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Prediction of pre-diabetes and type 2 diabetes nine years postpartum using serum metabolome in pregnant women with gestational diabetes requiring pharmacological treatment

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ABSTRACT

Aims: We examined the association between serum metabolome in women with pharmacologically treated gestational diabetes (GDM) and measures of glucose metabolism 9 years postpartum.
Methods: Serum targeted metabolome, adiponectin, inflammatory markers, and insulin-like growth factor-binding protein-1 phosphoisoforms were analyzed at the time of diagnosing GDM. Glucose metabolism and insulin resistance were assessed at 9 years postpartum. Data from 119 subjects were available for analyses. Associations between baseline measures and future measures of glycemia were examined with univariate regressions and multivariate prediction models. This is a secondary analysis of a previous prospective trial (NCT02417090).
Results: Baseline serum markers were most strongly related to measures of insulin resistance at 9-years follow-up. In multivariate analyses combination of IDL cholesterol, early gestational weight gain and in oral glucose tolerance test fasting and 2-h glucose predicted development of disorders of glucose metabolism (pre-diabetes and/or type 2 diabetes) better than clinical predictors alone (ROC-AUC 0.75 vs. 0.65, $p = 0.020$).
Conclusions: Serum metabolome in pregnancy in women with GDM is related to future glucose metabolism and insulin resistance. Compared to clinical variables alone metabolome might result in better prediction of future disorders of glucose metabolism and could facilitate personalized risk stratification for postpartum interventions and follow-up.

1. Introduction

Gestational diabetes (GDM) is a common disorder of pregnancy, and besides the association to short-term perinatal complications GDM is a known risk-factor for future type 2 diabetes (T2DM) in the mother.[1]

The risk of progression from GDM to T2DM after delivery may be

Abbreviations: BMI, body mass index; CI, confidence intervals; ELISA, enzyme-linked immunosorbent assay; FA, fatty acids; GDM, gestational diabetes; GlycA, glycoprotein acetyls; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA(2)-IR, homeostasis model assessment of insulin resistance (2); hsCRP, high-sensitivity C-reactive protein; IDL, intermediate-density lipoprotein; IGFBP-1, insulin-like growth factor-binding protein-1; IL-6, interleukin-6; LA, linoleic acid; LDL, low-density lipoprotein; MUFA, monounsaturated fatty acids; NMR, nuclear magnetic resonance; OGTT, oral glucose tolerance test; pre-DM, pre-diabetes; PUFA, polyunsaturated fatty acids; ROC-AUC, area under receiver operating characteristics curve; T2DM, type 2 diabetes; TG, triglycerides; VLDL, very-low-density lipoproteins.

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mitigated by interventions[2] that could preferably be focused for those at the greatest risk, based on accurate risk stratification. In non-pregnant population metabolomics have improved the prediction of future T2DM,[3] and similarly the accuracy of metabolome analyzed postpartum in predicting progression from GDM to T2DM exceeds more conventional risk factors.[4,5]

Predicting future morbidity with serum samples collected in pregnancy rather than postpartum, limits the possibility for the loss-of follow-up at postpartum visits. But also the physiologic insulin resistance in pregnancy could bring up metabolic features[6] that serve as predictive markers for impaired glucose metabolism in the future. The prediction of postpartum pre-diabetes (pre-DM) and/or T2DM using metabolome analyzed during pregnancy has been evaluated in only three recent studies in 2017,[7] 2021,[8] and 2023.[9] In two of them 3-hydroxybutyrate was found to predict future disorders of glucose metabolism.[7,8] However, in the first of these studies only 24 subjects with GDM were included and the follow-up was limited to three months postpartum.[7] In the second study, although large (n = 2290), GDM diagnosis was made only retrospectively in some of the participants.[8] In the most recent study a NMR metabolome was analyzed in pregnancy, and diagnosis of pre-diabetes was based on only fasting glucose at two years postpartum.[9] The authors found 3-hydroxybutyrate to be correlated with postpartum fasting glucose, but lipids in small high-density lipoprotein (HDL) particles were the best predictors of pre-diabetes.[9]

Besides metabolomics, markers of low-grade inflammation, insulin-like growth factor-binding proteins (IGFBP), and adiponectin have been related to persistent glycemia postpartum,[10–12] and could hence serve as predictors of progression into T2DM.

