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ORIGINAL RESEARCH ARTICLE

Metabolic profiling of pregnancies complicated by preeclampsia: A longitudinal study

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Abstract

Introduction: Preeclampsia is associated with maternal metabolic disturbances, but longitudinal studies with comprehensive metabolic profiling are lacking. We aimed to determine metabolic profiles across gestation in women who developed preeclampsia compared with women with healthy pregnancies. We also explored the respective effects of body mass index (BMI) and preeclampsia on various metabolic measures.

Material and methods: We measured 91 metabolites by high-throughput nuclear magnetic resonance spectroscopy at four time points (visits) during pregnancy (weeks 14–16, 22–24, 30–32 and 36–38). Samples were taken from a Norwegian pregnancy cohort. We fitted a linear regression model for each metabolic measure to compare women who developed preeclampsia ($n = 38$) and healthy controls ($n = 70$).

Results: Among women who developed preeclampsia, 92% gave birth after 34 weeks of gestation. Compared to women with healthy pregnancies, women who developed preeclampsia had higher levels of several lipid-related metabolites at visit 1, whereas fewer differences were observed at visit 2. At visit 3, the pattern from visit 1 reappeared. At visit 4 the differences were larger in most subgroups of very-low-density lipoprotein particles, the smallest high-density lipoprotein, total lipids and triglycerides. Total fatty acids were also increased, of which monounsaturated fatty acids and saturated fatty acids showed more pronounced differences. Concentration of glycine tended to be lower in pregnancies with preeclampsia until visit 3, although this was not significant after correction for multiple testing. After adjustment for age, BMI, parity and gestational weight gain, all significant differences were attenuated at visits 1 and 2. The estimates were less affected by adjustment at visits 3 and 4.

Conclusions: In early pregnancy, the metabolic differences between preeclamptic and healthy pregnancies were primarily driven by maternal BMI, probably representing the women's pre-pregnancy metabolic status. In early third trimester, several weeks before clinical manifestation, the differences were less influenced by BMI, indicating

Abbreviations: 95 CI, 95% confidence interval; GWG, gestational weight gain; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; NMR, nuclear magnetic resonance; SD, standard deviation; VLDL, very-low-density lipoprotein.

preeclampsia-specific changes. Near term, women with preeclampsia developed an atherogenic metabolic profile, including elevated total lipids, very-low-density lipoprotein, triglycerides, and total fatty acids.

KEYWORDS
high-risk pregnancy, hypertension in pregnancy, molecular biology, preeclampsia

1 | INTRODUCTION

Maternal metabolic changes throughout pregnancy are mostly normal physiological responses that support fetal development and growth. However, in some women, the changes may be related to adverse pregnancy complications such as preeclampsia and also have implications for future risk of cardiovascular disease.[1] Some authors therefore consider pregnancy a window to future health,[2] and gaining a more thorough understanding of metabolic changes throughout pregnancy may be of importance to fully understand the underlying metabolic pathways in the pathogenesis of preeclampsia.

Preeclampsia is a heterogeneous, pregnancy-specific syndrome that generally occurs after 20 weeks of gestation and most commonly near term.[3] An early-onset form is often defined as delivery prior to 34 weeks of gestation and is associated with fetal growth restriction. In late-onset preeclampsia, the neonates tend to have normal birthweight.[4]

There is evidence suggesting that early-onset preeclampsia develops due to suboptimal perfusion of the placenta resulting from abnormal remodeling of maternal spiral arteries.[5] In late-onset preeclampsia, placentation may be normal, but the condition is associated with placental dysfunction and endothelial activation. Diabetes mellitus, chronic hypertension, obesity and autoimmune disorders may be predisposing factors to preeclampsia by enhancing sensitivity of the maternal endothelial cells to inflammatory stimuli.[3,6,7]

Preeclampsia is associated with maternal dyslipidemia. Studies have reported changes in levels of fatty acids, cholesterol and triglycerides in early pregnancy in women who later develop preeclampsia,[8–10] suggesting that metabolic disturbances may precede disease onset. Elevated pre-pregnancy body mass index (BMI) is an independent risk factor for preeclampsia,[11] and dysregulated metabolism may mediate the association between overweight/obesity and risk of preeclampsia. It remains unclear to what extent metabolic differences between normal and preeclamptic pregnancies are a consequence of pre-pregnancy metabolic status or are directly related to underlying pathophysiological processes leading to preeclampsia.

Previous studies on metabolic changes in preeclamptic pregnancies are limited to only describing a few standard lipids. Longitudinal studies in unbiased cohorts of women with comprehensive metabolic profiling are lacking.

We aimed to compare metabolic profiles across gestation between healthy pregnancies and women who ultimately developed preeclampsia. In addition, we aimed to explore the respective effects of BMI and preeclampsia on various metabolic measures.

Key message

In late-onset preeclampsia, metabolic differences prior to pregnancy week 24 were attributed to maternal overweight/obesity, whereas from weeks 30–32 the metabolic alterations were more preeclampsia-specific. Near term, women with preeclampsia had elevated total lipids, VLDL, triglycerides, and total fatty acids.

