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Metabolomic Profiles of Nonobese and Obese Women With Gestational Diabetes

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Abstract

Context: In non-pregnant population, nonobese individuals with obesity-related metabolome have increased risk for type 2 diabetes and cardiovascular diseases. The risk of these diseases is also increased after gestational diabetes.

Objective: This work aimed to examine whether nonobese (body mass index [BMI] < 30) and obese (BMI ≥ 30) women with gestational diabetes mellitus (GDM) and obese non-GDM women differ in metabolomic profiles from nonobese non-GDM controls.

Methods: Levels of 66 metabolic measures were assessed in early (median 13, IQR 12.4-13.7 gestation weeks), and across early, mid (20, 19.3-23.0), and late (28, 27.0-35.0) pregnancy blood samples in 755 pregnant women from the PREDO and RADIEL studies. The independent replication cohort comprised 490 pregnant women.

Results: Nonobese and obese GDM, and obese non-GDM women differed similarly from the controls across early, mid, and late pregnancy in 13 measures, including very low-density lipoprotein-related measures, and fatty acids. In 6 measures, including fatty acid (FA) ratios, glycolysis-related measures, valine, and 3-hydroxybutyrate, the differences between obese GDM women and controls were more pronounced than the differences between nonobese GDM or obese non-GDM women and controls. In 16 measures, including HDL-related measures, FA ratios, amino acids, and inflammation, differences between obese GDM or obese non-GDM women and controls were more pronounced than the differences between nonobese GDM women and controls. Most differences were evident in early pregnancy, and in the replication cohort were more often in the same direction than would be expected by chance alone.

Conclusion: Differences between nonobese and obese GDM, or obese non-GDM women and controls in metabolomic profiles may allow detection of high-risk women for timely targeted preventive interventions.

Key Words: gestational diabetes, metabolomics, obesity

Abbreviations: AA, amino acid; BMI, body mass index; FA, fatty acid; GDM, gestational diabetes mellitus; HDL, high-density lipoprotein; IQR, interquartile range; ITU, InTraUterine Sampling in Early Pregnancy Study; LA, linoleic acid; NOGDM, nonobese with gestational diabetes mellitus; O, obese without gestational diabetes mellitus; OGDM, obese with gestational diabetes mellitus; PREDO, Prediction and Prevention of Pre-eclampsia and Intrauterine Growth Restriction study; PUFA, polyunsaturated fatty acid; RADIEL, Finnish Gestational Diabetes Prevention study; VLDL, very low-density lipoprotein.

Hyperglycemia in pregnancy affects globally 17% of pregnancies, with 80% being due to gestational diabetes mellitus (GDM) (1). GDM may lead to increased risk for several adverse perinatal outcomes, including cesarean delivery, macrosomia, shoulder dystocia, and neonatal hypoglycemia (2). GDM also poses long-term consequences both for mother and offspring. Not only are women with GDM at higher risk for developing subsequent type 2 diabetes, metabolic

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syndrome, and cardiovascular disorders (3), but also their offspring have an increased risk for obesity, type 2 diabetes, and neurodevelopmental and behavioral disorders (4).

Like type 2 diabetes (5, 6), GDM is a heterogeneous condition (7-10). Although body mass index (BMI) is a major risk factor for GDM, 20% to 66% of women with GDM are nonobese (BMI < 30) (11, 12). Nonobesity- and obesity-related GDM likely reflect differences in underlying pathophysiology, with insulin-secretion deficit characterizing more often those with nonobese GDM (NOGDM) (13) and insulin resistance obese (BMI ≥ 30) GDM (OGDM) (9). Even though obesity during pregnancy is associated with perturbations in the metabolome (14, 15), what remains unknown is whether women with NOGDM and OGDM differ in their metabolomic characteristics. Two recent studies in general populations have demonstrated that nonobese adults have a higher risk for type 2 diabetes and cardiovascular disease if their metabolomic profiles are unhealthy, and hence characteristic of obesity (16, 17). This suggests that the metabolomic profiles of women with NOGDM may instead resemble the profiles of women with OGDM, and obese without GDM (O), than differ from them.

Against this background we examined whether women with NOGDM, OGDM or O differed from the nonobese non-GDM controls in their metabolomic profiles in early, and across early, mid, and late pregnancy.

Materials and Methods

Participants

The study population consisted of 2 Finnish studies: the Prediction and Prevention of Pre-eclampsia and Intrauterine Growth Restriction (PREDO; ISRCTN.com registration no. ISRCTN14030412) study (18) and the Finnish Gestational Diabetes Prevention (RADIEL; ClinicalTrials.gov registration no. NCT01698385) study (19). A flowchart is presented in Supplementary Fig. S1 (20).

