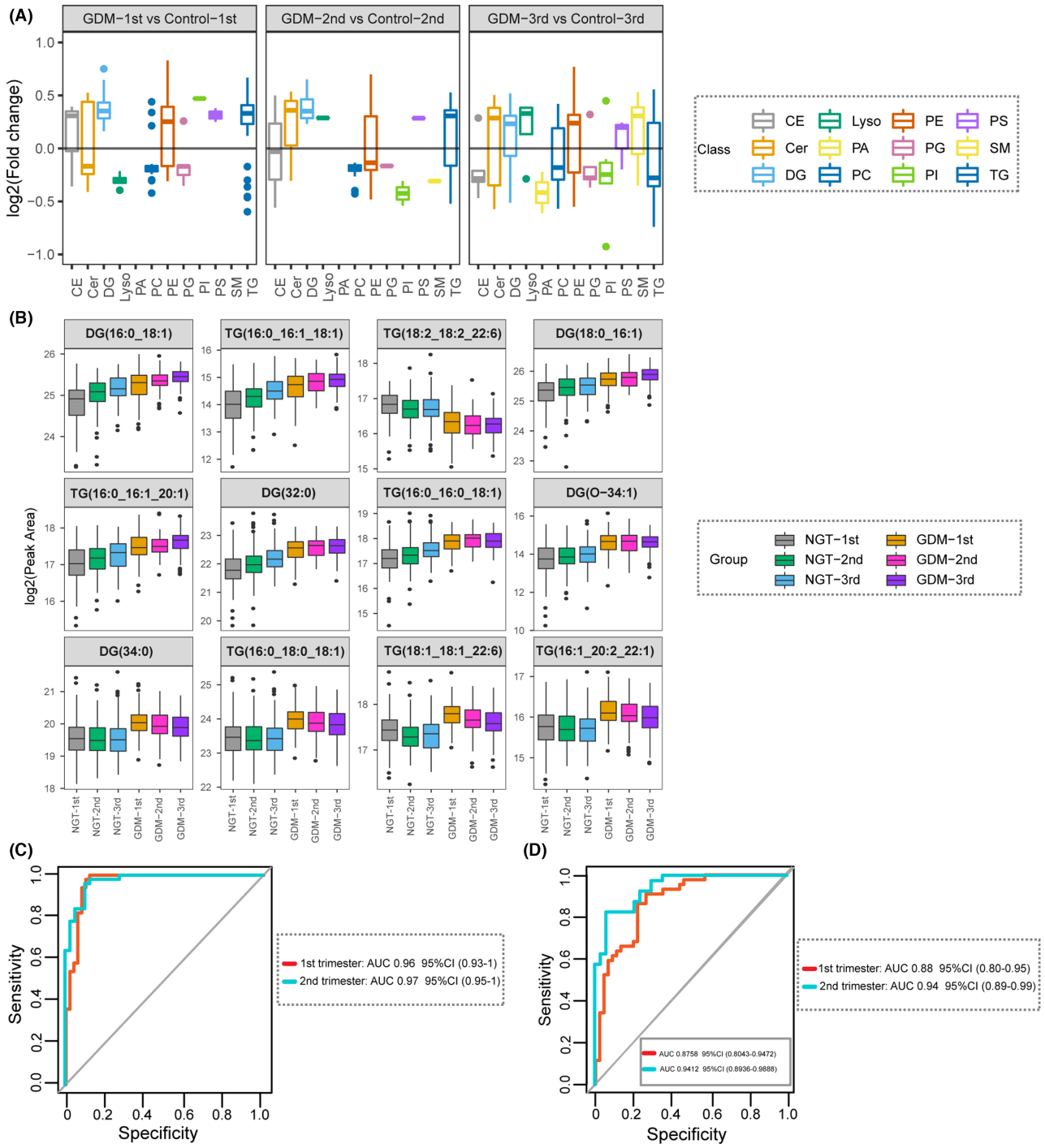

FIGURE 3 Evaluation of the 12 distinct lipid species selected to differentiate gestational diabetes mellitus (GDM) and normal glucose tolerance (NGT) groups. (A) Boxplot of differential lipid classes distribution for GDM versus NGT across three trimesters of pregnancy. (B) Boxplot showed the expression abundance (peak area of the corresponding peak area quantified by LC–MS) with different groups (two trimesters in NGT group, including NGT-1st, NGT-2nd and in GDM groups, including GDM-1st and GDM-2nd). Receiver operating characteristic (ROC) curves of the combination of 12 lipid markers at the first (red curve) and second (blue curve) trimesters of pregnancy in the training dataset (C) and validation dataset (D).

