Original Article
CLINICAL TRIALS AND INVESTIGATIONS

Obesity

Distinct Metabolomic Profile Because of Gestational Diabetes and its Treatment Mode in Women with Overweight and Obesity

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Objective: Whether the presence of gestational diabetes (GDM) and its treatment mode influence the serum metabolic profile in women with overweight or obesity was studied.
Methods: The serum metabolic profiles of 352 women with overweight or obesity participating in a mother-infant clinical study were analyzed with a targeted NMR approach (at 35.1 median gestational weeks). GDM was diagnosed with a 2-hour 75-g oral glucose tolerance test.
Results: The metabolomic profile of the women with GDM ($n=100$) deviated from that of women without GDM ($n=252$). Differences were seen in 70 lipid variables, particularly higher concentrations of very low-density lipoprotein particles and serum triglycerides were related to GDM. Furthermore, levels of branched-chain amino acids and glycoprotein acetylation, a marker of low-grade inflammation, were higher in women with GDM. Compared with women with GDM treated with diet only, the women treated with medication ($n=19$) had higher concentrations of several sizes of VLDL particles and their components, leucine, and isoleucine, as well as glycoprotein acetylation.
Conclusions: A clearly distinct metabolic profile was detected in GDM, which deviated even more if the patient was receiving medical treatment. This suggests a need for more intense follow-up and therapy for women with GDM during pregnancy and postpartum to reduce their long-term adverse health risks.

Study Importance

What is already known?

- Maternal obesity increases the risk for gestational diabetes.

What does this study add?

- Gestational diabetes and its treatment mode influence metabolic profile in women with overweight or obesity, calling for more intense follow-up, particularly in women treated with medication.

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Introduction

Pregnancy is a state in which a mother adapts to the demands of the fetus by altering her metabolism (1). Maternal overweight and obesity disturb the metabolic adaptation, and a loss of maternal metabolic plasticity increases the risk for pregnancy complications, the most common clinical condition being gestational diabetes (GDM) (2). There is evidence of differences in the metabolic profile of women with GDM in comparison with their healthy counterparts at the time of diagnosis (3,4) and even at early pregnancy, before the late GDM diagnoses (3,5). Further insights into the maternal metabolic profile related to GDM and its management, particularly medication, are needed when considering the likely adverse long-term health consequences of the metabolic aberrations to the mother and the child.

The common first-line approach to managing GDM is dietary modification with the aim to maintain the glucose levels within reference ranges and to promote fetal growth and development. Pharmacological treatment (insulin and/or metformin) is the second line of treatment when sufficient control of glucose balance is not obtained through dietary management (6). The extent to which GDM per se and its treatment by diet compared with pharmaceutical treatment is related to the metabolic

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profile is currently poorly understood. In patients with type 2 diabetes, metformin treatment was associated with lower levels of valine and carnitine and higher levels of lysophosphatidylethanolamines as well as elevated concentrations of leucine and isoleucine (7). Another study conducted in participants with coronary disease revealed a decrease in tyrosine and an increase in alanine in patients treated with metformin (8). Therefore, we hypothesized that the metabolic profile would be disturbed in GDM and that further aberrations could occur with regard to treatment mode.

To test our hypothesis, we investigated, in a clinical study setting, whether the metabolic profile of women with overweight and obesity would be divergent from those without GDM and whether the treatment mode of the GDM, i.e., dietary treatment compared with medication, contributed to the metabolic aberrations.

Methods

Design and participants

The serum samples were obtained from women who participated in a single-center mother-infant dietary intervention trial with recruitment between October 2013 and July 2017 (ClinicalTrials.gov, NCT01922791). The inclusion criteria for the study were overweight (self-reported prepregnancy BMI ≥ 25), early pregnancy (< 18 weeks of gestation), and availability of an oral glucose test (OGTT). The exclusion criteria were multi-fetal 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 further inclusion criterion for the current study was availability of a serum sample collected in late pregnancy; we had data on 352 women (out of a total trial population of 439) from a serum metabolomics analysis (at 35.1 median gestational weeks [interquartile range 34.6-35.9]). The intervention with dietary supplements (fish oil and probiotics) in the main trial had no influence on the incidence of GDM or glucose metabolism (9) but was nevertheless included as a covariate in the analysis.