The PREDO study recruited 1079 pregnant women with known risk factors for preeclampsia and intrauterine growth restriction between 12 and 14 weeks of gestation from 10 hospitals. A subgroup with a bilateral second-degree diastolic notch in the uterine blood flow were randomly assigned to receive low-dose aspirin (n = 61) or placebo (n = 60) to prevent preeclampsia. Blood samples were obtained at a median 13.0 (interquartile range [IQR] 12.6-13.4), 19.3 (19.0-19.7), and 27.0 (26.6-27.6) weeks of gestation from 425 women. In the PREDO cohort, those women who provided blood samples were younger (aged 32.5 vs 33.6 years; $P = .007$) and less likely to be obese (29.1% vs 39.3%; $P = .003$) than women who did not.

The RADIEL study recruited 720 women at high risk for GDM (BMI ≥ 30 or prior GDM or both) into a randomized clinical trial, for prevention of GDM by lifestyle intervention. These women were either planning a pregnancy or were at less than 20 weeks of gestation at enrollment. Blood samples were obtained from 339 women at a median 13.0 (IQR 11.9-14.3), 23.1 (22.6-24.1), and 35.1 (34.4-35.7) weeks of gestation. Of them, 177 were randomly assigned to the intervention group receiving advice on diet and physical activity, and 160 were in the control group (standard care). In the RADIEL cohort, those women who provided blood samples were less likely to be obese (14.0% vs 20.5%; $P = .04$) or to have GDM (27.9% vs 73.2%; $P < .0001$) or preeclampsia (3.3% vs 7.0%; $P = .04$) than women who did not.

In the combined PREDO-RADIEL cohort, no women eligible for data analyses had type 1 diabetes. The timing of blood samples in the combined cohort was at a median 13.0 (IQR 12.4-13.7), 20.0 (19.3-23.0), and 28.0 (27.0-35.0) weeks of gestation. Of the combined cohort of 755 women, 535 (70.9%) provided all 3 blood samples, 171 (22.7%) 2 samples, and 49 (6.5%) 1 sample, resulting in a sample size of 639, 679, and 678 women at the first, second and third time point (Supplementary Table S1) (20).

The replication cohort comprised a subsample of 490 pregnant women of the 943 pregnant women of the InTraUterine Sampling in Early Pregnancy Study (ITU) (21). These women provided one blood sample at a median 20.6 (IQR 20.1-23.4) gestational weeks. Women who provided the blood sample were less likely to be obese (3.4% vs 6.2%; $P = .006$) and more likely to have a tertiary education (83.7% vs 70.4%; $P < .0001$) than the women who did not.

All study participants signed an informed consent, and the study protocols were approved by the ethics committee of the Helsinki and Uusimaa Hospital District.

Methods

Metabolomic profiling

Venous blood was collected in all 3 cohorts between 7 and 10 AM after at least a 10-hour overnight fast. Plasma (in PREDO and ITU) and serum (in RADIEL) were separated immediately and stored at −80 °C until analysis. A high-throughput proton nuclear magnetic resonance metabolomics platform quantified 225 metabolic measures using the Nightingale Health Quantification Library 2020 (Nightingale Health Ltd). The analysis panel includes biomarkers of lipid and glucose metabolism, amino acids (AAs), fatty acids (FAs), ketone bodies, and a marker of low-grade inflammation. This method has been widely used in studies of pregnant and nonpregnant populations (22-25). Of all the metabolic measures, 37 have been validated against the standard clinical chemistry methods. Details of the experimentation are described elsewhere (26). Following the lead of earlier studies using the same metabolomics platform, 66 of these metabolic measures were considered appropriate to form an adequate picture of the systemic metabolism, and served as the primary outcomes (22, 23). Those measures not included in the analysis were composition within the various lipoprotein subclasses and relative lipoprotein lipid concentrations.

Gestational Diabetes Mellitus and Prepregnancy Nonobesity and Obesity

In all cohorts, the diagnosis of GDM came from medical records and in RADIEL and in PREDO was verified additionally by a jury composed of a research nurse and 2 or more medical doctors. Exceeding or equaling one or more of the plasma glucose thresholds (5.3, 10.0, and 8.6 mmol/L) in a 2-hour 75-g oral glucose tolerance test led to a diagnosis of GDM (27).

The Finnish Medical Birth Register, collecting data on prepregnancy weight and height, verified at the first visit to the antenatal clinics, provided information for calculating BMI. Among the participants recruited before pregnancy in the RADIEL study, we used prepregnancy weight and height measured at the last study visit before pregnancy. Obesity

was defined according to World Health Organization guidelines (28) as prepregnancy BMI of 30 or greater. Nonobesity was defined as prepregnancy BMI less than 30.