Metabolomic derangements precede the development of GDM,[13–15] and might be causally related to the pathogenesis. Strong correlations between maternal metabolome and insulin resistance exists,[6,16] and the same metabolites, e.g. increased branched-chain amino acids and triglycerides (TG), predict future T2DM.[17] Similarly low-grade inflammation, possibly driven by obesity,[18] has been associated with both GDM[19,20] and T2DM.[21] Adiponectin and IGFBP are additional circulating markers closely related to glucose homeostasis and altered in GDM,[10,22,23] and they have been found to predict T2DM.[10,11,24,25]

GDM is a heterogeneous disease[26] and therefore we examined a variety of factors known to be associated with glycemia, including amino acids,[7,8] lipids,[7,8] markers of low-grade inflammation,[12] IGFBP,[11] and adiponectin,[10] to evaluate their ability to predict disorders of glucose metabolism postpartum. With the ability to predict future morbidity already during pregnancy, more intense lifestyle interventions[2] could be focused on the high-risk individuals, and the follow-up of the low-risk individuals could be less strict. Another benefit of risk assessment already before delivery, would be the independency from postpartum visit, which some women choose not to attend.

In this longitudinal study, we examined the associations between serum metabolome assessed by nuclear magnetic resonance (NMR) spectroscopy, inflammatory markers, IGFBP-1, and adiponectin at the time of GDM diagnosis and measures of glucose metabolism up to 9 years postpartum in GDM patients requiring antihyperglycemic drug treatment. Furthermore, we studied the potential of serum metabolome measured in pregnancy, to predict disorders of glucose metabolism, i.e., pre-DM or T2DM in the future.

2. Material and methods

2.1. Study participants

This is a secondary analysis of a previous follow-up[27] of randomized trial.[28] The study design and outcomes have been reported previously.[27,28] Briefly, women with a singleton pregnancy and newly diagnosed GDM were included in a previous randomized trial comparing metformin and insulin treatments.[28] GDM diagnosis was based on at least two abnormal values in a 2-h 75 g oral glucose tolerance test (OGTT). Diagnostic cut-off values were fasting ≥4.8 mmol/l, 1-h ≥10.0 mmol/l, and 2-h ≥8.7 mmol/l, until the release of Finnish national guidelines 2008 and ≥5.3 mmol/l, ≥10.0 mmol/l, and ≥8.6 mmol/l thereafter. All the participants had recurrent hyperglycemia (fasting ≥5.5 and/or 1-h postprandial ≥7.8 mmol/l) in at-home glucose monitoring despite diet and lifestyle counselling and received pharmacological antihyperglycemic treatment. The participants were randomized to receive metformin (n = 110) or insulin (n = 107) treatment, and the intervention ended at delivery. The original trial was powered to prove non-inferiority of the primary outcome birth weight between the treatment groups. There were also no differences between the clinical baseline characteristics or other perinatal outcomes (incl. weight gain, gestational age at delivery, macrosomia, and neonatal hypoglycemia) between the treatment groups.[28]

Fasting serum samples were drawn at the time of recruitment (mean 30 gestational weeks). Glycated hemoglobin (HbA1c) and C-peptide were measured using routine laboratory methods, and additional samples were stored at −70 °C.

2.2. Postpartum follow-up

The subjects had a clinical evaluation at 6–8 weeks and 1 year after delivery with measurements of weight, HbA1c and an OGTT. Thereafter, no follow-up was organized between 1 and 9 years postpartum on behalf of this study.

The women were then asked to participate the follow-up study at 9 years postpartum.[27] At this follow-up visit the weight and body mass index (BMI) were recorded, HbA1c was measured and 2-h OGTT was performed. Additionally, fasting plasma C-peptide and insulin were measured.[27] Insulin resistance was estimated using homeostasis model assessment of insulin resistance 2 (HOMA2-IR)[29] with C-peptide as recommended.[30] HbA1c, OGTT glucose values, insulin, and C-peptide were not available at follow-up in women with T2DM diagnosed and treated prior to follow-up visit.

Pre-DM was defined as having either impaired fasting glucose (≥6.1 mmol/l), impaired glucose tolerance (OGTT 2-h glucose ≥7.8 mmol/l), and/or elevated HbA1c (42–48 mmol/mol [6.0–6.5 %]). T2DM was defined as either pre-diagnosed prior to the 9-years follow-up, or fasting glucose ≥7.0 mmol/l, OGTT 2-h glucose >11.0 mmol/l or HbA1c ≥48 mmol/mol (6.5 %) at the follow-up.

The study participants signed an informed consent. Both the original trial and the follow-up study were approved by the ethics committee of the Hospital District of Southwest Finland and are registered at ClinicalTrials.gov (NCT01240785 and NCT02417090).

