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See corresponding commentary on page 8.

Distinct Metabolic Profile in Early Pregnancy of Overweight and Obese Women Developing Gestational Diabetes

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ABSTRACT

Background: Reliable biomarkers for gestational diabetes mellitus (GDM) would be beneficial in the early prevention of adverse metabolic outcomes during pregnancy and beyond.

Objectives: The objective of this study was to investigate whether the early pregnancy serum metabolic profile differs in women developing GDM from those remaining healthy. Furthermore, we evaluated the potential of these metabolites to act as predictive markers for GDM.

Methods: This was a prospective study investigating overweight and obese [prepregnancy BMI (in kg/m^2) ≥25 and >30, respectively] pregnant women (prepregnancy median BMI: 28.5; IQR: 26.4–31.5; $n = 357$). Fasting serum samples were analyzed with a targeted NMR approach in early pregnancy (median: 14.3 weeks of gestation). GDM was diagnosed on the basis of a 2-h, 75-g oral-glucose-tolerance test at a median of 25.7 weeks of gestation.

Results: In early pregnancy, 78 lipid metabolites differed in women who later developed GDM ($n = 82$) compared with those who remained healthy ($n = 275$) (ANCOVA, adjusted for confounding factors and corrected for multiple comparisons; false discovery rate <0.05). Higher concentrations of several-sized VLDL particles and medium- and small-sized HDL particles, and lower concentrations of very large-sized HDL particles, were detected in women developing GDM. Furthermore, concentrations of amino acids including 2 branched-chain amino acids, isoleucine and leucine, and GlycA, a marker for low-grade inflammation, were higher in women who developed GDM. Receiver operating characteristic analysis revealed that the most predictive marker for GDM was a higher concentration of small-sized HDL particles (AUC: 0.71; 95% CI: 0.67, 0.77; $P < 0.001$).

Conclusions: We identified a distinct early pregnancy metabolomic profile especially attributable to small HDL particles in women developing GDM. The aberrant metabolic profile could represent a novel way to allow early identification of this most common medical condition affecting pregnant women. This trial was registered at clinicaltrials.gov as NCT01922791. *J Nutr* 2020;150:31–37.

Keywords: gestational diabetes, metabolomics, overweight, obesity, prediction, HDL

Introduction

The prevalence of gestational diabetes mellitus (GDM) is increasing in parallel with the epidemic of overweight and obesity, currently affecting up to 28% of women (1). The International Association of Diabetes in Pregnancy Study Groups has recommended universal screening of women between 24 and 28 weeks of pregnancy [with an oral-glucose-tolerance test (OGTT)] (2). Because GDM may predispose both the mother and the child to clinically significant metabolic complications during pregnancy, deeper insights into the pathophysiology of GDM (i.e., signs already evident during early pregnancy) could provide novel tools to allow the early identification of women at increased risk for GDM as well as targets for early prevention of GDM. They could also help identify women at an increased risk for postpartum metabolic aberrations, including type 2 diabetes.

Enlightening insights into the association between metabolism and clinical conditions, such as GDM, may be gained by adopting a high-technology metabolomics approach that allows the simultaneous evaluation of a multitude of metabolites. In a previous study using a targeted NMR-based approach, an increase in several lipoprotein-related variables and both increases and decreases in specific amino acids and fatty acids in pregnant compared with nonpregnant women were reported (3). A range of metabolic markers involved in GDM pathogenesis have been identified at various gestational

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ages (4, 5), although the results have been inconsistent. This may be due to factors such as variations in the timing of sample withdrawal and performance of the OGTT test during pregnancy, fasting or nonfasting conditions, or nutritional status of the women, which we have standardized in this study. We investigated whether there are differences in the concentrations of specific metabolites in early pregnancy between women who develop GDM and those who remain healthy. This was done by utilizing a targeted NMR-based metabolomics approach analyzing fasting serum samples from overweight and obese pregnant women, excluding high-risk women who had tested positive for GDM in early pregnancy. Furthermore, we evaluated the potential of the metabolites, individually or in combination, to act as predictive markers for the development of GDM.