Covariates

We adjusted for cohort (PREDO, RADIEL) and weeks of gestation at time of blood sampling (all cohorts). The other covariates were chosen based on the literature, and included maternal age (years) (22), parity (primiparous vs multiparous) (22, 23), and smoking (no vs yes) (23), which were drawn from the Medical Birth Register; education (basic/secondary vs tertiary) (22) and alcohol use (no vs yes) (23) were reported in early pregnancy (all cohorts).

Statistical Analysis

To study whether women with NOGDM, OGDM, or O differed from the controls in the metabolic measures during pregnancy, we applied an individual-participant data meta-analytic approach by using mixed-model regression analyses (PREDO, RADIEL). The repeated metabolic measures represented the within-person outcome variables, and gestational week at blood sampling the time-varying within-person predictor variables in these analyses. The groups of women—NOGDM, OGDM, O, and the controls (referent)—and the other covariates were included in these models as between-person fixed effects. We included interaction effects of NOGDM, OGDM, and O (with controls as the referent) × gestational week at blood sampling into the models to study whether women with NOGDM, OGDM, or O differed from controls in the change in the metabolic measures during pregnancy.

We defined unstructured covariance and first-order autoregressive error covariance matrices and allowed random effects to account for individual differences in the intercept. As the mixed models allow missing data, we did not impute missing values on metabolic measures (missingness per metabolic measure is shown in Supplementary Table S1) (20). However, if the measure was below detection level, we used a value equivalent to 0.9 multiplied by the nonzero minimum value of that measurement. For the between-groups fixed factors, missingness was minimal, and we conducted complete case analyses, except for smoking and alcohol consumption during pregnancy for which missing values were coded in a separate category.

We log-transformed the metabolic measures to normalize their distributions and analyzed the values in cohort-specific standardized units. As the metabolic measures are highly correlated, the Bonferroni-correction for multiple testing may be overly conservative and raise the risk of type II error (29). To reduce this risk, principal components analysis has been applied as a multiple testing correction method for correlated data to identify the effective number of independent tests (30, 31). We identified 25 principal components, which explained more than 99% of the variation in the 66 metabolic measures that we used as the primary outcomes. Therefore, 2-sided P less than .002 (.05/25) was used to infer statistical significance.

In the analysis of the replication cohort, we used linear regression analysis in which the metabolic measures served as dependent variables, and women with NOGDM, OGDM, or O were compared to controls in models adjusted for the covariates. We used the Fisher's exact test to study whether the group differences found in the combined PREDO-RADIEL cohort were more often in the same direction in the ITU replication cohort than what would be expected by chance alone.

As effect size indicators we report estimates and their 99.8% CIs (mixed models in PREDO-RADIEL) and unstandardized β coefficients and their 95% CIs (linear regression models in ITU). The estimates and unstandardized regression coefficients represent mean differences (grand mean of the early, mid, and late pregnancy values; and mean differences in the change [estimate representing slope]) in the metabolic measures in SD units of women with NOGDM, OGDM, or O, with controls as the referent in all analyses. To ensure that missing data would not influence the findings on change, we report the results of change also among women who provided metabolomic data at all 3 time points during pregnancy.

Statistical analyses were performed with SAS 9.4 (SAS Institute Inc).

Results

Background Characteristics

Table 1 presents the characteristics of the control group (n = 312) and of the 3 study groups: women with NOGDM (n = 96), with OGDM (n = 89), and with O (n = 258). The results for all 66 metabolic measures across pregnancy and in early pregnancy are presented in Supplementary Fig. S2 and S3 (20).

Metabolic Measures in Which Differences of Nonobese With Gestational Diabetes Mellitus, Obese With Gestational Diabetes Mellitus, and Obese Without Gestational Diabetes Mellitus From Controls are Similar

Among these 66 metabolic measures, there were 13 in which all study groups—women with NOGDM, OGDM, or O—differed significantly from the controls and in which, in effect size, the mean differences of these groups from controls were similar (Fig. 1; numeric values in Supplementary Table S2, Panel A (20)). These 13 metabolic measures included very small to large, and mean diameter of very low-density lipoprotein (VLDL), total cholesterol in VLDL, triglycerides in VLDL, LDL, and high-density lipoprotein (HDL), ratio of apolipoprotein B to apolipoprotein A-I, and monounsaturated and saturated FAs. The differences in all these measures were statistically significant also at the early pregnancy measurement point (see Fig. 1B). In small and medium LDL, remnant cholesterol, and apolipoprotein B, women with NOGDM, OGDM, or O also differed from controls in a similar manner, but all the differences were statistically significant only at the early pregnancy measurement point (see Fig 1B). In the analysis of the change, during pregnancy there were no metabolic measures in which the 3 study groups differed from the controls significantly, and in which, in effect size, the differences in change were similar (Supplementary Fig. S4 (20)).