GDM was diagnosed on the basis of a 2-hour 75-g OGTT if one or more values were at or above the following threshold levels: 0 hours ≥ 5.3, 1 hour ≥ 10.0, and 2 hours ≥ 8.6 mmol/L (10). OGTT was conducted between gestational weeks 24 and 28 ($n = 326$) and in high-risk women (BMI ≥ 35, previous GDM, glucosuria, polycystic ovarian syndrome, or family risk of diabetes, $n = 105$) between gestational weeks 12 and 16 in accordance with the national care guidelines. For all the women with GDM, a dietary therapy is the first-line option based on Finnish Current Care guidelines for dietary treatment of GDM. The dietary guidelines include recommendations on intakes of carbohydrates with high fiber content; fat, including two-thirds of mono- and polyunsaturated fatty acids; and increased intakes of fruits and vegetables.

Prepregnancy BMI was calculated by dividing self-reported weight in kilograms, obtained from welfare clinic records, by height in meters squared measured with a wall stadiometer to the nearest 0.1 cm in early pregnancy. The characteristics of the women, including age, education, GDM in previous pregnancy, smoking, and a diagnosis of diabetes or metabolic syndrome in the mother's parents, were collected by questionnaires (Table 1).

This study was conducted according to the guidelines laid down in the Declaration of Helsinki as revised in 2013, and all procedures that involved human participants were approved by the Ethics Committee of the Hospital District of Southwest Finland (permission number 115/180/2012). Written informed consent was obtained from all women.

Primary and secondary outcomes

The primary outcome was the difference in late pregnancy serum metabolic profile in women with overweight or obesity diagnosed with

TABLE 1 Baseline characteristics of participants

	Women without GDM, $n = 252$	Women with GDM, $n = 100$	All, $n = 352$	*P* value
Age	30.2 (27.1-33.4)	31.0 (28.8-34.8)	30.4 (27.6-34.0)	0.092
Prepregnancy BMI	28.4 (26.3-31.2)	30.1 (27.2-33.6)	28.7 (26.5-31.9)	0.001
Obesity	34% (86/252)	48% (48/100)	61% (214/352)	0.002
Primipara	49% (123/252)	47% (47/100)	48% (170/352)	0.759
College or university education	67% (164/246)	56% (55/99)	63% (219/345)	0.053
Mother's parents have diabetes or metabolic syndrome	18% (43/238)	32% (31/98)	22% (74/336)	0.006
Smoked during pregnancy	5% (11/246)	4% (4/99)	4% (15/345)	0.859
Mother has had GDM in a previous pregnancy	5% (13/252)	19% (19/100)	9% (32/352)	< 0.001
Smoked before pregnancy	22% (55/248)	15% (15/99)	20% (70/347)	0.141
Weeks between early pregnancy OGTT and blood sampling	20.9 (19.1-22.6), $n = 79$	19.0 (18.3-21.9), $n = 26$	20.6 (18.9-22.2), $n = 105$	0.063
Weeks between blood sampling and late pregnancy OGTT	9.2 (8.1-10.4), $n = 252$	9.0 (7.4-10.0), $n = 74$	9.1 (8.0-10.4), $n = 326$	0.104

Values are median (interquartile range) for continuous variables and percentage for categorized variables.
Mann-Whitney *U* test used for continuous variables between women without and with GDM, and χ^2 used for categorized variables between women without and with GDM.
GDM, gestational diabetes; OGTT, oral glucose tolerance test.

GDM compared with women without GDM. The secondary outcome was the difference in the serum metabolic profile in women with GDM treated with either diet alone or medication.