Methods

Study participants and design

In this follow-up study, serum metabolomic profiles were analyzed in women participating in a mother–infant dietary single-center intervention trial (clinicaltrials.gov, NCT01922791) being conducted in southwest Finland. The inclusion criteria for the study were overweight [self-reported prepregnancy BMI (in kg/m^2) ≥25] and early pregnancy (<18 weeks of gestation). The exclusion criteria were GDM diagnosed during the current pregnancy; multifetal pregnancy; and the presence of metabolic or inflammatory diseases, including type 1 and type 2 diabetes, celiac disease, and inflammatory bowel disease, but the presence of allergy was allowed. A total of 439 women were recruited in the clinical trial, but we excluded women in whom GDM had been diagnosed during early pregnancy or if their blood samples were unavailable for analysis. This resulted in a total of 357 women with both serum metabolomics data during early pregnancy [median (IQR) 14.3 (12.8–15.6) weeks of gestation] and the OGTT result in late pregnancy [25.7 (IQR: 25.0–27.1) weeks of gestation] (**Supplemental Figure 1**). Prepregnancy BMI was calculated by dividing self-reported weight in kilograms, obtained from women's welfare clinic records, by height measured with a wall stadiometer to the nearest 0.1 cm in early pregnancy. The characteristics of the women (**Table 1**), including age, education, GDM in a previous pregnancy, smoking status, and a diagnosis of diabetes or metabolic syndrome in the mother's parents, were collected by questionnaires.

This study was conducted in accordance with the Declaration of Helsinki as revised in 2013. All procedures that involved human subjects were approved by the Ethics Committee of the Hospital District of Southwest Finland (permission number 115/180/2012), and all participants provided written informed consent.

Primary outcome

The primary outcome was the difference in serum metabolites in early pregnancy in women who developed GDM in later pregnancy compared with women who remained unaffected by GDM during pregnancy.

Blood sampling and analytical methods

Fasting (10-h minimum) blood samples were drawn from the antecubital vein, and the serum was separated and frozen in aliquots at −80°C until analyzed by serum metabolomics. A high-throughput proton NMR metabolomics platform (Nightingale) was used to analyze the serum metabolic profile as described elsewhere (6). The analysis platform comprises 228 variables, including biomarkers of lipid and glucose metabolism; amino acids; ketone bodies; and glycoprotein acetylation (GlycA), a novel marker of low-grade inflammation. GlycA consists of a complex heterogeneous NMR signal originating from the *N*-acetyl sugar groups on multiple acute-phase glycoproteins present in the circulation: α_1-acid glycoprotein, haptoglobin, α_1-antitrypsin, α_1-antichymotrypsin, and transferrin (7). The intake of fat was calculated from 3-d food records.

Diagnosis of GDM

GDM was diagnosed on the basis of a 2-h, 75-g OGTT if ≥1 values were at or above the threshold levels: 0 h ≥5.3, 1 h ≥10.0, and 2 h ≥8.6 mmol/L (8). In addition, early pregnancy OGTT was conducted in high-risk women (BMI ≥35, previous GDM, glucosuria, polycystic ovarian syndrome, or family risk of diabetes) between 12 and 16 weeks of gestation in accordance with the national care guidelines.

Statistical analysis

The normality of the distributions of the data was analyzed by visual inspection of histograms and using skewness as a test of normality. Because several subjects had zero values in extremely large, extra-large, and large VLDL particles, the values of these variables were excluded from the analysis according to the instructions from the analyzer. The differences in the baseline characteristics between the women developing GDM and those remaining healthy were evaluated using 2-sample *t* test for normally distributed variables or by Mann–Whitney *U* test for non-normally distributed continuous variables and the Pearson chi-square test for categorical variables.