Metabolic Measures in Which Differences of Obese With Gestational Diabetes Mellitus From Controls Were More Pronounced Than Were the Differences of Nonobese With Gestational Diabetes Mellitus and Obese Without Gestational Diabetes Mellitus From Controls

Among these 66 metabolic measures, there were 6 in which women with OGDM differed significantly from the controls

Table 1. Characteristics of the 755 women with either nonobesity and no gestational diabetes mellitus, nonobesity and gestational diabetes mellitus, obesity and gestational diabetes mellitus, or obesity and no gestational diabetes mellitus

Variable	Nonobese non-GDM (controls) (n = 312)	Nonobese GDM (NOGDM) (n = 96)	P^b	Obese GDM (OGDM) (n = 89)	P^b	Obese non-GDM (O) (n = 258)	P^b
Cohort, n (%)			<.0001		<.0001		<.0001
PREDO	248 (79%)	44 (46%)		45 (51%)		79 (31%)	
RADIEL	64 (21%)	52 (54%)		44 (49%)		179 (69%)	
Maternal age (mean, SD), y	32.6 (5.3)	33.9 (4.4)	.02	34.1 (4.6)	.009	32.5 (4.8)	.99
BMI (mean, SD)	23.4 (3.1)	25.0 (2.7)	<.0001	35.8 (4.3)	<.0001	34.3 (3.8)	<.0001
Parity, n (%)			.002		.67		<.0001
Primiparous	83 (27%)	11 (11%)		26 (29%)		126 (49%)	
Multiparous	228 (73%)	86 (89%)		63 (71%)		132 (51%)	
Education level, n (%)			.34		.001		<.0001
Secondary or lower	143 (46%)	51 (53%)		59 (66%)		178 (69%)	
Tertiary	161 (52.0%)	46 (47%)		28 (31%)		79 (31%)	
Data not available[a]	7 (2.3%)	0		2 (2.2%)		1 (0.4%)	
Smoking or alcohol use during pregnancy, n (%)			.60		.83		.38
No	236 (84.0%)	80 (87.0%)		65 (83.3%)		218 (87.2%)	
Smoked/used alcohol at any time during pregnancy	45 (16.0%)	12 (13.0%)		13 (16.7%)		32 (12.8%)	
Data not available	30 (9.7%)	5 (5.2%)		11 (12.4%)		8 (3.1%)	

Abbreviations: BMI, body mass index; GDM, gestational diabetes mellitus; PREDO, Prediction and Prevention of Pre-eclampsia and Intrauterine Growth Restriction; RADIEL, Finnish Gestational Diabetes Prevention.
[a]Unless indicated otherwise, no missing data.
[b]Calculated for the difference between study group and controls.