3.4 | Characterising lipids related to BMI, ethnicity and maternal age

Considering that GDM is associated with BMI, maternal age and ethnicity, we systematically evaluated the difference of lipid abundance mainly affected by these variables. SMs were significantly associated with BMI adjusted for GDM, ethnicity and maternal age (Figure 4A). Lipid classes including Lyso-PC, PA, PC, PS and TG were significantly associated with ethnicity adjusted for GDM, BMI and maternal age (Figure 4B). No lipids were significantly associated with maternal age adjusted for GDM, BMI and ethinity (Figure 4C). Amongst the above 12 lipid markers, most were associated with GDM, with the exception of DG (18:0_16:1)

BJOG An International Journal of Obstetrics and Gynaecology

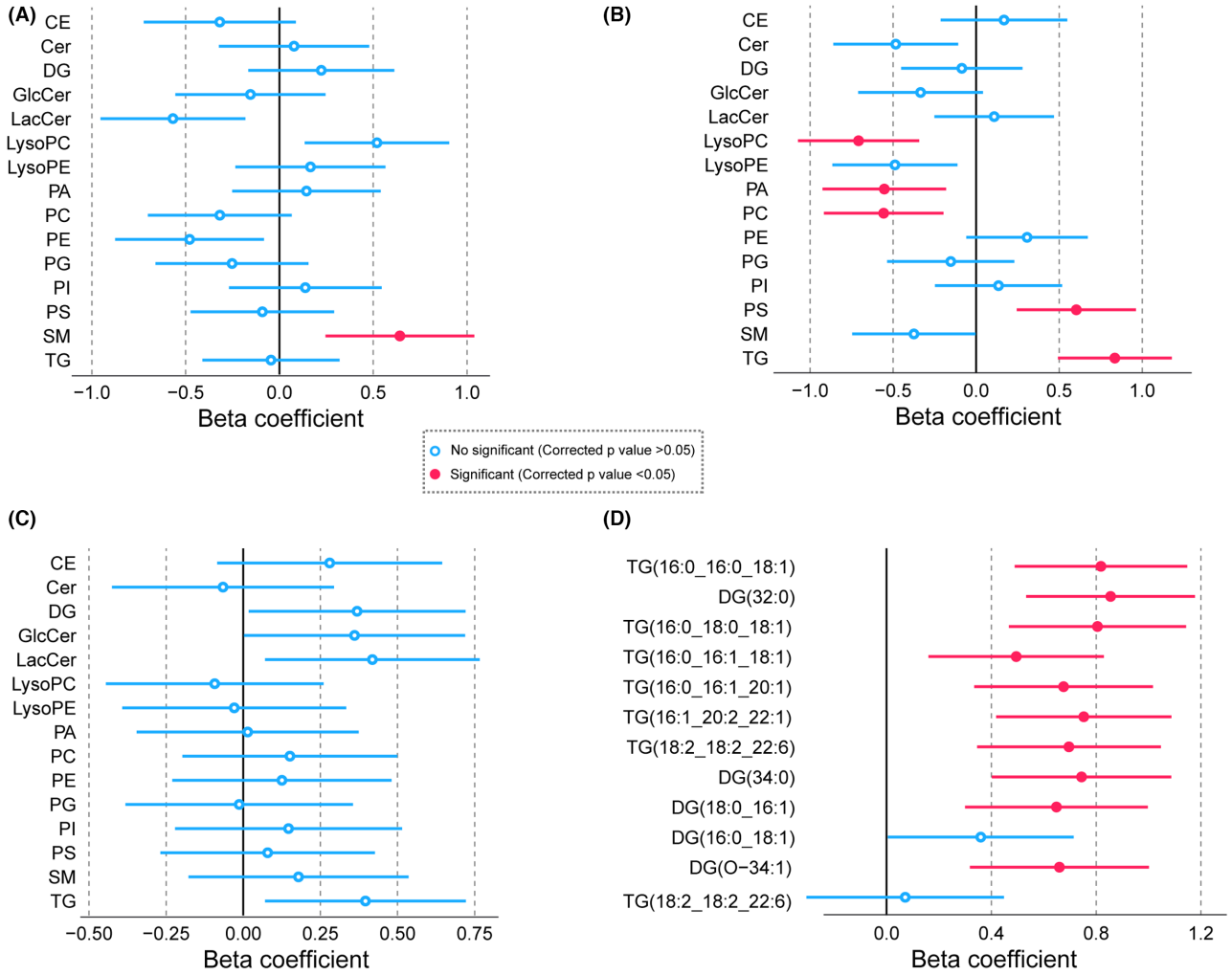

FIGURE 4 Phenotype-dependent associations of lipid classes/subclasses with maternal BMI, ethnicity and maternal age. Adjusted β-coefficient with 95% CI of lipid classes regressed on level of BMI group with adjusting gestational diabetes mellitus (GDM), ethnicity and maternal age (A), ethnicity group with adjusting GDM, BMI and maternal age (B), maternal age group with adjusting GDM, BMI and ethnicity (C) and GDM group with adjusting BMI, ethnicity and maternal age (D). Red solid circles show the association was significantly (corrected $p < 0.05$). Open circles represent nonsignificant classes (corrected $p > 0.05$). BMI, body mass index; CE, cholesteryl ester; Cer, ceramide; DG, diacylglycerol; LPC, lysoalkenylphosphatidylcholine; LPE, lyso-phosphatidylethanolamines; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; TG, triacylglycerol.

and TG (18:2_18:2_22:6), when adjusted for BMI, ethnicity and maternal age (Figure 4D). The combination of these 12 lipid markers with BMI and ethnicity could predict GDM and NGT in the first and second trimesters with an AUC of 0.89 and 0.92, indicating that these lipid markers could be potential signatures for GDM prediction in the early stage of pregnancy.

4 | DISCUSSION