2 | MATERIAL AND METHODS

2.1 | Design and study population

The STORK study was a prospective longitudinal cohort study in which 1031 healthy women of Scandinavian heritage were followed throughout their pregnancy and gave birth at Oslo University Hospital in 2002–2008.[12] The exclusion criteria included multiple pregnancies, known pre-gestational diabetes and any severe chronic diseases. Each pregnant woman had four study-related antenatal visits at weeks 14–16, 22–24, 30–32 and 36–38. A total of 38 women (3.7%) developed preeclampsia in this cohort. The current study includes a subset of the STORK sample, consisting of the 38 preeclamptic women and 70 randomly chosen women from the total cohort of healthy women, not imposing differences other than whether or not they developed preeclampsia.

2.2 | Data collection

Clinical data were collected at each antenatal visit and obtained from hospital records after birth. BMI was calculated based on height and weight measured at the first visit. Gestational weight gain (GWG) was calculated as weight at current visit minus weight at first visit. Venous blood was drawn into tubes with EDTA additives in the morning between 07:30 hours and 08:30 hours after an overnight fast, centrifuged for 25 min at 3000 ***g*** at 4°C, separated and stored at −80°C until analyzed.

2.3 | Definition of variables

Diagnosis of gestational diabetes mellitus was defined as fasting blood glucose ≥7.0 mmol/L or blood glucose ≥7.8 mmol/L, 2 hours after an oral glucose tolerance test measured at visit 1 (gestational weeks 14–16) and visit 3 (gestational weeks 30–32). These were the diagnostic criteria at the time of the study.[13]

The clinical diagnostic criteria for preeclampsia at the time of the study were defined as elevated blood pressure (≥140/90 mmHg) and proteinuria. This information was obtained from the medical charts.

2.4 | Metabolic profiling

The EDTA plasma samples were analyzed by high-throughput nuclear magnetic resonance (NMR) spectroscopy at the accredited laboratory Nightingale Health, Finland. From the full selection of metabolic measures, we analyzed 91 metabolic measures that were sufficient to form an adequate picture of the systemic metabolism. Composition within the various lipoprotein subclasses and relative lipoprotein lipid concentrations were not included. Our measures represent a snapshot of each woman's systemic metabolism and include lipid concentration and composition of 14 subclasses of lipoproteins as well as fatty acids, amino acids, glycolysis-related metabolites and ketone bodies. The 14 subclasses of lipoproteins consist of four major subclasses: very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), intermediate-density lipoprotein (IDL) and high-density lipoprotein (HDL). These are further divided into subgroups based on their size: XXL-VLDL, XL-VLDL, L-VLDL, M-VLDL, S-VLDL, and XS-VLDL; IDL; L-LDL, M-LDL and S-LDL; XL-HDL, L-HDL, M-HDL and S-HDL. NMR-based metabolomics has previously been applied in multiple large-scale epidemiologic and genetic studies, and the method is described elsewhere.[14]

2.5 | Statistical analysis

Sex- and gestational age-specific standardized birthweight (ie *z*-score) was calculated based on reference values from a Norwegian population-based study.[15]

Skewness of all metabolites (within group and visit) was computed by the *skewness* function in the *e1071* package in R,[16] and 30 measures with skewness >1.5 were $\log_e$-transformed. All measures were then scaled to number of standard deviations (SD).

We used a modified Bonferroni method to correct for multiple testing. Due to metabolic data being strongly correlated, we performed principal component analysis (PCA) across all four visits to determine the number of independent tests. Eight principal components explained more than 90% of the variation in the metabolic data. After correcting for multiple testing, the statistical significance level was set at 0.006 (0.05/8). This method is commonly used within metabolomics.[17,18]

Crude linear regression models were fitted for each metabolic measure at each antenatal visit, with preeclampsia (yes/no) as the explanatory variable. We then adjusted for maternal age (continuous), BMI (continuous), parity (dichotomous) and GWG (continuous). To enable comparison across the multiple metabolic measures in a single figure, all measures were scaled before analysis. Results are reported as difference in SD units between pregnancies complicated by preeclampsia and healthy pregnancies. All point estimates are given with associated 95% confidence intervals (95% CI). Absolute values and number of samples per visit are given in Table S1.

In addition, we performed an analysis of the correlation between the respective effects of preeclampsia and BMI on all metabolic measures. We plotted crude differences in SD units between preeclamptic and healthy pregnancies against crude differences in SD units between overweight/obese and underweight/normal-weight women. In this analysis, BMI was treated as a dichotomous variable with a cut-off value of 25 kg/m^2.

We performed a sensitivity analysis restricted to women attending visit 4 (26 women with preeclampsia) in order to determine whether this selection had an impact on the remaining model (Figure S1). We also compared the healthy women in our sample with the underlying STORK cohort to assure that the sample was representative.

All statistical analyses and visualizations were performed in R (version 4.1.1) using RSTUDIO (version 1.4.1717).

2.6 | Ethics statement

Written informed consent was obtained from all study participants. All clinical investigations were conducted in accordance with the principles in the Declaration of Helsinki. The study was approved by the Regional Committees for Medical Research Ethics South East Norway (reference number S-01191) on February 23, 2022.