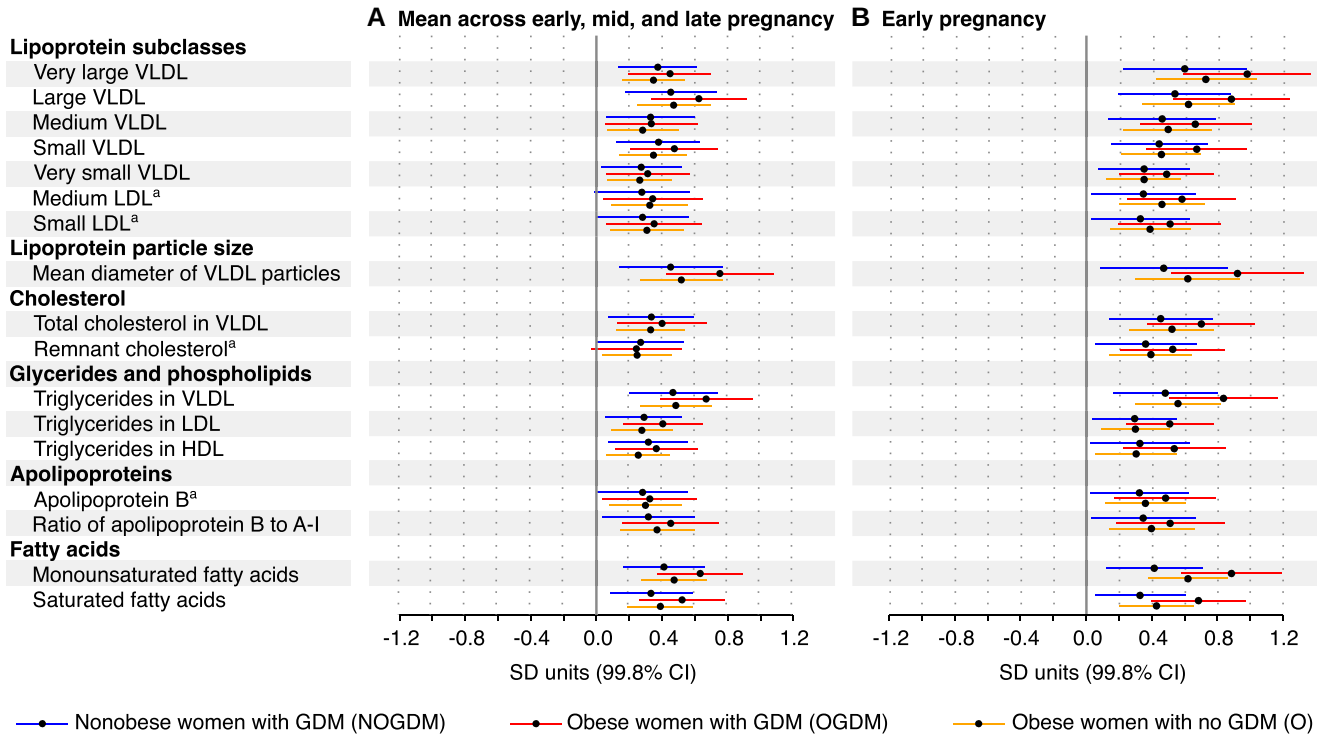

Figure 1. Mean differences and 99.8% CIs in those metabolic measures in which nonobese women with GDM (NOGDM), obese women with GDM (OGDM), and obese women with no GDM (O) differed in a similar manner from nonobese controls with no GDM across A, early, mid-, and late pregnancy measurement points and B, in early pregnancy. Associations adjusted for cohort, gestational week at blood sampling, maternal age, parity, education, and smoking and alcohol use during pregnancy. [a]Statistically significant only in early pregnancy.

and in which, in effect size, the differences from controls were more pronounced in women with OGDM than in women with NOGDM or O (Fig. 2, numeric values in Supplementary Table S2, Panel B (20)). These 6 measures included linoleic acid (LA) to total FAs, and polyunsaturated fatty acids (PUFA) to total FA ratios, glucose, citrate, valine, and 3-hydroxybutyrate. At the early pregnancy measurement point, differences between women with OGDM and controls were more pronounced in both of the FA ratios, and glucose, and additionally, in alanine (Fig. 2B).

In the analysis of change during pregnancy, there were 7 metabolic measures in which women with OGDM differed significantly from controls, and in which, in effect size, the differences in change from controls were more pronounced in women with OGDM than in women with NOGDM or O (Supplementary Fig. S4 and S5(20)). These measures included intermediate-density lipoprotein, small HDL, several cholesterol-related measures, total cholines, and sphingomyelin (all increased less during pregnancy, except for sphingomyelin, which decreased more in women with OGDM).

Metabolic Measures in Which Differences of Obese With Gestational Diabetes Mellitus and Obese Without Gestational Diabetes Mellitus From Controls Were More Pronounced Than Differences of Nonobese With Gestational Diabetes Mellitus From Controls

Among these 66 metabolic measures, there were 16 in which obese groups—women with OGDM, or O—differed significantly from the controls and in which, in effect size, the mean differences of these obese groups from controls were similar (see Fig. 3; numeric values in Supplementary Table S2C (20)). In these metabolic measures, women with NOGDM differed less (on 5 of these measures) or did not differ significantly (on 11 of these measures) from controls (see Fig. 3). These measures included several HDL-related measures, different FA ratios, some AAs, and glycoprotein acetylation. Differences in 13 of these measures, and additionally in small HDL particles and in the docosahexaenoic to total FA ratio, were statistically significant also at the early pregnancy measurement point (see Fig 3B).

In the analysis of change during pregnancy, there were 24 metabolic measures in which women with OGDM or O differed significantly from controls, and in which, in effect size, the differences in change from controls were similar in women with OGDM or O, whereas women with NOGDM differed less in change from controls or did not differ significantly (Supplementary Fig. S4 and S5 (20)). These measures included most sizes of VLDL and LDL, total cholesterol in VLDL, remnant cholesterol, triglycerides, apolipoprotein B, many FAs, some AAs, and glycoprotein acetylation (all increased less during pregnancy, except for tyrosine, which decreased more in women with OGDM or O).

Replication of the Results in the InTraUterine Sampling in Early Pregnancy Study Cohort

In ITU, findings concerning the differences of NOGDM, OGDM, and O from the controls were tested for replication. Even though not all the differences replicated or reached statistical significance in ITU, they were in the same direction more often than would be expected by chance alone ($P < .001$ from Fisher's exact test) (Supplementary Table S2 (20)). Of the metabolic measures in which mean differences (across early, mid, and late pregnancy or in early pregnancy) of NOGDM, OGDM, and O from controls were similar, 80% were in the same direction in ITU; in which mean differences of OGDM from controls were more pronounced than differences of NOGDM or O from controls, 71% were in the same direction in ITU; and in which mean differences of OGDM and O from controls were more pronounced than differences of NOGDM from controls, 76% were in the same direction in ITU (see Supplementary Table S2 (20)).