The study has demonstrated that, first, in both the NGT and GDM groups, the expression levels of Cers, CEs, SMs, PAs, PCs, PEs, PGs and PSs increase with increasing gestation, whereas Lyso-PCs and Lyso-PEs decrease with increasing gestation. Secondly, 12 lipid species with the most prominent expression differences could be used as potential discriminators for the prediction of GDM in early pregnancy, achieving high AUCs in the first and second trimesters of pregnancy. Finally, lipid profiling during pregnancy is significantly influenced by maternal racial origin.

It is known that major changes of lipid metabolism occur during pregnancy, leading to an increase in lipid synthesis and adipose tissue mass at the initial stage of pregnancy, as well as maternal hyperlipidaemia at the later stage of pregnancy.[38–40] In this study, major lipid classes have demonstrated consistent profile changes with increasing gestation in both the NGT and GDM groups. In addition to the gestational impact on lipid metabolism, we have identified a subset of lipid species showing significant expression differences between the NGT pregnancies and women with GDM. In particular, the expression of TGs and DGs was significantly higher in women with GDM than NGT women,

especially in early pregnancy. We have shown that the expression changes of five DG and seven TG signatures could be used to predict GDM in the first and second trimesters of pregnancy (AUC 0.9644 with 95% CI 0.9283–1, and AUC 0.9735 with 95% CI 0.9469–1), respectively. Lu et al. have reported the use of an unbiased lipidomics approach to identify four serum lipid biomarkers, including TGs and PCs, to predict GDM in the second trimester, achieving an AUC of 0.71.[40] Liu et al. have analysed serum metabolites of GDM women and healthy controls in late third trimester using an UPLC/Q-TOF-MS-based approach, and have identified nine metabolites, including glucosamine, 1-methyladenosine, L-tyrosine and others, for the prediction of GDM, achieving an AUC of 0.7.[25] Our lipid biomarkers have achieved a higher predictive power than previous studies. Hence, our 12 lipid signatures may serve as potential predictors for GDM before the manifestation of the disorder. However, the methodology used to measure the potential lipid predictors of GDM is more expensive than testing haemoglobin A1C (HbA1C) and OGTT in clinical practice. Other than prospective validation, there will be a need to develop a targeted approach to measure the lipid predictors for affordable GDM screening in early pregnancy.