3 | RESULTS

Characteristics of the 108 study participants are given in Table 1. Women with preeclampsia (n = 38) were younger, were more frequently nulliparous, had higher BMI and were more commonly diagnosed with gestational diabetes mellitus than women with healthy pregnancies (n = 70). Only three women gave birth before 34 weeks of gestation (all women with preeclampsia); hence, the majority of women had late-onset preeclampsia.

Associations between preeclampsia and the metabolic measures are displayed in Figures 1 and 2.

3.1 | Lipoprotein particle concentration and lipid-related measures

The longitudinal pattern revealed relatively large metabolic differences between the two groups at visit 1, which were reduced at visit

TABLE 1 Characteristics of the study participants, $n = 108$

Characteristics	All $n = 108$[a]	Healthy $n = 70$ (65%)[a]	Preeclampsia $n = 38$ (35%)[a]	P[b]
Age, years	31.4 (3.8)	32.2 (3.7)	30.0 (3.8)	**0.005**
Married/cohabiting	105 (97%)	70 (100%)	35 (92%)	**0.041**
Higher education	89 (82%)	58 (83%)	31 (82%)	0.5
Smoking				
Current smoker	1 (0.9%)	1 (1%)	0 (0%)	0.7
Quit during pregnancy	21 (19%)	15 (21%)	6 (16%)	
Nulliparous	61 (56%)	34 (49%)	27 (71%)	**0.024**
Gestational diabetes Missing, *n*	7 (6.7%)	1 (1.4%)	6 (17%) 3	**0.005**
BMI, kg/m^2 Missing, *n*	25.0 (3.9)	24.1 (2.9)	26.9 (4.9) 1	**0.002**
Overweight/obesity[d]	48 (45%)	24 (34%)	24 (65%)	**0.002**
Gestational weight gain visit 1–2, kg Missing, *n*	3.6 (1.7)	3.7 (1.5) 1	3.3 (2.0) 2	0.4
Gestational weight gain visit 1-3, kg Missing, *n*	7.9 (2.9)	7.7 (2.8)	8.2 (2.9) 4	0.2
Gestational weight gain visit 1–4, kg Missing, *n*	10.9 (4.0)	10.3 (3.9) 2	12.4 (3.8) 10	**0.013**
Male sex	59 (55%)	39 (56%)	20 (53%)	0.8
Gestational age at birth, weeks	39.7 (2.0)	40.3 (1.2)	38.5 (2.5)	**<0.001**
Preterm birth < 34, weeks	3 (2.8%)	0 (0%)	3 (7.9%)	**0.041**
Birthweight, g	3,487 (624)	3,551 (466)	3,369 (837)	0.3
Standardized birthweight[c]	−0.06 (1.02)	−0.15 (0.93)	0.11 (1.16)	0.3
Birthweight category				
Large for gestational age	13 (12%)	6 (9%)	7 (18%)	0.2
Small for gestational age	11 (10%)	7 (10%)	4 (11%)	
Placental weight, g Missing, *n*	696 (152)	699 (148) 4	689 (161) 1	0.8

Note: No missing data unless stated otherwise.

[a]Mean (standard deviation) for continuous variables; *n* (%) for categorical variables.

[b]Wilcoxon rank sum test; Fisher's exact test; Pearson's chi-square test.

[c]Standardized birthweight (*z*-score) was based on reference values to adjust for gestational age and sex.

[d]BMI ≥25 kg/m^2.

2 (Figure 1). At visit 3, the diverging pattern reappeared, reaching statistical significance for a wide range of metabolic measures at visit 4.

At visits 1–3, triglycerides were trending towards increased levels among women with preeclampsia overall, but the differences were not statistically significant after correction for multiple testing. At visit 4, triglycerides were all significantly increased in women with preeclampsia.

After adjusting for BMI, age, parity and GWG, none of the 14 subclasses of lipoproteins or lipid-related metabolites was significantly associated with preeclampsia ($P \geq 0.006$) at visits 1–3. There was, however, a trend towards higher levels of the largest VLDL subclasses (XXL, XL, and L), VLDL size, and triglycerides in VLDL at visit 3. At visit 4, VLDL particles, total lipids, VLDL cholesterol, VLDL lipids, VLDL free cholesterol, VLDL phospholipids and most triglycerides were significantly increased in preeclamptic pregnancies compared with healthy pregnancies.

3.2 | Fatty acids

Fatty acids tended to be higher in preeclamptic pregnancies than in healthy pregnancies at visit 1 (Figure 2) but the estimates were attenuated after adjustment for BMI, age, parity and GWG. At visit 4, there were significantly increased levels of total fatty acids, monounsaturated fatty acids and saturated fatty acids, also in the adjusted analyses.