The differences in serum metabolomics (total 228 variables) between the women developing GDM and those remaining healthy were analyzed using the Mann–Whitney *U* test (**Supplemental Table 1**). The lipid variables ($n = 208$) were adjusted for multiple comparison using the Benjamini–Hochberg (BH) procedure. After the procedure, 83 of the 90 statistically significant ($P < 0.05$ for all) lipids remained statistically significant (false discovery rate <0.05) (Supplemental Table 1). These 83 lipid variables and 8 other statistically significant variables (out of 20) were further analyzed with ANCOVA (**Supplemental Table 2**). In these analyses, the variables skewed to the right (skewness >1) were transformed with natural logarithms. Furthermore, to have comparable values, all metabolic measures chosen for ANCOVA were divided by their SD (9).

To take into account the possible confounding factors, ANCOVA was adjusted with factors differing between the groups—that is, prepregnancy BMI as a categorical variable and dietary factors that were estimated to contribute to GDM (absolute intake of dietary fat) and also the intervention groups in the original intervention setting. The lipid variables were further adjusted for multiple comparison using the BH procedure, resulting in 78 statistically significant lipid variables. These are presented as a mean difference in SD units between the women who developed GDM and those who remained healthy. To evaluate the possible metabolic pathways associated with the incidence of GDM, we performed a principal component analysis (PCA). In this, we included prepregnancy BMI, concentrations of lipoproteins and amino acids that differed according to GDM status, glucose, pyruvate, and GlycA. Receiver operating characteristic (ROC) curve analyses were performed to investigate the predictive value of specific serum metabolites alone and in combination with other measures—that is, metabolites that

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The study sponsors were not involved in the design of the study; the collection, analysis, and interpretation of data; writing the report; or the decision to submit the report for publication.
Data sharing: Publicity about the data sets generated during the current study would limit the ongoing study; therefore, the data are only available from the corresponding author upon reasonable request.
Supplemental Figures 1 and 2 and Supplemental Tables 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
Address correspondence to KM (e-mail: kamamo@utu.fi).
Abbreviations used: BCAA, branched-chain amino acid; BH, Benjamini–Hochberg; GDM, gestational diabetes mellitus; GlycA, glycoprotein acetylation; OGTT, oral-glucose-tolerance test; PCA, principal component analysis; ROC, receiver operating characteristic.

TABLE 1 Characteristics of the pregnant women[1]

Characteristics	Women remaining healthy		Women who developed GDM		All	*P* value
	Values	*n*	Values	*n*		
Age, y	30.3 ± 4.4	275	31.1 ± 4.6	82	30.5 ± 4.4	0.137
Prepregnancy BMI, kg/m^2	28.4 (26.3–31.3)	275	29.8 (26.8–32.0)	82	28.5 (26.4–31.5)	0.078
Obese (BMI >30), %	34.6	95/275	57.3	47/82	39.8 (142/357)	0.02
Blood pressure, mm Hg						
Systolic	115.5 (110.0–122.5)	273/275	116.0 (111.0–122.6)	82	115.5 (110.0–122.5)	0.489
Diastolic	75.5 (70.0–80.0)	273/275	76.5 (71.5–82.6)	82	76.0 (70.5–81.0)	0.131
Pregnant woman's parents have DM or metabolic syndrome, %	17.9	45/252	27.9	22/79	20.2 (67/331)	0.054
Expecting her first child, %	48.4	133/275	50.0	41/82	48.9 (174/357)	0.795
GDM in previous pregnancy, %	5.1	14/275	9.8	8/82	6.2 (22/357)	0.123
Gestational weeks	14.1 (12.7–15.4)	275	14.6 (13.1–15.6)	82	14.3 (12.8–15.6)	0.252
Gestational weeks at OGTT	25.9 (25.0–27.0)	275	25.8 (21.5–27.5)	82	25.7 (25.0–27.1)	0.35
Education (college or university), %	67.3	175/260	55.0	44/80	64.4 (219/340)	0.07
Smoked before pregnancy, %	23.3	61/262	15.0	12/80	21.4 (73/342)	0.114
Smoked during pregnancy, %	4.6	12/260	2.5	2/80	4.1 (14/340)	0.405