The hyperglycaemia of GDM is associated with impaired glucose tolerance due to pancreatic β-cell dysfunction on a background of chronic insulin resistance. We found that in women with GDM, six TG species had elevated expression and one TG species had reduced expression throughout pregnancy. Previously, increased TG levels in the plasma of GDM women compared with NGT women has also been reported, closely correlated with plasma glucose and insulin concentrations.[41,42] The reason for the elevated TG expression in GDM is believed to be related to lipoprotein lipase deficiency as a result of an increase in fat intake, in addition to worsening insulin resistance.[41] However, most previous studies have only quantified the total TG level using enzymatic assays instead of assessing the expression level of individual TG signatures. Using fold-change analysis of different TG species, we have found that not all, but only a proportion (25.1 and 12.0% in the first and second trimesters) of TG lipids have an elevated expression in GDM women. Furthermore, we have shown that instead of increased expression, at least one TG signature has a significantly reduced expression in GDM pregnancy. Thus, it is possible that only a subset of total TGs has altered expression related to the development of GDM, and the total TG level measured by conventional enzymatic assays may not have sufficient discriminatory power in predicting GDM.

As one of the best-studied mediators of lipid-induced insulin resistance, DGs are well-known for their potential mechanistic roles in hepatic insulin resistance.[43] Our results have confirmed the important role of DGs in the development of GDM. DGs are key intermediates in maintaining intracellular TG balance by acyltransferases or phosphohydrolases.[44] In type 2 diabetes mellitus, increased levels of DGs have been linked to impaired protein kinase C activation and insulin signalling.[45–47] In normal liver, mature human insulin receptor B isoform (INSR Thr^{1160}) does not interfere with insulin receptor kinase (IRK) activation, and downstream signalling proceeds normally upon insulin binding. In nonalcoholic fatty liver disease, DGs accumulation promotes membrane translocation of protein kinase C epsilon type, which in turn phosphorylates INSR Thr^{1160} to impair IRK activity and thereby induces hepatic insulin resistance.[48] Genetically modified mouse models have been generated to examine the DGs hypothesis of lipid-induced hepatic insulin resistance. Mice with loss-of-function of mitochondrial acyl-CoA:glycerol-sn-3-phosphate acyltransferase, a major hepatic glycerol-3-phosphate acyltransferase, accumulate less intrahepatic DGs and TGs than controls on high-fat feeding, associated with protection from hepatic insulin resistance.[49] Recently, two separate lipidomics studies have demonstrated that GDM is associated with the elevation of TGs and DGs in the third trimester, as well as many other lipids; though no lipid markers have been evaluated for the prediction of GDM.[50,51]

There are recognised racial/ethnic differences in the prevalence of GDM. Women with normal BMI of African, Caribbean and South Asian racial origins are at a higher risk of developing GDM, in comparison with white women.[52] In this study, we have shown that racial origin is a major factor that influences lipid expression. Interestingly, a meta-analysis of maternal lipid levels during pregnancy has demonstrated that in comparison with NGT women, women with GDM in the USA tend to have reduced levels of total cholesterol and low-density lipoprotein cholesterol (LDLC), and increased levels of non-LDLC, whereas women with GDM in other countries tend to show no altered levels of lipids.[41] Our results suggest that racial origin should be considered when identifying biomarkers, especially metabolomic markers, for the prediction of GDM in early pregnancy in an unselected population.

The strengths of this study include the well-defined diagnosis of GDM in a prospective population with longitudinal maternal plasma samples collected in all three trimesters of pregnancy. This study also benefits from a relatively large sample size of GDM cases, compared with previous metabolomics studies, and appropriately identified NGT controls.

There are several limitations to our study. First, although we validated our candidate lipid biomarkers using a predefined subset, these biomarkers were not externally validated in large cohorts. Secondly, biochemical results of HbA1C, total TGs and high-density lipoprotein cholesterol were not acquired by additional testing due to the limited sample volume, or by clinical records as they are not routinely measured as part of antenatal care. Thus, association analysis of the lipidomics discovery in this study with biochemical results could not be performed. Thirdly, we only selected 12 lipid features for univariate analysis, which might have been an overly conservative approach. Lastly, the efficacy of our prediction model may be overestimated due to the case–control study design.