3.3 | Other metabolites and inflammatory markers related to preeclampsia

In the adjusted analyses, citrate, glutamine and creatinine were significantly increased in pregnancies with preeclampsia compared

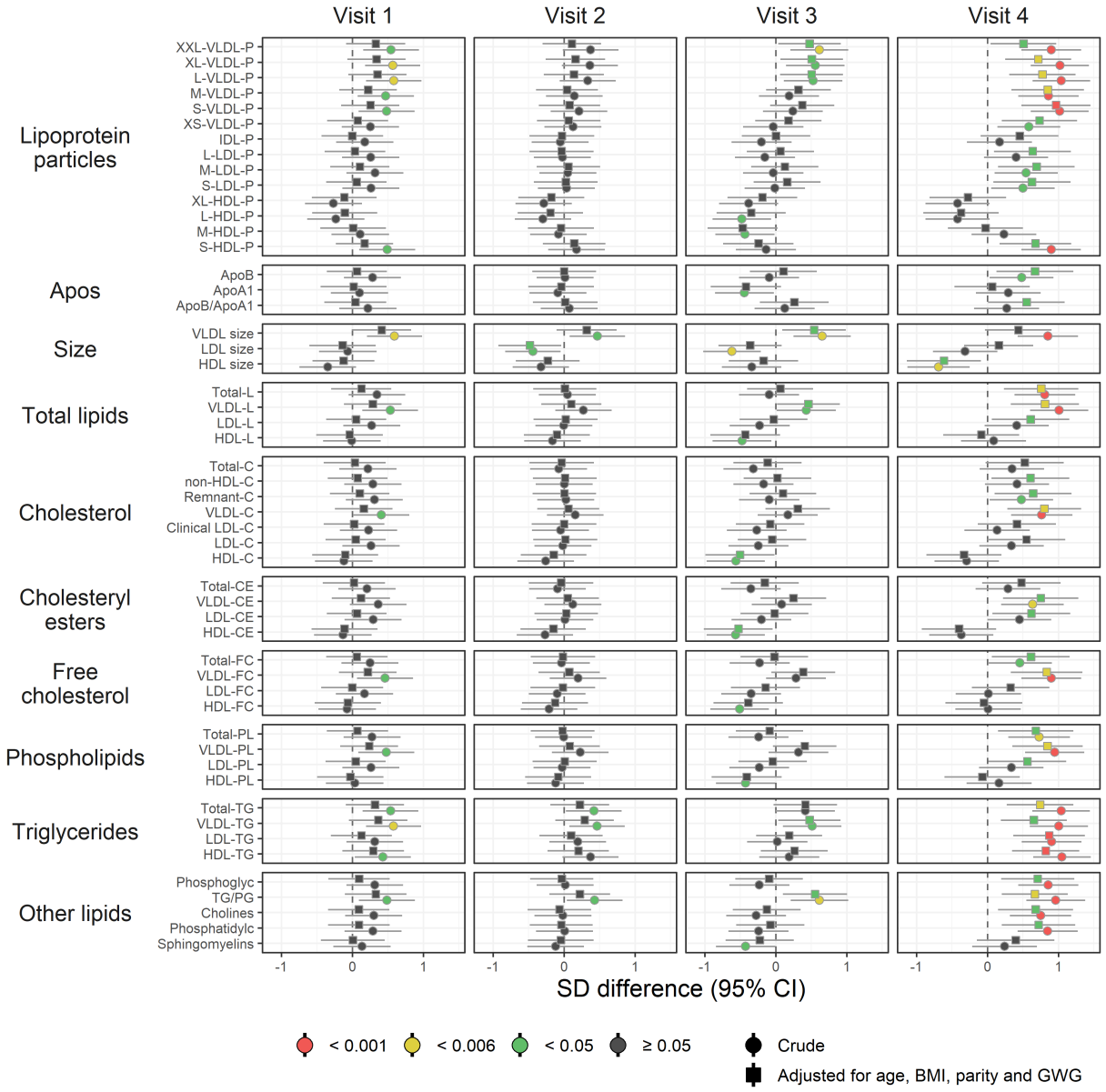

FIGURE 1 Forest plot illustrating longitudinal associations between preeclampsia and the metabolic measures (visits 1–4). Pregnancies complicated by preeclampsia ($n = 38$) were compared with healthy pregnancies ($n = 70$). Point estimates denote the scaled differences in standard deviation (SD) units with 95% CI. Circles indicate crude estimates, and squares indicate estimates adjusted for age, BMI, parity and GWG. Robustness of statistical significance is indicated by color: red indicates a *P*-value <0.001; yellow a *P*-value <0.006 (significance level after correction for multiple testing); green a *P*-value <0.05; dark gray a *P*-value ≥0.05. Abbreviations: HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation; VLDL, very-low-density lipoprotein.

with healthy pregnancies at visit 4 (Figure 2). Although glycine was not associated with preeclampsia after correction for multiple testing ($P = 0.026$ at visit 1, $P = 0.008$ at visit 2, and $P = 0.024$ at visit 3), it is worth noting that the estimates were almost unaffected by the adjustment and occurred repeatedly. This can also be observed in Figure 3, displaying the same associations with trend lines for some of the most pronounced metabolites from the full analysis (Figure 3).

Sensitivity analyses for all four visits restricted to women attending visit 4 (26 women with preeclampsia) revealed only minor changes in the effect estimates (Figure S1).