[1] Values are medians (IQRs), means ± SDs, or percentages. Statistics: Mann–Whitney *U* test, 2-sample *t* test, or Pearson chi-square test. DM, diabetes mellitus; GDM, gestational diabetes mellitus; OGTT, oral-glucose-tolerance test.

showed the clearest differences between women who developed GDM and those who remained healthy were chosen. Statistical analyses were performed with SPSS for Windows, version 24 (IBM).

Results

Baseline characteristics

The prepregnancy median BMI value for all women was 28.5 (IQR: 26.4–31.5); ~4 out of 10 (39.8%) were obese (BMI ≥30). Prepregnancy BMI as a continuous variable did not differ according to the GDM status, but 34.6% of the women without GDM were obese compared to 57.3% of those who developed GDM ($P = 0.02$). GDM was diagnosed in 82 women in late pregnancy, whereas the other 275 remained healthy. There were no further statistically significant differences in baseline characteristics between the women developing GDM and those remaining healthy (Table 1).

Difference in the serum metabolic profile in women developing GDM compared with unaffected women

A total of 78 lipid variables at early pregnancy differed according to their GDM status in late pregnancy (adjusted for confounding factors and multiple testing) (Supplemental Table 2). In women who developed GDM, the concentrations of all-sized VLDL particles were higher compared with those of the unaffected women (**Figure 1**A). The higher concentration of VLDL particles was attributable to the higher lipid content within the particles, consisting mainly of phospholipids and triglycerides, and also of cholesterol in large VLDL particles. In HDL particles, the difference in the concentrations between the women developing GDM and those remaining healthy was dependent on the size of the HDL particles: The concentrations of medium- and small-sized HDL particles were higher, whereas the concentrations of very large HDL particles were lower in the women who developed GDM. The elevated concentrations of medium- and small-sized HDL particles were attributable to higher total lipids consisting of phospholipids, triglycerides, and cholesterol, whereas very large-sized HDL particles comprised phospholipids and cholesterol. In addition, in women who developed GDM, lower concentrations of total cholesterol and cholesterol ester as well as a higher free cholesterol to total lipid ratio were analyzed in VLDL particles (Figure 1B). In contrast, in very large-sized HDL particles, higher concentrations of total cholesterol and cholesterol ester and a lower free cholesterol to total lipid ratio were detected. Furthermore, the estimated degree of unsaturation of fatty acids, the ratio of n–6 long-chain PUFAs and PUFAs to total fatty acids, was lower in women who developed GDM. In addition, the concentrations of triglycerides as well as the ratio of MUFAs to total fatty acids were elevated in women developing GDM compared to unaffected women.

In addition to lipids, higher concentrations of glucose, lactate, pyruvate, as well as 2 branched-chain amino acids (BCAAs)—isoleucine and leucine—alanine, and the aromatic amino acid phenylalanine and an elevated concentration of GlycA were observed in women who developed GDM compared to women who remained healthy. The higher concentrations of all these metabolites, with the exception of lactate, remained statistically significant after adjusting for potential confounding factors in ANCOVA (Figure 1C).

In PCA, a clear pattern of a metabolic profile was observed between the women who developed GDM and unaffected women. In particular, the concentration of very large HDL particles clustered in the unaffected women, but also a clear separation of the amino acids isoleucine and leucine and GlycA was observed according to the women's GDM status (**Supplemental Figure 2**).