5 | CONCLUSIONS

There are prominent changes in lipid metabolism across the three trimesters of pregnancy associated with the up-regulation of Cers, CEs, SMs, PAs, PCs, PEs, PGs and PSs, and the down-regulation of Lyso-PCs and Lyso-PEs. We have demonstrated that the development of GDM is associated with an altered expression of a subset of TGs and DGs, instead of total TGs and DGs. The involvement of this subset of TGs and DGs in the development of GDM is independent of maternal BMI and racial origin, suggesting their potential role in the prediction of GDM in the first and second trimesters of pregnancy.

AUTHOR CONTRIBUTIONS

Conceptualisation: KHN and FC. Methodology: GH, YR, YG and LCP. Investigation: LCP, AS, JG, LL, JZ, FXS and WX. Resources: LCP and AS. Formal analysis: GH, CZ and BW. Supervision: KHN, YG and FC. Funding acquisition: KHN. Writing – Original Draft: GH, CZ and YG. Writing – Review & Editing: KHN, LCP, and FC.

ACKNOWLEDGEMENTS

We thank all the physicians who facilitated the recruitment of individuals at the King's College Hospital and all the participants who agreed to take part in this study.

FUNDING INFORMATION

This work was supported by the Fetal Medicine Foundation (UK Charity No: 1037116).

CONFLICT OF INTERESTS

None declared. Completed disclosure of interest forms are available to view online as supporting information.

DATA AVAILABILITY STATEMENT

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper have been deposited into CNGB Sequence Archive (CNSA) of China National GeneBank DataBase (CNGBdb) with accession number CNP0001246.

ETHICS APPROVAL

This study was approved by the Ethics Committee of the King's College Hospital (REC Reference.02-03-033) and the Institutional Review Board of BGI-Shenzhen (BGI-IRB 19175-T2).

ORCID

Ya Gao https://orcid.org/0000-0002-5094-9842
Fengxia Su https://orcid.org/0000-0003-0724-6284

REFERENCES

1. No authors. The global challenge of diabetes. Lancet. 2008;371(9626):1723.
2. Sacks DA, Hadden DR, Maresh M, Deerochanawong C, Dyer AR, Metzger BE, et al. Frequency of gestational diabetes mellitus at collaborating centers based on IADPSG consensus panel-recommended criteria: the hyperglycemia and adverse pregnancy outcome (HAPO) study. Diabetes Care. 2012;35(3):526–8.
3. HAPO Study Cooperative Research Group, Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008;358(19):1991–2002.
4. Bellamy L, Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. Lancet. 2009;373(9677):1773–9.
5. Metzger BE. Long-term outcomes in mothers diagnosed with gestational diabetes mellitus and their offspring. Clin Obstet Gynecol. 2007;50(4):972–9.
6. National Collaborating Centre for Women's and Children's Health (UK). Diabetes in pregnancy: management of diabetes and its complications from preconception to the postnatal period. London: National Institute for Health and Care Excellence (UK); 2015.
7. Walker JD. NICE guidance on diabetes in pregnancy: management of diabetes and its complications from preconception to the postnatal period. NICE clinical guideline 63. London, March 2008. Diabet Med. 2008;25(9):1025–7.
8. Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn. 2011;31(1):7–15.
9. Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco MC, et al. Aspirin versus placebo in pregnancies at high risk for preterm preeclampsia. N Engl J Med. 2017;377(7):613–22.
10. Powe CE. Early pregnancy biochemical predictors of gestational diabetes mellitus. Curr Diab Rep. 2017;17(2):12.
11. Lorenzo-Almoros A, Hang T, Peiro C, Soriano-Guillen L, Egido J, Tunon J, et al. Predictive and diagnostic biomarkers for gestational diabetes and its associated metabolic and cardiovascular diseases. Cardiovasc Diabetol. 2019;18(1):140.
12. Saravanan P, Diabetes in Pregnancy Working G, Maternal Medicine Clinical Study G, Royal College of O, Gynaecologists UK. Gestational diabetes: opportunities for improving maternal and child health. Lancet Diabetes Endocrinol. 2020;8(9):793–800.
13. Bain JR, Stevens RD, Wenner BR, Ilkayeva O, Muoio DM, Newgard CB. Metabolomics applied to diabetes research: moving from information to knowledge. Diabetes. 2009;58(11):2429–43.
14. Bentley-Lewis R, Huynh J, Xiong G, Lee H, Wenger J, Clish C, et al. Metabolomic profiling in the prediction of gestational diabetes mellitus. Diabetologia. 2015;58(6):1329–32.
15. Huynh J, Xiong G, Bentley-Lewis R. A systematic review of metabolite profiling in gestational diabetes mellitus. Diabetologia. 2014;57(12):2453–64.
16. Han X. Lipidomics for studying metabolism. Nat Rev Endocrinol. 2016;12(11):668–79.
17. Carpenter MW. Gestational diabetes, pregnancy hypertension, and late vascular disease. Diabetes Care. 2007;30(Suppl 2):S246–50.
18. O'Gorman N, Wright D, Syngelaki A, Akolekar R, Wright A, Poon LC, et al. Competing risks model in screening for preeclampsia by maternal factors and biomarkers at 11-13 weeks gestation. Am J Obstet Gynecol. 2016;214(1):103.e1–12.
19. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements—a prospective study. Am J Obstet Gynecol. 1985;151(3):333–7.
20. Robinson HP, Fleming JE. A critical evaluation of sonar "crown-rump length" measurements. Br J Obstet Gynaecol. 1975;82(9):702–10.
21. World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. [cited 2022 Oct 1]. Available from: https://www.who.int/publications/i/item/definition-and-diagnosis-of-diabetes-mellitus-and-intermediate-hyperglycaemia
22. Alberti KG, Zimmet PZ. Definition, diagnosis, and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998;15(7):539–53.
23. Dunn WB, Broadhurst D, Begley P, Zelena E, Francis-McIntyre S, Anderson N, et al. Procedures for large-scale metabolic profiling of

serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. Nat Protoc. 2011;6(7):1060–83.
24. Sarafian MH, Gaudin M, Lewis MR, Martin FP, Holmes E, Nicholson JK, et al. Objective set of criteria for optimization of sample preparation procedures for ultra-high throughput untargeted blood plasma lipid profiling by ultra performance liquid chromatography-mass spectrometry. Anal Chem. 2014;86(12):5766–74.
25. Liu T, Li J, Xu F, Wang M, Ding S, Xu H, et al. Comprehensive analysis of serum metabolites in gestational diabetes mellitus by UPLC/Q-TOF-MS. Anal Bioanal Chem. 2016;408(4):1125–35.
26. Zeng C, Wen B, Hou G, Lei L, Mei Z, Jia X, et al. Lipidomics profiling reveals the role of glycerophospholipid metabolism in psoriasis. GigaScience. 2017;6(10):1–11.
27. Wishart DS, Feunang YD, Marcu A, Guo AC, Liang K, Vazquez-Fresno R, et al. HMDB 4.0: the human metabolome database for 2018. Nucleic Acids Res. 2018;46(D1):D608–D17.
28. Fahy E, Sud M, Cotter D, Subramaniam S. LIPID MAPS online tools for lipid research. Nucleic Acids Res. 2007;35:W606–12.
29. Wen B, Mei Z, Zeng C, Liu S. metaX: a flexible and comprehensive software for processing metabolomics data. BMC Bioinformatics. 2017;18(1):183.
30. Kultima K, Nilsson A, Scholz B, Rossbach UL, Falth M, Andren PE. Development and evaluation of normalization methods for label-free relative quantification of endogenous peptides. Mol Cell Proteomics. 2009;8(10):2285–95.
31. Rusilowicz M, Dickinson M, Charlton A, O'Keefe S, Wilson J. A batch correction method for liquid chromatography-mass spectrometry data that does not depend on quality control samples. Metabolomics. 2016;12:56.
32. Kumar L, Futschik ME. Mfuzz: A software package for soft clustering of microarray data. Bioinformation. 2007;2(1):5–7.
33. Kuhn M. Building predictive models in R using the caret package. J Stat Softw. 2008;28(5):1–26.
34. Wickham H. ggplot2: elegant graphics for data analysis. 2nd. ed. New York: Springer International Publishing; 2016.
35. Chong J, Soufan O, Li C, Caraus I, Li S, Bourque G, et al. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. Nucleic Acids Res. 2018;46(W1):W486–W94.
36. Poon LC, Tan MY, Yerlikaya G, Syngelaki A, Nicolaides KH. Birth weight in live births and stillbirths. Ultrasound Obstet Gynecol. 2016;48(5):602–6.
37. Seghieri G, Anichini R, De Bellis A, Alviggi L, Franconi F, Breschi MC. Relationship between gestational diabetes mellitus and low maternal birth weight. Diabetes Care. 2002;25(10):1761–5.
38. Villar J, Cogswell M, Kestler E, Castillo P, Menendez R, Repke JT. Effect of fat and fat-free mass deposition during pregnancy on birth weight. Am J Obstet Gynecol. 1992;167(5):1344–52.
39. Alvarez JJ, Montelongo A, Iglesias A, Lasuncion MA, Herrera E. Longitudinal study on lipoprotein profile, high density lipoprotein subclass, and postheparin lipases during gestation in women. J Lipid Res. 1996;37(2):299–308.