3.4 | Metabolic association between BMI and preeclampsia

Figure 4 illustrates the correlation between the crude effects of preeclampsia and overweight/obesity (BMI ≥25 kg/m^2) on the various metabolic measures (results of crude analyses for preeclampsia are shown in Figures 1 and 2).

Overall, there was a strong correlation (R^2 ranging between 0.85 and 0.69 at various visits) (Figure 4). In general, the effect of preeclampsia increased with time, and the magnitude of the SD difference was larger at visit 4 than at the previous visits. As seen

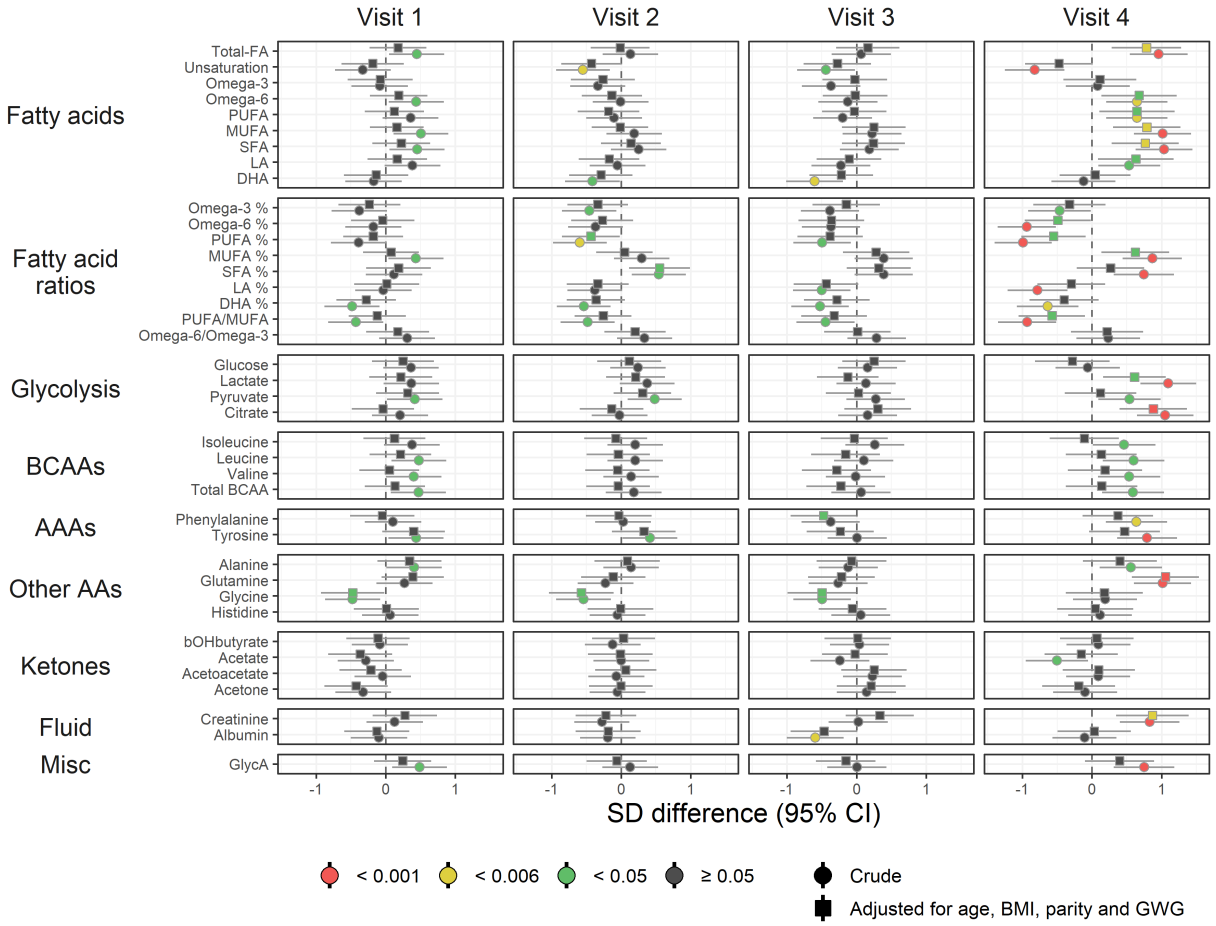

FIGURE 2 Forest plot illustrating longitudinal associations between preeclampsia and the metabolic measures (visits 1–4). Pregnancies complicated by preeclampsia ($n = 38$) were compared with healthy pregnancies ($n = 70$). Point estimates denote the scaled differences in standard deviation (SD) units with 95% CI. Circles indicate crude estimates, and squares indicate estimates adjusted for age, BMI, parity and GWG. Robustness of statistical significance is indicated by color: red indicates a *P*-value <0.001; yellow a *P*-value <0.006 (significance level after correction for multiple testing); green a *P*-value <0.05; dark gray a *P*-value ≥0.05. Abbreviations: AA, amino acids; AAA, aromatic amino acids; BCAA, branched-chain amino acids.

from the fitted linear regression lines, overweight/obesity seemed to have a larger effect on the metabolic measures compared with preeclampsia at visit 1 and partly at visit 2. At visit 3, the red and black lines were almost parallel, indicating that the effects of preeclampsia and overweight/obesity were more alike. At visit 4 the regression line indicated a stronger effect of preeclampsia. Glycine and tyrosine were more affected by preeclampsia than were overweight/obesity at visits 1 and 2, whereas HDL size was more affected by preeclampsia than by overweight/obesity at visit 4.