Prediction of GDM by ROC analysis

The variable that was most predictive of the incidence of GDM was the concentration of small-sized HDL particles, followed by concentrations of glucose, GlycA, leucine, and pyruvate (**Table 2**, **Figure 2**A), as demonstrated by the AUC from the ROC analysis. When these variables with the highest predictive values were combined in the same model, the AUC was enhanced, with the combination of glucose, GlycA, leucine, and small-sized HDL particles having the highest predictive accuracy for GDM (Table 2, Figure 2B).

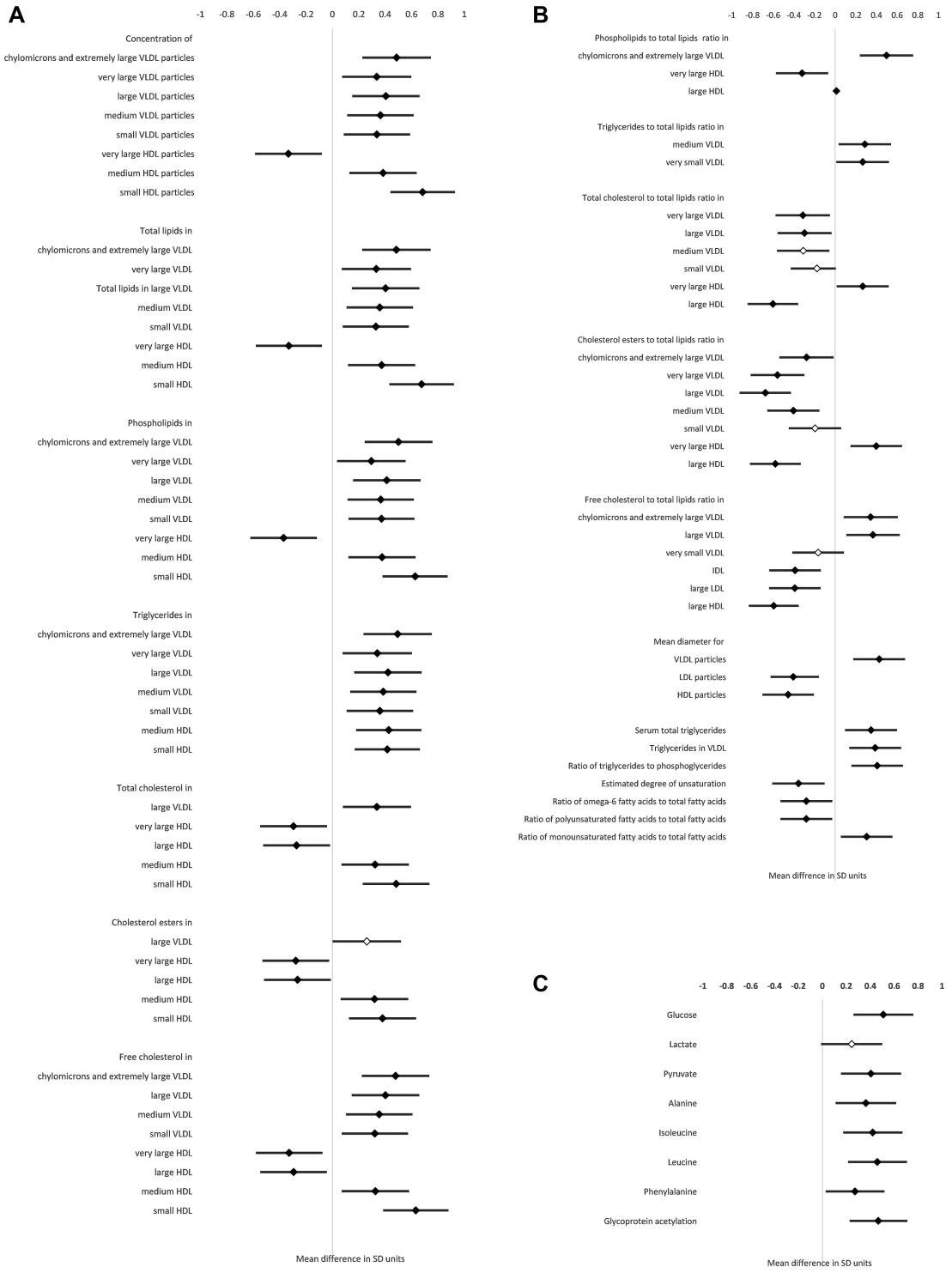

FIGURE 1 The mean differences in serum metabolites between women who develop GDM and women who remain healthy. Values describe the mean differences in SD units, with positive values indicating higher concentrations in women who developed GDM. Black diamonds indicate statistically significant differences in women developing GDM compared with those remaining healthy, white diamonds indicate non-significant differences. In panels A and B, the black diamonds indicate statistically significant differences when the *P* values from ANCOVA were corrected for multiple testing (FDR <0.05), whereas in panel C, the *P* values were not corrected for multiple testing. FDR, false discovery rate; GDM, gestational diabetes mellitus.

Discussion

It was found that the metabolic profile of women who would develop GDM in late pregnancy was already abnormal in early pregnancy in comparison with that of women who remained healthy. Interestingly, the single variable that was most predictive for the GDM diagnosis was a high concentration of serum small-sized HDL particles, although its predictive value increased when combined with other early pregnancy biomarkers, primarily GlycA (i.e., low-grade inflammation), glucose, and the amino acid leucine.

TABLE 2 Prediction of GDM in the pregnant women using AUC from the ROC analysis[1]