Discussion

The main findings of our study are 3-fold. First, our findings demonstrated that—when compared to controls (nonobese non-GDM)—women with NOGDM, OGDM, or O differed significantly in 13 metabolomic measures. These differences were similar in effect size, suggesting that women with NOGDM, OGDM, or O display similar metabolomic profiles. Second, in 6 of the metabolomic measures, differences from the controls of women with OGDM were more pronounced in effect size than were the differences from the controls of women with NOGDM or O. These differences suggest that across these metabolic measures, such differences reflect the nonobesity- and obesity-related pathophysiology of GDM. Finally, we identified 16 additional metabolomic measures in which the differences of women with OGDM or O from the controls were more pronounced in effect size than were the differences of women with NOGDM from controls, the latter differences being mostly not statistically significant. These differences suggest that across these 16 metabolomic measures, such differences reflect the pathophysiology of nonobesity and obesity, rather than nonobesity- and obesity-related pathophysiology of GDM. Our findings are supported by the replication in an independent sample of pregnant women, in which the results were in the same direction in 77%, more often than would be expected by chance alone.

Our first finding, similar adverse metabolomic profiles with perturbations in VLDL-related measures, triglycerides in VLDL, LDL, and HDL, ratio of apolipoprotein B to A-I, and some FAs among women with NOGDM, OGDM, or O is in parallel with others' study findings demonstrating similar perturbations in obesity and GDM (15, 22). The metabolic perturbations in women with NOGDM highlights the metabolic burden of GDM even in the absence of obesity. In our earlier study (7), lean women with GDM carried an increased risk for subsequent diabetes and had a surprisingly high body fat percentage 5 years post partum despite their seemingly nonobese BMI. Another longitudinal Finnish birth cohort study (32) demonstrated risk for type 2 diabetes to be markedly increased also among normal-weight women with GDM; the hazard ratio for diabetes 20 years after pregnancy was more than 10-fold. Among women with prepregnancy overweight, or with concomitant overweight and GDM, the corresponding figures exceeded 12 and 47. That many of the metabolic aberrations we detected were already evident in early pregnancy may reflect that the underlying disease process was already present weeks or months before GDM diagnosis, and may offer identifiable biomarkers for detecting women at high risk. This would be crucial especially for nonobese women, as they frequently remain undetected, and their identification is of great clinical relevance and offers substantial potential for prevention.

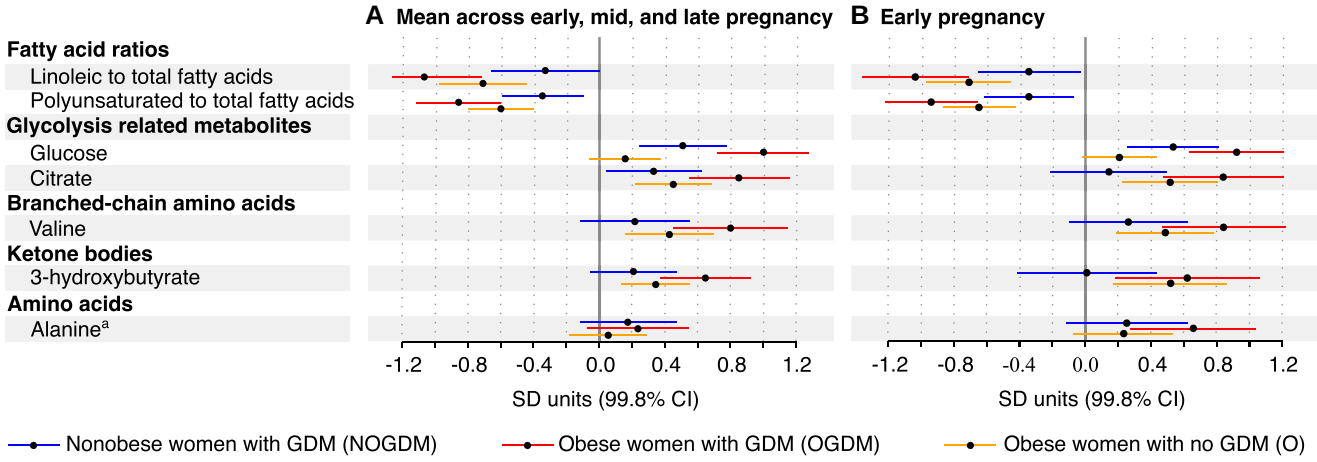

Figure 2. Mean differences and 99.8% CIs in those metabolic measures in which differences of obese women with GDM (OGDM) from controls (nonobese with no GDM) are more pronounced than are the differences of nonobese women with GDM (NOGDM), and of obese women with no GDM (O) from controls across A, early, mid-, and late pregnancy measurement points and B, in early pregnancy. Associations adjusted for cohort, gestational week at blood sampling, maternal age, parity, education, and smoking and alcohol use during pregnancy. [a]Statistically significant only in early pregnancy.