Blood sampling and analytical methods

Fasting (10 hours minimum) blood samples were drawn from the antecubital vein (at 35.1 median gestational weeks), and the serum was separated and frozen in aliquots at −80°C until being analyzed for serum metabolomics. A high-throughput proton nuclear magnetic resonance (NMR) metabolomics platform (Nightingale, Helsinki, Finland) was used to analyze the serum metabolic profile as described earlier (11). The analysis platform assesses 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 present on multiple acute-phase glycoproteins in the circulation, including α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin, and transferrin (12). The intake of energy (kilojoules), fat, and protein as a percentage of energy intake was calculated from 3-day food diaries and used as confounding factors in ANCOVA.

Statistics

The normality of the distributions of the data was analyzed by visual inspection of histograms and by using skewness as a test of normality. As not all variables were normally distributed, Mann-Whitney *U* test was used to analyze the difference in serum metabolites between women with and without GDM and between the women with GDM treated either with medication (insulin, metformin, or both) or dietary therapy. The statistically significant lipid variables were corrected for multiple testing by using Benjamini-Hochberg procedure (false discovery rate < 0.05). Those lipid variables that remained statistically significant and other statistically significant variables were further analyzed by ANCOVA and adjusted with potential confounding factors, including prepregnany BMI (< 30 or ≥ 30); intake of energy (kilojoules), fat, and protein as a percentage of energy intake; intervention group; previous GDM; education; and mother's parents with diabetes mellitus or metabolic syndrome ($n = 324$ women). After ANCOVA, the lipids were again adjusted for multiple testing with the Benjamini-Hochberg procedure (false discovery rate < 0.05). In ANCOVA, the nonparametric variables were logarithm transformed. In addition, all variables in the ANCOVA model were divided by their SD to enable a comparison of the variables with different scales.

Statistical analyses were performed with SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, New York).

Results

GDM was diagnosed in 100 out of a total of 352 women (31%) (Table 1). All were treated with diet in line with the current care guidelines for GDM in well-women clinics, but 21 women were also administered medications, including 12 metformin, 4 insulin, and 5 both insulin and metformin. Prepregnancy BMI differed according to the GDM status, being higher in women with GDM (Table 1), whereas no difference in prepregnancy BMI was observed between the treatment mode of the women with GDM ($P = 0.127$). There were some further statistically significant differences between the study groups, i.e., a family history of diabetes mellitus or metabolic syndrome and previous GDM, which were taken into account as confounding factors in ANCOVA.

Serum metabolic profiles according to GDM status

Metabolic profile differed according to the GDM status. In summary, 137 metabolites were changed in women with GDM; of these, 128 were lipid variables and the other 9 included amino acids and markers of glucose metabolism and ketone bodies (Supporting Information Table S1). Of the lipid variables, 121 lipids were still statistically significantly different even after correcting for multiple testing. Furthermore, after adjusting for possible confounding factors, 70 lipid variables remained statistically significant (Figure 1A-1B, Supporting InformationTable S2). These were mainly attributable to all-sized very low-density lipoprotein (VLDL)-related particles, which were higher in women with GDM (Figure 1A-1B). The differences in VLDL particles were mostly due to the increased levels of lipids, including phospholipids and triglycerides within particles, but also the levels of cholesterol and free cholesterol were elevated in nearly all VLDL-related particles. Furthermore, the women with GDM had more triglycerides in their medium- and small-sized high-density cholesterol (HDL) particles. In addition, higher serum concentrations of triglycerides and triglycerides in VLDL and HDL particles (a trend, $P = 0.05$) were present in the women with GDM. There were also seven other variables that were higher in women with GDM, including the concentrations of citrate, an intermediate of the citric acid cycle; two branched-chain amino acids (BCAAs), isoleucine and leucine; phenylalanine; one ketone body (acetoacetate); and GlycA (Figure 1C).