	AUC (95% CI)	*P* value
Prepregnancy BMI	0.563 (0.482, 0.634)	0.084
Very large HDL particles	0.610 (0.540, 0.681)	0.002
Chylomicrons and extremely large VLDL particles	0.631 (0.562, 0.699)	0.001
Isoleucine	0.641 (0.575, 0.707)	<0.0001
Pyruvate	0.644 (0.574, 0.715)	<0.0001
Leucine	0.644 (0.576, 0.712)	<0.0001
GlycA	0.644 (0.577, 0.71)	<0.0001
Glucose	0.682 (0.613, 0.751)	<0.0001
Small HDL particles	0.714 (0.656, 0.772)	<0.0001
GlycA and glucose	0.712 (0.646, 0.777)	<0.0001
GlycA, glucose, and small HDL	0.712 (0.646, 0.777)	<0.0001
GlycA, glucose, small HDL, and pyruvate	0.713 (0.648, 0.777)	<0.0001
GlycA, glucose, small HDL, and leucine	0.719 (0.654, 0.784)	<0.0001
GlycA, glucose, small HDL, leucine, and pyruvate	0.717 (0.651, 0.782)	<0.0001

[1] GDM, gestational diabetes mellitus; GlycA, glycoprotein acetylation; ROC, receiver operating characteristic.

Our finding of a higher concentration of small- and also medium-sized HDL particles in women who developed GDM is of importance; in particular, small-sized HDL particles have been consistently related with the risk of cardiovascular diseases, whereas larger sized HDL particles exhibit inverse relationships with these conditions (10). In addition to being a potential predictor for GDM, small-sized HDL particles are thought to be a contributor to the heightened risk for postpartum cardiovascular disease, and the risk for these disorders is known to be elevated in women with GDM (11). Although controversy exists regarding which HDL subpopulation particles have higher capability to promote cholesterol efflux and thus higher HDL functionality, one interpretation based on our findings is that larger HDL particles associate with higher HDL functionality. Furthermore, the finding of increased concentrations of VLDL particles and triglycerides already in early pregnancy in women who developed GDM is of importance regarding their later risk for cardiovascular diseases (12).