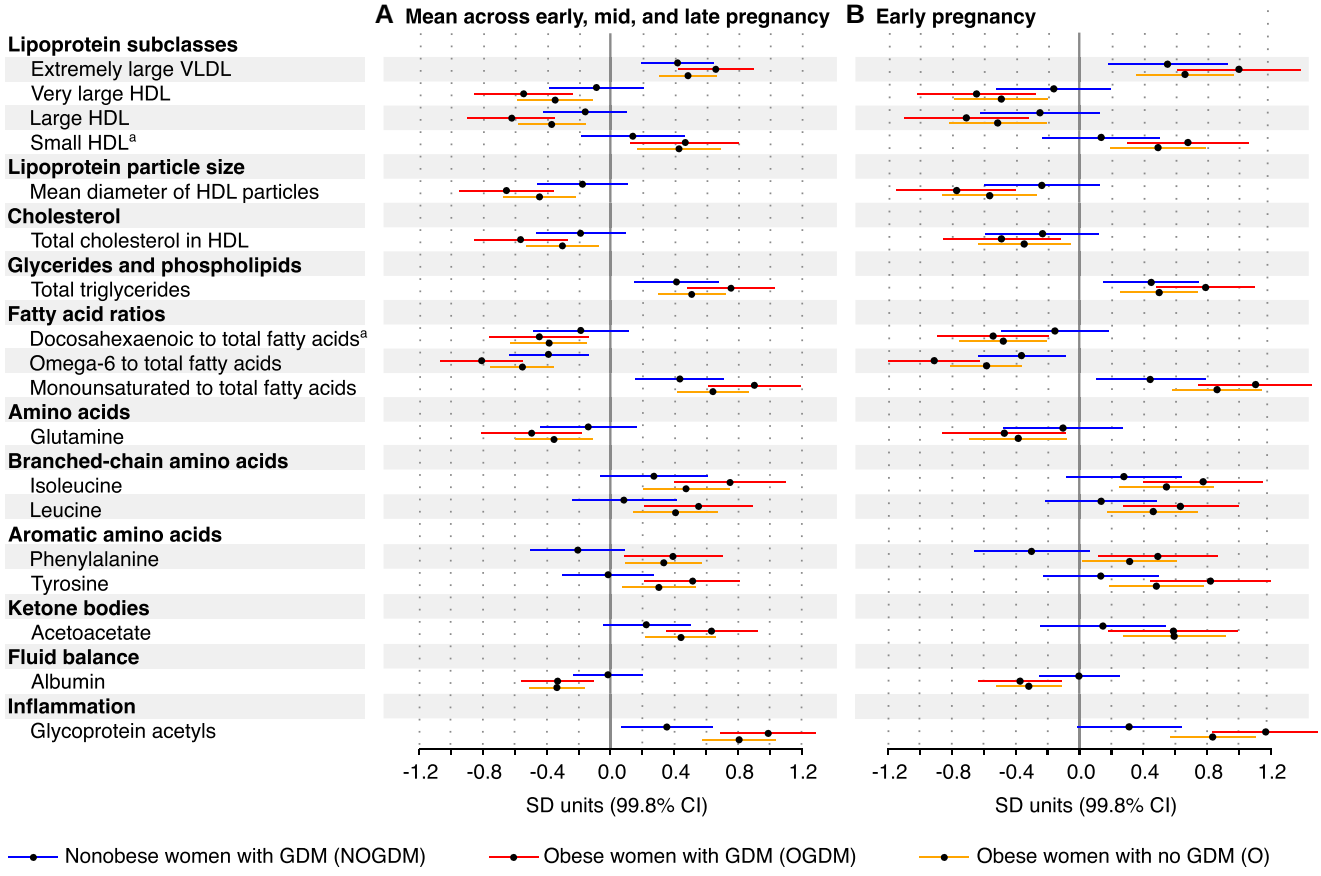

Figure 3. Mean differences and 99.8% CIs in those metabolic measures in which differences of obese women with GDM (OGDM), and of obese women with no GDM (O) from controls (nonobese with no GDM) are more pronounced than are the differences of nonobese women with GDM (NOGDM) from controls across A, early, mid-, and late pregnancy measurement points and B, in early pregnancy. Associations adjusted for cohort, gestational week at blood sampling, maternal age, parity, education, and smoking and alcohol use during pregnancy. [a]Statistically significant only in early pregnancy.