Serum metabolomic profile according to GDM treatment

The GDM treatment mode of the GDM also altered the metabolic profile in the comparison between women treated with diet only and women treated with medication. A total of 75 lipid variables (after correcting for multiple testing and adjusting for possible confounding factors of the statistically significant 104 lipid variables) and 4 other variables, including amino acids and GlycA (after adjusting for possible confounding factors of the 7 statistically significant variables), remained statistically significant (Figure 2, Supporting Information Tables S3-S4). Women treated with medication had higher concentrations of several sizes of VLDL particles, total lipids, and cholesterol in their VLDL particles and lower concentrations of cholesterol in the small HDL particles. Furthermore, there were elevations in VLDL diameter and the triglycerides in VLDL particles, and the serum levels of leucine, isoleucine, and GlycA were higher, as were serum triglycerides, in women whose GDM was treated with medication. It is noteworthy that the aberrant metabolic profile in the women treated with medication was evident despite the fact that there were no differences in their serum glucose levels attributable to the treatment mode (Supporting Information Table S4, Figure S2C). This suggests that even though both modes (i.e., diet and medication) managed to balance glucose metabolism, the treatment by medication conferred no benefit to correct the disturbed lipids and amino acid metabolic profile.

Discussion

In this group of women with overweight or obesity, we observed a distinct metabolomic profile in women with GDM compared with healthy