2.3. Analysis of baseline serum markers

The following analyses were performed using the samples collected at recruitment and stored at −70 °C. A targeted metabolome including amino acids, fatty acids (FA), lipoproteins, phospholipids, glycolysis-related metabolites, and ketones (142 metabolites in total, Supplementary Table 1) was analyzed using high-throughput ^{1}H NMR spectroscopy (Nightingale Health, Helsinki, Finland).[31] This NMR method was chosen due to it being scalable and reproducible.[31] Several prior publications have applied this method in describing metabolomic alterations in GDM[14,15] and metabolomic derangements preceding T2DM.[17,32] High-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), and adiponectin were measured using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions [human C-reactive protein (CRP) ELISA kit; interleukin-6 (IL-6) ELISA kit; Quantikine ELISA Human Total Adiponectin/ACRP30, R&D Systems, Minneapolis, USA]. Non-phosphorylated IGFBP-1 (non-pIGFBP-1), low-pIGFBP-1, and high-pIGFBP-1 were analyzed by ELISA as described previously.[33] Glycoprotein acetyls (GlycA) was measured along the targeted NMR metabolome.[31]

2.4. Statistical analysis

Those women who were diagnosed with autoimmune diabetes, did not have baseline serum sample for metabolome analysis, had undergone bariatric surgery prior to follow-up, or did not attend the follow-up visit were excluded from all analyses.

Descriptive statistics of the clinical characteristics at baseline and at the 9-years follow-up were calculated. The clinical characteristics of the participants who had pre-DM or T2DM at the 9-years follow-up were compared to those of normoglycemic participants using the unpaired *t*-test, the Mann-Whitney *U* test, the χ^2 test, or the Fisher's exact test. *P*-value below 0.05 was considered statistically significant. The participants with T2DM diagnosed prior to follow-up visit were excluded from the comparison of OGTT, HbA1c, insulin, C-peptide, HOMA2-IR, IFG, IGT, and elevated HbA1c at follow-up.

2.4.1. Univariate analyses

The associations between individual serum metabolites at baseline and outcome variables (OGTT fasting and 2-h glucose values, HbA1c, fasting C-peptide, fasting insulin, and HOMA2-IR) at 9-years follow-up were studied with linear regression. Regression analyses were run both unadjusted and adjusted for a priori selected confounding factors as follows: weight gain (from first antenatal visit to 9-years follow-up), age, nulliparity (at baseline), and BMI category (normal weight: <25 kg/m^2, overweight 25–30 kg/m^2, or obese ≥30 kg/m^2) according to BMI at the first antenatal visit (pre-pregnancy BMI). Continuous variables were centered and scaled prior to analyses and the outcome variables with skewed distribution, assessed by histograms, were log-transformed. To account for multiple testing in highly intercorrelated metabolome data an alpha threshold of was 0.01 selected.

The women who had been diagnosed with T2DM prior to follow-up did not undergo OGTT and were thus excluded from these univariate analyses (Fig. 1), (but were included in the subsequent multivariate prediction analysis).

2.4.2. Prediction of future pre-DM or T2DM at 9 years postpartum

The aim of this analysis was to evaluate the potential of combination of serum biomarkers and clinical variables to predict disorders of glucose metabolism 9 years after the index pregnancy. The following clinical predictors were initially included: OGTT fasting, 1-h and 2-h glucose, HbA1c, fasting C-peptide, HOMA2-IR, and age at baseline, gestational weeks at OGTT, pre-pregnancy BMI, and early gestational weight gain (weight gain preceding baseline serum sampling). The binary outcome was presence or absence of pre-diabetes or type 2 diabetes.

First, univariate associations between clinical predictors, serum biomarkers (including NMR metabolome, inflammatory markers, IGFBP-1 phosphoisoforms, and adiponectin) and the binary outcome was assessed by logistic regression. All predictors were centered and scaled. Clinical variables with $p < 0.1$ in the univariate analysis were included in the multivariate models. Of all serum biomarker predictors, those with the lowest *p*-values (within the 5th centile) in univariate