4 | DISCUSSION

In the present study, we compared systemic metabolites in women with preeclamptic pregnancies and healthy pregnancies across gestation. Most of the observed differences prior to pregnancy week 24 were strongly influenced by BMI and thereby pre-pregnancy status. In the third trimester of pregnancy, metabolites were less affected by adjustment for age, BMI, parity and GWG. We interpret this as preeclampsia-specific alterations. Near term, women with preeclampsia developed significantly higher levels of total lipids, VLDL particles, triglycerides and fatty acids compared with women with healthy pregnancies. Level of glycine was lower in weeks 14–16 among women who subsequently developed preeclampsia than in women with healthy pregnancies, and this difference held until weeks 30–32. Our findings are important to reach a broader understanding of the physiological changes in preeclamptic pregnancies across gestation.

To our knowledge, this is the first study to describe comprehensively the metabolic profiles in preeclamptic pregnancies across gestation. In a meta-analysis including 74 studies, Spracklen et al. found a statistically significant association between preeclampsia and total cholesterol, triglycerides, non-HDL cholesterol and HDL cholesterol.[19] We did not replicate the association with total cholesterol, and the associations with non-HDL cholesterol and HDL cholesterol were only trending towards statistical significance at visits 3 and 4, respectively. The results from Spracklen et al. and the present study are not directly comparable

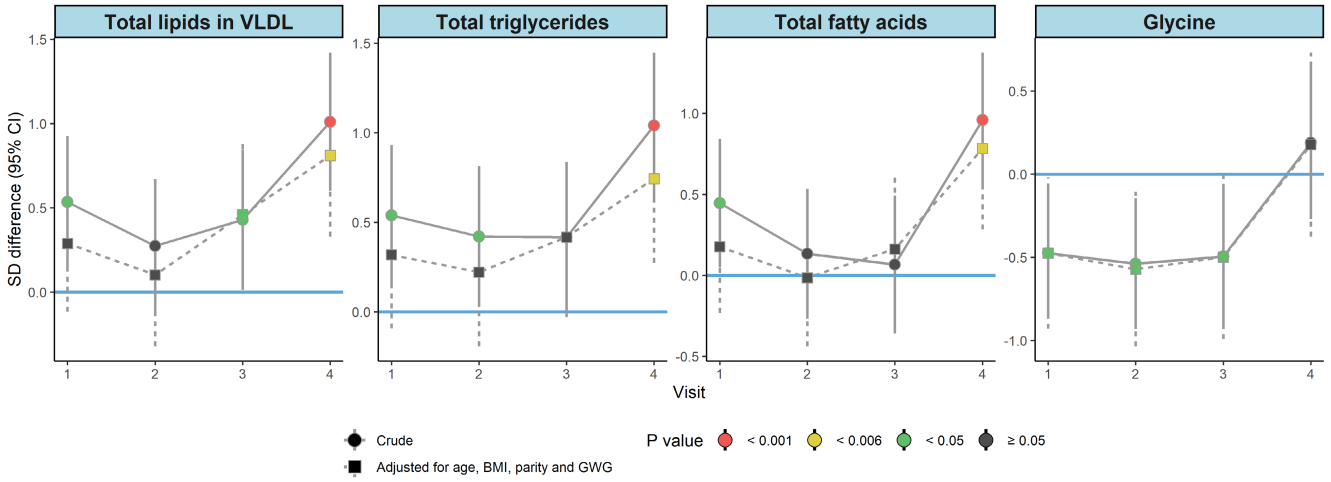

FIGURE 3 Trend plot illustrating longitudinal associations between preeclampsia and the four most pronounced metabolites from the full analysis shown in Figures 1 and 2. Pregnancies complicated with preeclampsia ($n = 38$) are compared to healthy pregnancies ($n = 70$). Healthy pregnancies are indicated by the blue line. Point estimate denote the scaled difference in standard deviation (SD) units with 95% confidence intervals (CI). Circles indicate crude estimates, and squares indicate estimates adjusted for age, BMI, parity and GWG. Robustness of statistical significance is indicated by color: red indicates a *P*-value <0.001; yellow a *P*-value <0.006 (significance level after correction for multiple testing); green a *P*-value <0.05; dark gray a *P*-value ≥0.05. Abbreviations: VLDL, very-low-density lipoprotein.