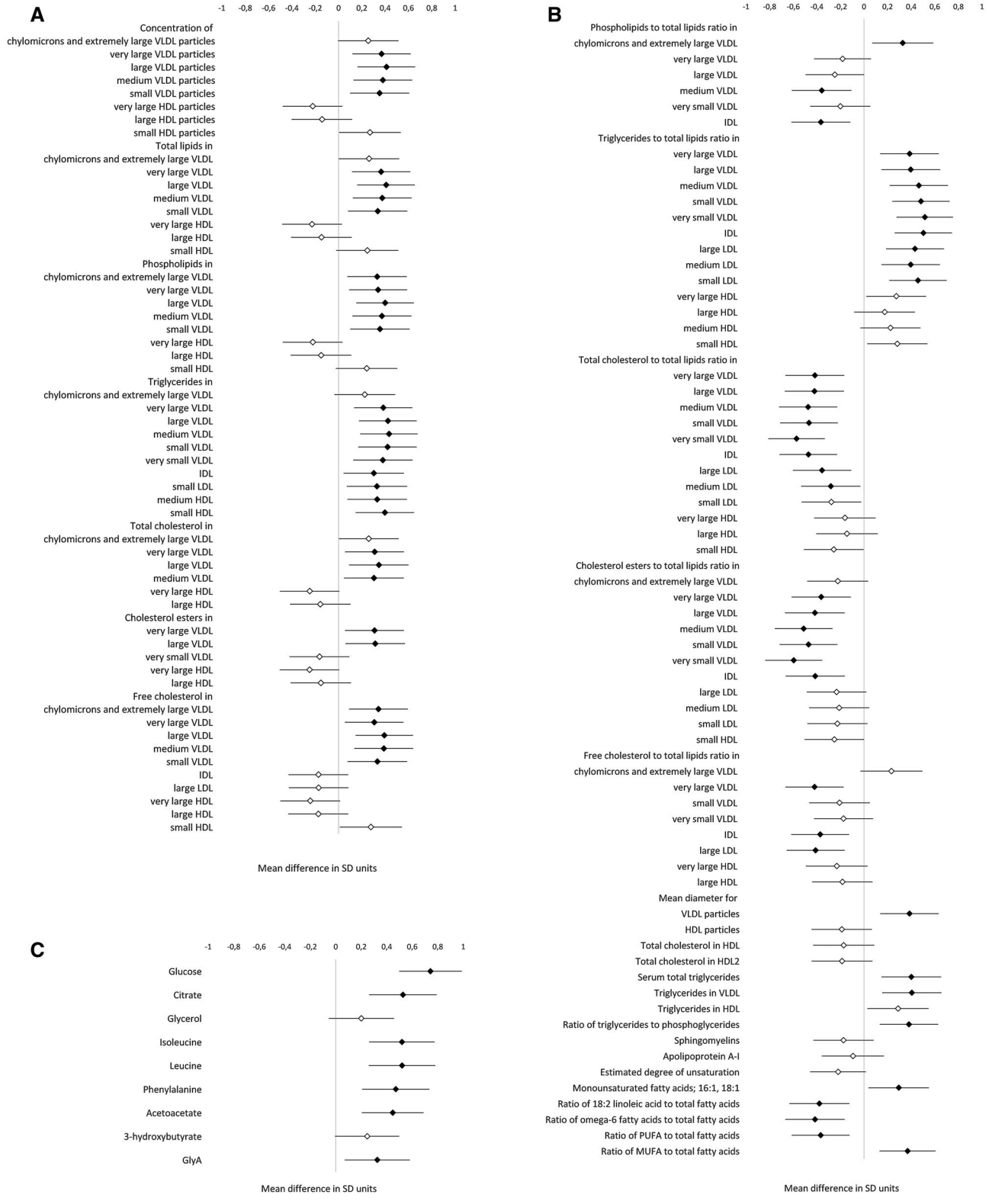

Figure 1 Mean differences in serum metabolites between women with GDM and healthy women. (**A**) Lipid variables, (**B**) lipid variables, and (**C**) other variables. Values describe mean differences in SD units, and positive values indicate higher concentrations in women with GDM. Black squares indicate statistically significant differences after adjusting for multiple comparisons (lipids) (false discovery rate < 0.05). $n = 95$ women with GDM; $n = 229$ women without GDM. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