Our findings of the serum lipoprotein constituents are partly in line with those of a previous study (9) that demonstrated increased lipids in VLDL and in small-sized HDL particles. The discrepancies observed here in comparison to the previous investigation seem to be related to total cholesterol and cholesterol esters. White et al. (9) detected elevations in various-sized VLDL particles in women with GDM, whereas in our study, a higher concentration of total cholesterol was only observed in the large VLDL particles of the women who developed GDM. Furthermore, in our study, higher concentrations of total cholesterol and cholesterol esters in women who developed GDM were observed in various-sized HDL particles, at odds with the previous report (9). It is noteworthy that we collected fasting blood samples, whereas the serum samples assayed in the previous study were withdrawn during nonfasting conditions (9), thus highlighting the benefits of analyzing fasting samples to exclude the potential problems with nonfasting samples. Importantly, in contrast to the study by White et al. (9), we excluded the high-risk women who had already been diagnosed with GDM in early pregnancy to increase the predictive value of the early metabolic profiles with regard to the onset of GDM.

According to our results, the serum concentrations of 2 BCAAs, isoleucine and leucine, as well as phenylalanine and alanine, were higher already in early pregnancy in women who developed GDM. Similar findings have been reported previously: elevated BCAA concentrations, also including valine and phenylalanine but not alanine, were detected in obese women with GDM (9). Instead, investigations of women with heterogeneous BMI have reported an increase in early pregnancy concentrations of valine (13) and alanine (13, 14) or no alterations in BCAAs (14, 15) in association with the onset of GDM. It is noteworthy that the OGTT in 3 of these studies (9, 13, 14) was performed in the second trimester, and therefore it is plausible that women with a diagnosis of GDM in early pregnancy were included in these studies. If this were the case, then it would be a clear confounding factor when interpreting the findings. Based on our findings and those of others, and also considering that amino acids have the capacity to induce insulin resistance (16), it can be proposed that these amino acids very likely play a role in the onset of GDM. Indeed, a meta-analysis of studies in nonpregnant populations revealed that BCAAs were associated with insulin resistance (17). On the other hand, although pregnancy is considered to be a condition manifested by insulin resistance, the concentrations of BCAAs have been reported to remain unchanged (3) or even decrease (18) during the course of a healthy pregnancy. Another study found higher concentrations only of the nonessential amino acid ornithine in maternal plasma of women with GDM at the time of delivery compared with women without GDM (19). However, in the same study, elevated concentrations of several amino acids, such as ornithine, and BCAAs isoleucine and leucine were observed in the umbilical venous plasma in women with GDM (19), suggesting alterations in fetal and placental amino acid metabolism in association with GDM. Obesity is an acknowledged risk factor for GDM. In a recent study, compared to normal-weight pregnant women, the placentas of obese women were shown to have higher concentrations of amino acids, including the BCAAs isoleucine and leucine (20). When comparing the placental amino acids between the women with GDM and those without GDM, no differences were observed (20). It is possible that during a healthy pregnancy, amino acids including BCAAs are consumed during gluconeogenesis and for the production of ketone bodies (in response to the reduced glucose concentrations and increased insulin resistance) or utilized as a nitrogen source for the fetus during the pregnancy (21). However, in pathological conditions such as GDM and obesity, these adaptations may somehow become disturbed. Clearly, there