40. Lu L, Koulman A, Petry CJ, Jenkins B, Matthews L, Hughes IA, et al. An unbiased Lipidomics approach identifies early second trimester lipids predictive of maternal glycemic traits and gestational diabetes mellitus. Diabetes Care. 2016;39(12):2232–9.
41. Ryckman KK, Spracklen CN, Smith CJ, Robinson JG, Saftlas AF. Maternal lipid levels during pregnancy and gestational diabetes: a systematic review and meta-analysis. BJOG. 2015;122(5):643–51.
42. Bao W, Dar S, Zhu Y, Wu J, Rawal S, Li S, et al. Plasma concentrations of lipids during pregnancy and the risk of gestational diabetes mellitus: a longitudinal study. J Diabetes. 2018;10(6):487–95.
43. Petersen MC, Shulman GI. Roles of diacylglycerols and ceramides in hepatic insulin resistance. Trends Pharmacol Sci. 2017;38(7):649–65.
44. Eichmann TO, Lass A. DAG tales: the multiple faces of diacylglycerol – stereochemistry, metabolism, and signaling. Cell Mol Life Sci 2015;72(20):3931–52.
45. Chibalin AV, Leng Y, Vieira E, Krook A, Bjornholm M, Long YC, et al. Downregulation of diacylglycerol kinase delta contributes to hyperglycemia-induced insulin resistance. Cell. 2008;132(3):375–86.
46. Moro C, Galgani JE, Luu L, Pasarica M, Mairal A, Bajpeyi S, et al. Influence of gender, obesity, and muscle lipase activity on intramyocellular lipids in sedentary individuals. J Clin Endocrinol Metab. 2009;94(9):3440–7.
47. Szendroedi J, Yoshimura T, Phielix E, Koliaki C, Marcucci M, Zhang D, et al. Role of diacylglycerol activation of PKCtheta in lipid-induced muscle insulin resistance in humans. Proc Natl Acad Sci U S A. 2014;111(26):9597–602.
48. Petersen MC, Madiraju AK, Gassaway BM, Marcel M, Nasiri AR, Butrico G, et al. Insulin receptor Thr1160 phosphorylation mediates lipid-induced hepatic insulin resistance. J Clin Invest. 2016;126(11):4361–71.
49. Neschen S, Morino K, Hammond LE, Zhang D, Liu ZX, Romanelli AJ, et al. Prevention of hepatic steatosis and hepatic insulin resistance in mitochondrial acyl-CoA:glycerol-sn-3-phosphate acyltransferase 1 knockout mice. Cell Metab. 2005;2(1):55–65.
50. Liu H, Pan LL, Lv S, Yang Q, Zhang H, Chen W, et al. Alterations of gut microbiota and blood lipidome in gestational diabetes mellitus with hyperlipidemia. Front Physiol. 2019;10:1015.
51. He Q, Zhu S, Lin M, Yang Q, Wei L, Zhang J, et al. Increased GPR120 level is associated with gestational diabetes mellitus. Biochem Biophys Res Commun. 2019;512(2):196–201.
52. Yuen L, Wong VW. Gestational diabetes mellitus: challenges for different ethnic groups. World J Diabetes. 2015;6(8):1024–32.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Hou G, Gao Y, Poon LC, Ren Y, Zenga C, Wen B, et al. Maternal plasma diacylglycerols and triacylglycerols in the prediction of gestational diabetes mellitus. BJOG. 2023;130(3):247–256. https://doi.org/10.1111/1471-0528.17297