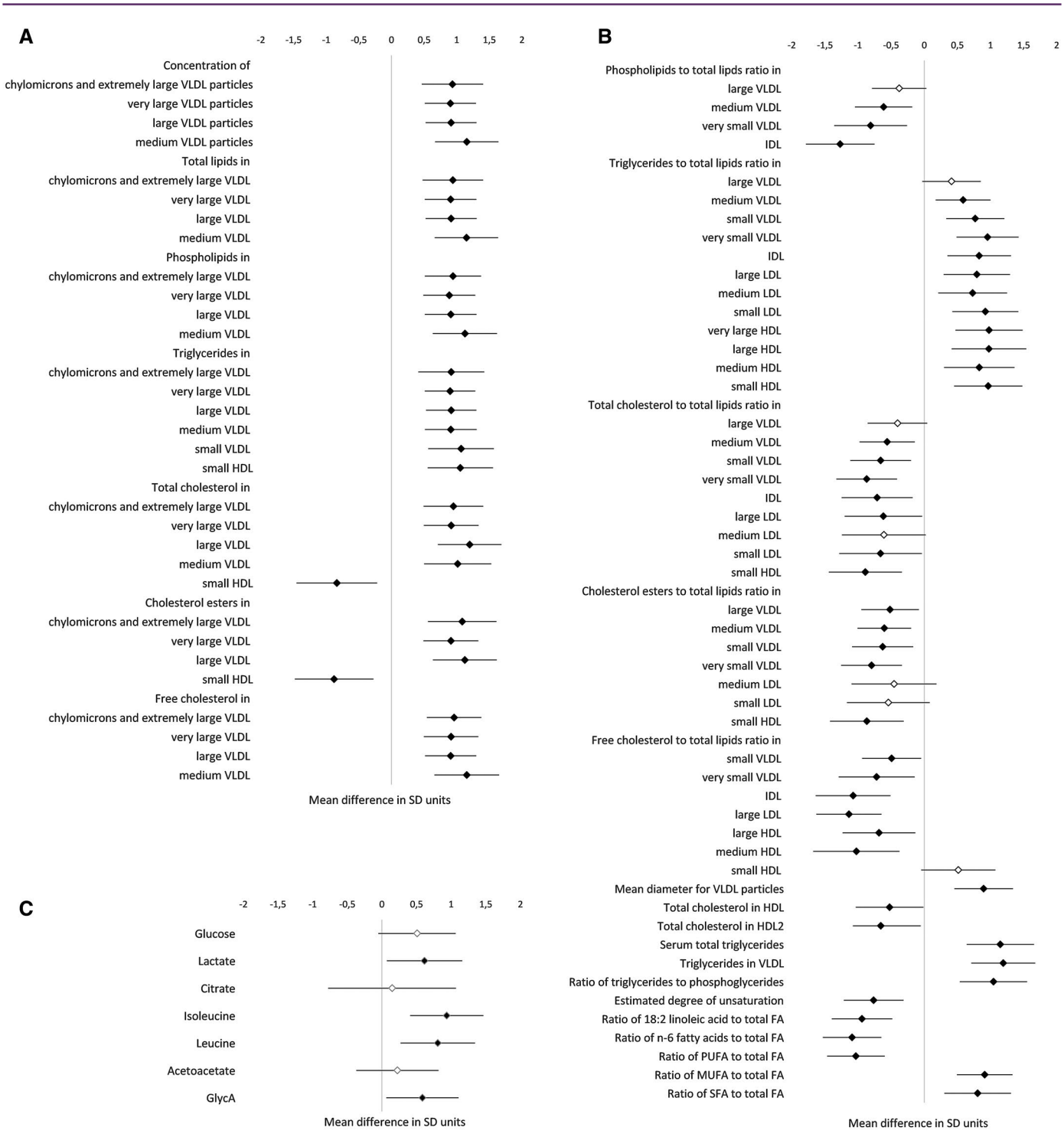

Figure 2 Mean differences in serum metabolites between women with diet only and women administered medication. (**A**) Lipid variables, (**B**) lipid variables, and (**C**) other variables. Values describe mean differences in SD units, and positive values indicate higher concentrations in women receiving medication. Black squares indicate statistically significant differences after adjusting for multiple comparisons (lipids) (false discovery rate < 0.05). $n = 19$ women with medication; $n = 76$ women with diet. FA, fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

women. This was attributable to the elevated concentrations of several lipids, particularly VLDL particles and their components. In addition, levels of BCAAs (isoleucine and leucine), a ketone body (acetoacetate), and a marker of low-grade inflammation (GlycA) were higher in women with GDM. Importantly, the new finding here, when compared with previous studies investigating the metabolomics in women with

GDM, is that more pronounced disturbances in the metabolomic profile and low-grade inflammation were observed in the women treated with medication compared with those treated with diet alone. Based on our findings, we propose that the aberrant metabolic profile is related to GDM, and regardless of the ongoing treatment, this poses a high risk for long-term metabolic complications.

The elevated amounts of several sizes of VLDL particles indicate that the women with GDM were carrying an increased metabolic burden in their late pregnancy, even though their GDM was treated according to the treatment guidelines. The increase in VLDL particles, also previously shown to associate with GDM (3,4,5,13,14), may reflect a similar interaction between lipid and glucose metabolism as occurs in type 2 diabetes (15). Nonetheless, an increase in levels of VLDL-related particles is not an exclusive sign of diabetic status because it may also reflect pregnancy-induced metabolic alterations; i.e., higher levels have also been observed in pregnant women unaffected by GDM compared with nonpregnant women (16). Previously, similar findings related to VLDL particles were detected by using the same NMR method in women recently diagnosed with overweight and obesity (3). However, some deviations were observed between these two studies in the lipid content of extremely large VLDL particles and very large and large HDL particles as well as in serum amino acids concentrations. For example, in our study, no differences in the lipids in extremely large VLDL and in very large and large HDL particles were detected according to the GDM status. Whereas in the previous study, the samples were provided at the time of OGTT, in our trial, the women had been already treated for GDM for approximately 10 weeks, which may have somewhat improved the metabolic variables.