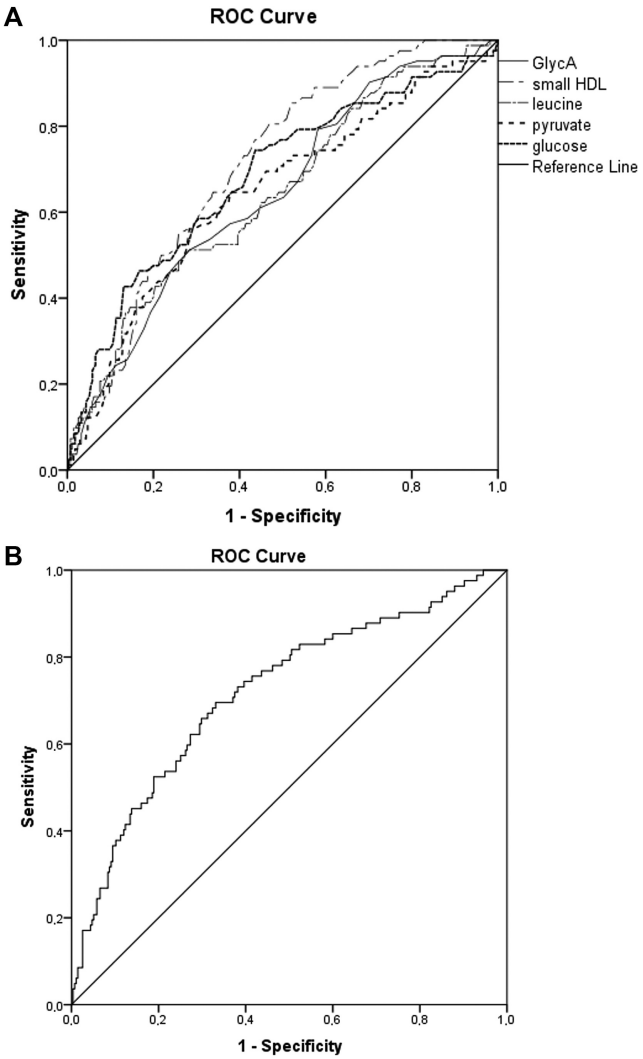

FIGURE 2 ROC curves of GDM prediction: the specific serum metabolites alone (A) and combination of the variables with highest predictive values (B). GDM, gestational diabetes mellitus; GlycA, glycoprotein acetylation; ROC, receiver operating characteristic.

is a need for further exploration of the changes occurring in amino acids during pregnancy, particularly the physiological alterations.

One important finding is that the observed elevations in the circulating lipids in women who develop GDM may be related to low-grade inflammation, a condition generally recognized as a risk factor for many metabolic and clinical disorders. In the current study, GlycA, a novel measure of low-grade inflammation, was found to be a predictor for GDM. Thus, according to ROC analysis, we propose that GlycA exerts a pathogenic role either individually or together with other metabolites in the development of GDM, particularly in overweight and obese women. In a smaller study, we previously demonstrated a correlation between GlycA concentrations and markers of glucose and lipid metabolism in overweight and obese women (22). In nonpregnant populations, increased concentrations of GlycA have been observed in association with metabolic disturbances such as type 2 diabetes and cardiovascular disease (23, 24).

Our study has several strengths. All the samples were collected in the fasting condition, and all were handled and analyzed using the same protocol. We also excluded the high-risk women who had been tested positive for GDM in early pregnancy, thus providing comprehensive data on the early metabolic profile of the pre-GDM condition. Furthermore, the carefully collected background data of the women allowed us to perform robust statistical analyses, taking into account clinically and statistically relevant potential confounding factors. Our study is not without limitations, and further studies are needed to evaluate whether the serum metabolomic profile differs even prior to pregnancy in women who will be diagnosed with GDM and if there is a difference in the metabolomic profile between women receiving an early or a late pregnancy GDM diagnosis. In addition, the pathophysiology of GDM may be influenced by maternal BMI: it needs to be clarified whether a different metabolomic profile underlies the GDM in normal-weight and overweight individuals and obese subjects.

In conclusion, we identified a distinctive early pregnancy metabolic profile between women with GDM developing in late pregnancy and pregnant women who did not become diabetic. In particular, the concentrations of small- and medium-sized HDL particles and several-sized VLDL particles were enhanced in women who developed GDM, with the small-sized HDL particles having the strongest predictive value for GDM. Similar to the observed increases in small-sized HDL particles and VLDL particles, elevated concentrations of BCAAs and GlycA have been associated with poorer metabolic outcomes, including increased risk for cardiovascular disease. Thus, these findings may provide a target for the prevention of both GDM and postpartum metabolic manifestations in the early phase of pregnancy.

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