Second, we identified 6 metabolic measures distinguishing GDM subtypes: NOGDM and OGDM, across pregnancy, with differences not reflecting nonobesity and obesity. The first ones were LA to total FAs, and PUFA to total FAs, and previous studies, without separately studying the GDM subtypes, have reported lower ratios (22) among women with

GDM. Our finding of lower ratios of these FAs among women with OGDM, presumably insulin resistant, than among women with NOGDM is consistent with findings of an inverse association between circulating PUFAs, LA, and insulin resistance (33). The level of valine, a branched-chain amino acid with a well-established association with insulin resistance (34), was lower in women with NOGDM than in women with OGDM (9). Two of the measures distinguishing women with NOGDM and OGDM were glycolysis related, that is, glucose and citrate, and higher levels may reflect a poorer balance of glycemic control among women with obesity. The sixth metabolic measure distinguishing NOGDM from OGDM was a ketone body, 3-hydroxybutyrate, suggested in one study as a prognostic metabolic biomarker for GDM (35). In the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study (36), elevated levels of 3-hydroxybutyrate were observable in women with GDM at 28 weeks of gestation after adjustment for BMI. In our study, only women with OGDM, not NOGDM, had significantly higher 3-hydroxybutyrate levels, both in early and across pregnancy, than did controls. Separate analyses of nonobese and obese women in earlier studies could have possibly revealed new information about the usefulness of 3-hydroxybutyrate as a biomarker for GDM across the full spectrum of BMI.

The obesity-related perturbations we detected among women with O or OGDM, but not NOGDM, included lower levels of many HDL-related measures, higher levels of total triglycerides, relatively higher levels of saturation of FAs, higher levels of many insulin resistance–associated (34, 37) measures like branched-chain and aromatic amino acids, and higher level of inflammation. For women with NOGDM, preventing excessive weight gain in pregnancy and maintaining normal BMI afterward may be important for sustaining levels comparable to controls in these obesity-related measures, and thus avoiding morbidity associated with many of these metabolic perturbations. Moreover, these perturbations may be important in explaining differences in risk for macrosomia or cesarean delivery between GDM subtypes (9). Low HDL cholesterol has already been associated with accelerated epigenetic aging of the placenta (38) and higher offspring birth weight (39, 40). The obesity-related metabolic perturbations in our study may be one mechanism connecting the intrauterine environment and offspring outcomes and warrant further studies.

We have earlier reported smaller change across pregnancy in many of the metabolic measures among obese compared to normal-weight, and among GDM compared to non-GDM women (15). The findings in this study indicate that the difference in change from controls is evident only among women with OGDM, not with NOGDM, and thus, may be associated with the differences in the pathophysiology of GDM among obese and nonobese women.

Among the strengths of our study is its longitudinal design, allowing us to identify both the mean levels of metabolic measures across pregnancy as well as early pregnancy levels. The metabolic panel was targeted and has been widely used in previous studies. Many of the metabolic measures have been validated against conventional laboratory techniques. Our sample included a large number of nonobese and obese women, with and without GDM, thus providing ample statistical power. In addition, we performed a replication study in another independent cohort, providing robust evidence of perturbations in the lipid profiles of the study groups. The relatively small sample size in the replication cohort may explain our inability to replicate all results. One limitation of our study is the possible effect of the original study interventions. We have, however, already shown (15) that interventions were not associated with differences in metabolomic profiles. Plausible bias due to different samples, plasma and serum, in 2 cohorts, seems minimal (41) and our statistical methods, with SD scaling and adjustment for cohort, were designed to address this issue. An additional limitation is that our study included women from an ethnically homogeneous, high-resource Nordic setting, which limits the generalizability of our results.

In conclusion, our study highlights the fact that women with NOGDM, OGDM, or O differ from controls by displaying similar metabolomic profiles, by displaying differences that reflect nonobesity- and obesity-related pathophysiology of GDM, and by displaying differences that are obesity driven. These findings may allow identification of women at risk for GDM, and at long-term risk for GDM- and obesity-related health adversities and allow tailoring of timely targeted preventive interventions.

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Author Contributions

K.R., S.B.K., P.V.G., H.S.H., and E.H. designed the research. K.R., S.B.K., P.V.G., H.S.H., and E.H. conducted the study. P.V.G. and H.S.H. performed the statistical analyses. H.S.H., E.H., K.R., and S.B.K. drafted the manuscript and had primary responsibility for the final content. All authors contributed to the interpretation of the findings and critically reviewed, commented on, read, and approved the final manuscript. K.R., S.B.K., and P.V.G. are the guarantors of this work.

Disclosures

The authors have nothing to disclose.

Data Availability

Data sets generated during the presents study are not publicly available but will be made available on reasonable request. Requests are subject to further review by the national register authority and by the ethical committees.

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