Interestingly, we observed that the women treated with medication had more pronounced aberrations in their metabolic profile compared with women treated with diet alone. The differences between the two treatment modes were mostly related to concentration and lipid content of all-sized VLDL particles, which were higher in the women treated with medication. Furthermore, lower levels of total cholesterol and cholesterol esters in small HDL particles and higher serum levels of lactate, leucine, isoleucine, and GlycA were found in women treated with medication. This may have consequences with respect to their later risk for developing metabolic complications, such as insulin resistance and cardiovascular disease (17-21).

With respect to glucose metabolism, the level of citrate, an intermediate in the tricarboxylic acid cycle that is synthetized from acetyl-CoA and oxaloacetate, was higher in women with GDM. Citrate is an important regulator of energy metabolism; it inhibits glycolysis and conversely stimulates gluconeogenesis and lipid synthesis (22), i.e., energy storage. Thus, in addition to its relation to GDM, it may be one determinant of the adiposity of women with overweight and obesity, as observed in a study with nonpregnant participants with obesity (23). Interestingly, we also found an increased level of acetoacetate, a ketone body, in women with GDM. It is known that in the latter half of normal pregnancy, maternal metabolism is converted to a catabolic state, during which the increased lipolysis provides fatty acids to be utilized in ketogenesis (24). The ketone bodies produced are used as a maternal energy source in place of glucose, which is mostly utilized by the fetus (25). Previous studies have also detected elevated levels in ketone bodies in pregnant women with high fasting glucose concentrations (26) and in association with GDM (3,4,27). The reason for the increased levels of both citrate and ketone bodies in women with GDM in our study is not clear. Nevertheless, the excess amount of citrate inhibits energy production, and on the other hand, ketogenesis is promoted, which is a normal physiological response to starving. The occurrence of these two antagonistic physiological conditions may originate from the disturbed energy metabolism and the dysregulation of glucose metabolism associated with GDM.

In addition to alterations in lipid and glucose metabolism, there was also evidence of disturbances in some amino acids; levels of two BCAAs, isoleucine and leucine, as well as phenylalanine were higher in women with GDM. Other studies have reported increases in BCAAs and also other amino acids, such as alanine (28) and leucine (7), or decreases in, for example, L-valine (29) in women with GDM at the time of OGTT. Furthermore, higher levels of leucine and isoleucine have been reported in association with obesity in nonpregnant and pregnant participants (30-32). It is proposed that the by-products of BCAA catabolism may contribute to insulin resistance (30) (e.g., the incomplete oxidation of BCAAs may induce mitochondrial dysfunctions) by altering the balance between anaplerosis and cataplerosis. This imbalance may thus influence the citric acid cycle (21), the capacity of which was shown to be lowered in women with type 2 diabetes (33). Another suggested mechanism associating BCAAs to insulin resistance relates to the capability of BCAAs to activate mammalian target of rapamycin complex 1, which by phosphorylation of insulin receptor substrate 1, interferes with insulin signaling (21). Low-grade inflammation, particularly that indicated by the novel inflammatory marker GlycA, has been associated with BMI, severe metabolic complications such as type 2 diabetes, and cardiovascular diseases (17,18,34). In our study, as in previous studies (3,4,5) the concentration of GlycA was higher in the women with GDM, suggesting an involvement of low-grade inflammation in the pathophysiology of GDM. It is concerning that GlycA remained elevated in women with GDM regardless of the treatment and that it was even higher in women treated with medication, i.e., the women with the most severe disease profile.