given that the meta-analysis included sampling from all trimesters, fasting status was not described for all included studies, and the authors were not able to adjust for BMI. The quantification method was also different. In a validation study of NMR-based metabolomics, McBride et al. found that VLDL (size ranging from S to XL) and triglycerides in VLDL were predictors of hypertensive disorders in pregnancy when combined with risk factors.[20] Their NMR analysis was conducted in pregnancy weeks 26–28, which is comparable to our visit 3 (weeks 30–32). We found the same associations as McBride et al., although they were not significant after correction for multiple testing. The mechanisms behind this finding cannot be elucidated based on our study. However, we speculate that elevated levels of VLDL and triglycerides may be related to oxidative stress through systemic endothelial dysfunction in preeclampsia.[21] The enzyme lipoprotein lipase is located in the endothelial cells and central in lipolytic removal of plasma triglycerides.[22] This implies that lipid changes associated with preeclampsia may be caused by endothelial dysfunction, in contrast to the pathogenesis of atherosclerosis, where the lipids are considered the origin of the endothelial dysfunction. In addition, preeclampsia is associated with systemic inflammation,[23] which may enhance adipocyte lipolysis and cause increased release of free fatty acids and triglycerides.

We also found a lower level of glycine. Although this was not statistically significant after correction for multiple testing, the finding occurred repeatedly and thereby indicates robustness. Austdal et al. have previously demonstrated a lower level of glycine in placental dysfunction,[24] although this was close to delivery. Glycine is a precursor to glutathione, which plays an important role in protection against oxidative stress.[25] Glycine is also related to insulin resistance, and is inversely associated with prediabetes and diabetes type 2.[26] As the lower level already occurred in weeks 14–16 in our data, and endogenous formation is exceedingly higher than dietary intake, we can only speculate that there is a genetic component behind the mechanism.

According to our findings, there are overlapping metabolic profiles between overweight/obesity and late-onset preeclampsia. These findings shed light on metabolic risk factors for hypertensive pregnancy complications. Overweight and obese women should possibly be offered tailored preconception counseling to identify metabolic abnormalities such as increased levels of glucose, insulin and lipids, and receive advice and support regarding lifestyle interventions. Women with metabolic abnormalities in early pregnancy may be at increased risk of preeclampsia. If we are able to predict preeclampsia earlier in pregnancy, we may be able to identify women that should be offered closer surveillance and possibly preventive treatment. The current recommendations for prevention of preeclampsia with aspirin in high-risk women were not implemented at the time of the study; hence, none of the participants received aspirin.

Further studies are warranted to explore the role of endothelial dysfunction in relation to preeclampsia-specific lipid changes. The identified metabolites should be evaluated in terms of their ability to predict late-onset preeclampsia in validation cohorts.

The main strengths of this study are the broad molecular profiling of preeclampsia and the prospective longitudinal design, allowing measurements at four time points during pregnancy. With the NMR method, we were provided with reliable measures of absolute concentrations that represent useful clinical units. The STORK cohort consisted of women of Scandinavian heritage who were healthy prior to pregnancy. This may limit generalization to other populations. The proportion of women with preeclampsia was 3.7%, which is in line with other population-based cohort studies.[27,28] We also performed a sensitivity analysis to ensure that