A previous study in pregnant women (Hyperglycemia and Adverse Pregnancy Outcome Study) reported a relationship between maternal BMI and lipids and amino acids, including BCAAs. Furthermore, BCAAs and their metabolites were associated with insulin resistance (31). In another study with the same study population, several associations between metabolites and glucose were detected, including lipids and amino acids and their metabolites. In this study, the association between serum glucose and the BCAAs leucine and isoleucine was attenuated when adjusted for BMI (32). In our study, even after adjusting for prepregnancy BMI, higher concentrations of BCAAs were detected in women with GDM. In 734 women with GDM from different geographical locations, similar associations between GDM and lipid profile including VLDL lipoproteins were reported (4). Furthermore, a positive association between GDM and GlycA was detected (4). By utilizing a metabolomics approach, we could obtain a comprehensive metabolic profile, and it was evident that women with GDM had an aberrant metabolic profile, which was even more pronounced in those women treated with medication. There was no difference in serum glucose concentrations between the treatment modes, but based on our findings, those women who were treated with medication had a higher metabolic burden, and it would be predicted that they carry a higher risk for metabolic complications, even in the long term. Here, the women with medication consumed insulin and/or metformin. While insulin is an anabolic hormone, i.e., it stimulates energy storage by increasing glucose uptake and triglyceride

synthesis, several mechanisms of how metformin improves both glucose and lipid metabolism have been reported, including increased insulin receptor activity, suppression of hepatic gluconeogenesis, reduction of hypertriglyceridemia, increase in clearance of VLDL particles, and inhibition of lipolysis. The molecular mechanism underlying these effects has been associated with the activation of adenosine monophosphate-activated protein kinase (35,36). Because of these partly different modes of action, the treatment mode of these therapies may have a distinct influence on the metabolomic profile and this needs further evaluation.

As GDM has been consistently linked to adverse postpartum metabolic conditions, new means should be devised focusing on the metabolic profile. For example, in a recent study in women with GDM, 19% had type 2 diabetes and 19% had impaired glucose tolerance 6 years postpartum (37). The well-known Hyperglycemia and Adverse Pregnancy Outcome Study reported that about 52% of the women with GDM compared with 20% of women without GDM developed a disorder in glucose metabolism during the median follow-up of 11.4 years (38). A large cohort study including 10,234 women with GDM with long-term follow-up data up to 19 years postpartum reported a higher risk for dyslipidemia in women with GDM compared with those without (out of a total of 160,527 women) (39). Furthermore, the levels of BCAAs have been shown to associate with insulin resistance (21), elevated levels of VLDL and lower HDL with the risk for cardiovascular diseases (19,20), and that of GlycA with several metabolic aberrations (17,18). Because these variables have been linked with multiple metabolic complications, their potential as diagnostic tools should be evaluated.

The strength of our study lies in the high number of study participants and the timing of the sample withdrawal; i.e., these analyses conducted in late pregnancy provide novel information on the extent of the metabolic aberrations taking place regardless of GDM treatment. We also controlled for the possible role of dietary intake in the metabolomics results by including intakes of energy as well as fat and protein as a proportion of the energy intake as possible confounding factors in the analysis. One limitation is that we have no data on the postpartum metabolic profile (i.e., if the metabolic profile remained disturbed, thus increasing the postpartum risk for metabolic complications); this topic should be elucidated in further studies. Furthermore, there may be differences because of the actual form of the medical treatment (i.e., insulin vs. metformin) (40).

Conclusion

In women with overweight or obesity, a distinct metabolic profile was identified between women with GDM and women without GDM. The profile was characterized by higher levels of several VLDL-related particles, BCAAs, and GlycA. The adverse metabolic profile, particularly in relation to VLDL particles and low-grade inflammation, was more pronounced in those women treated with medication compared with those undertaking only dietary changes. Our findings provide important insights into the metabolism of women with overweight or obesity. Increased metabolic burden of women with GDM emphasizes the need for improved GDM treatment approaches, particularly those targeting lipids and amino acids. Furthemore, a more intensive follow-up of these women postpartum is needed to prevent the potential complications of these metabolic disturbances.O

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Author contributions: KL and KM designed the study; KL organized the data collection; KM and TV conducted the statistical analyses; KM analyzed the data; KM and KL interpreted the data and wrote the manuscript. NH and EK contributed to data collection and revised the manuscript. KL is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Supporting information: Additional Supporting Information may be found in the online version of this article.

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