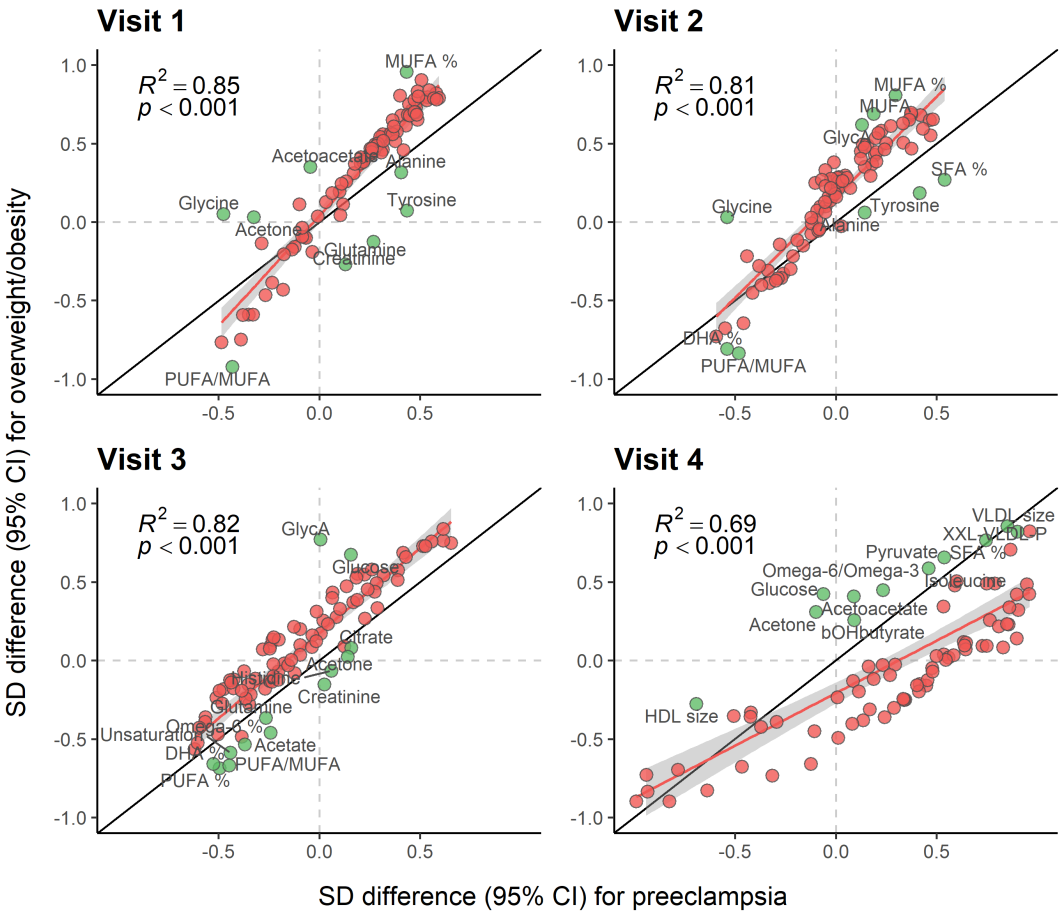

FIGURE 4 Scatter plot of the crude effects (differences in SD units) of preeclampsia (x-axis) and overweight/obesity (y-axis) on the metabolic measures. Each point represents a single metabolic measure. Metabolic measures ≤1.5 SD from the fitted regression line (red) are given in red, whereas those >1.5 SD from the regression line are given in green with annotation. A linear fit for the overall proportion of variance in overweight/obesity and preeclampsia is denoted by R^2 as a measure of goodness-of-fit. The black line is a reference line corresponding to a perfect 1:1 relation (intercept = 0, slope = 1); a slope of ±~1 and R^2~1 would indicate equal crude effects of preeclampsia and overweight/obesity on the metabolic measures. Abbreviations: DHA, docosahexaenoic acid; GlycA, glycoprotein acetyl; LA, linoleic acid; LDL, low-density lipoprotein; MUFA, monounsaturated fatty acids; PG, phosphoglycerides; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acid; TG, triglycerides; VLDL, very-low-density lipoprotein.

the sample did not differ from the underlying cohort in any clinical characteristics. Only three women gave birth before 34 weeks of gestation; hence, our findings are limited to late-onset preeclampsia. Sensitivity analysis excluding the three cases did not change the estimates (data not shown). As women with gestational diabetes mellitus who also developed preeclampsia could potentially have exacerbated the metabolic differences, and we therefore performed a sensitivity analysis excluding women with gestational diabetes mellitus (data not shown). There were only minor changes in estimates, although the smaller sample affected the statistical power of the analysis. BMI was based on height and weight measured at visit 1. We chose these measures, as self-reported weight in pregnancy is prone to underestimation, especially among obese women.[29] Both visceral and subcutaneous fat contribute to BMI and we did not have data to discriminate between these in our analyses. We emphasize that the metabolic differences observed from weeks 30–32, and exacerbated near term, were weeks prior to a clinical diagnosis of preeclampsia. Given that the diagnostic criteria at the time of the study included both hypertension and proteinuria, the number of patients with preeclampsia according to the current updated definition[30] might have been underestimated. However, none of the participants attending the antenatal visits were hospitalized. We acknowledge that women who developed preeclampsia and were still able to attend visit 4 comprise women with late-onset preeclampsia not requiring delivery. Hence, women with a more severe preeclamptic phenotype who had already given birth at visit 4, may have had even more pronounced metabolic disturbances. The sensitivity analysis restricted to the women attending visit 4 revealed only minor changes in the effect estimates.

To correct for multiple testing, we performed principal component analyses across all metabolites for each visit to determine the appropriate number of independent tests. The variation was not the same for all visits, and the highest number of principal components were chosen to set one level of significance across all four visits. The level of significance of 0.006 may be regarded as too strict in some tests, increasing the probability of a type 2 error.

5 | CONCLUSION

Women who developed preeclampsia had significantly elevated levels of atherogenic metabolic measures including total lipids, VLDL, triglycerides and total fatty acids compared to women with healthy pregnancies. This may potentially explain the increased risk of cardiovascular disease observed later in life in women who had a preeclamptic pregnancy. Most of the metabolic differences observed in early pregnancy among women who later developed preeclampsia were related to overweight and obesity, representing the women's pre-pregnancy metabolic status. From early third trimester, several weeks before clinical manifestation, the metabolic differences were gradually more related to the development of preeclampsia. This indicates that the atherogenic metabolic profile may be a part of the pathogenesis of preeclampsia.

AUTHOR CONTRIBUTIONS

HNS: conceptualization, methodology, software, formal analysis, writing-original draft, visualization. JJC: methodology, software, validation, formal analysis, visualization, writing- review & editing. NG: methodology, validation, formal analysis, writing- review & editing. KBH: conceptualization, methodology, writing- review & editing. TL: data curation, writing- review & editing. TH: funding acquisition, methodology, writing- review & editing. TMM: conceptualization, funding acquisition, methodology, project administration, supervision, writing- review & editing. MCPR: conceptualization, methodology, data curation, resources, supervision, funding acquisition, writing- review & editing. All authors read and approved the final version of the manuscript.

FUNDING INFORMATION

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CONFLICT OF INTEREST

None.

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SUPPORTING INFORMATION

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

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