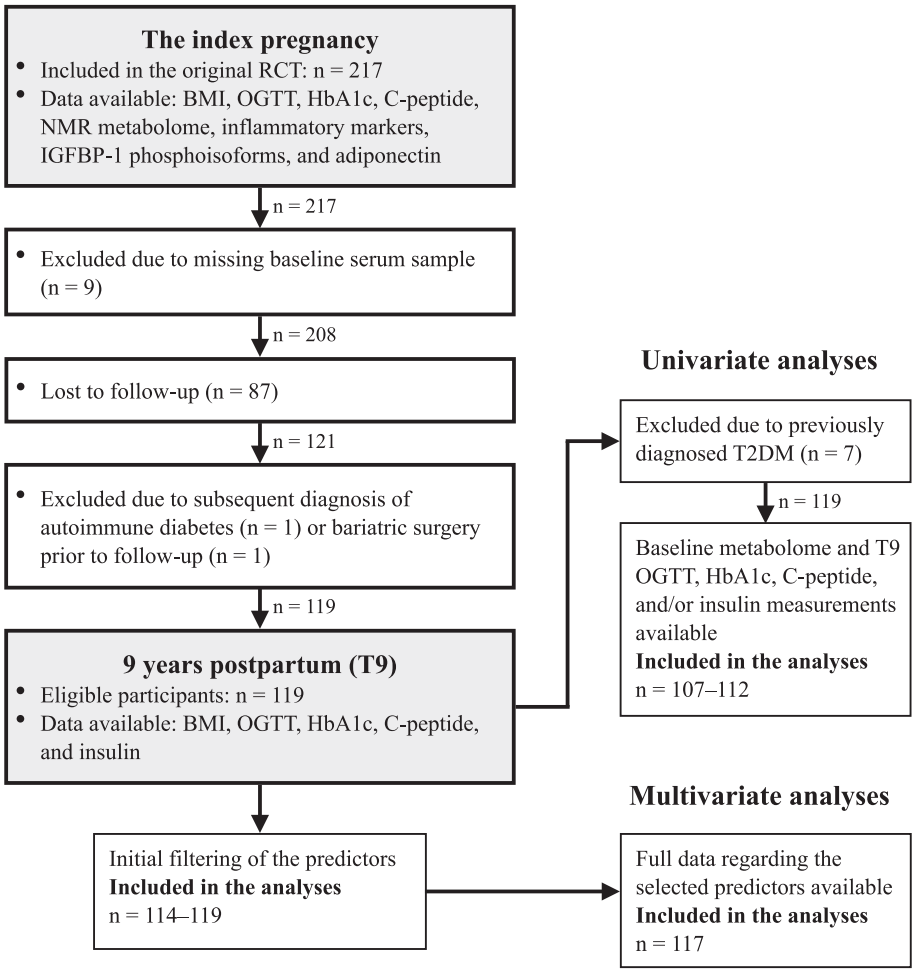

Fig. 1. Study flowchart.
BMI: body-mass index, HbA1c: glycated hemoglobin, IGFBP-1: insulin-like growth factor-binding protein-1, NMR: nuclear magnetic resonance, OGTT: oral glucose tolerance test, RCT: randomized controlled trial.

analyses were first included, and subsequentially the most intercorrelated variables (cut-off 0.99) were filtered out. Multivariate regression models were then built with stepwise selection based on Akaike information criterion using R-packages *caret* (version 6.0–86, https://CRAN.R-project.org/package=caret) and *MASS*.[34] Two predictions models were built with the following sets of predictors: 1) clinical variables only and 2) clinical variables and serum biomarker predictors together. Performance of the final models was evaluated by area under receiver operating characteristics curve (ROC-AUC), and the ROC-AUC values were compared between the two models by the deLong method using R-package *pROC*.[35]

2.4.3. Additional analyses

To address possible selection bias due to loss to follow-up, we compared the baseline clinical characteristics between the subjects included in the analyses vs. those excluded for any reason. As the participants were randomized to metformin and insulin treatments in the index pregnancy, we also compared the baseline clinical characteristics of the subjects included in the present study between the two treatment groups.

All analyses were performed in R statistical software (version 4.0.3).

3. Results

Two-hundred-seventeen subjects were randomized at baseline, of whom nine had baseline serum sample missing and 87 (40.1 %) were lost to follow-up. One subject was excluded due to subsequently diagnosed autoimmune diabetes and one due to bariatric surgery prior to follow-up, leaving 119 (54.8 %) eligible subjects at 9-years follow-up (Fig. 1). Seven women with T2DM diagnosed prior to 9-years follow-up were further excluded from the univariate analyses. Due to few missing values in some of the predictors and covariates 102–112 (47.0–51.6 %) subjects were included in the univariate analyses. One-hundred-nineteen (54.8 %) participants were eligible for multivariate analyses of which 117 (53.9 %) had complete baseline data regarding the selected predictors and were included in the multivariate prediction analysis.

The subjects included in this present study were older at baseline (33.0 vs. 30.9 years, $p = 0.0029$) and had lower HbA1c at the time of GDM diagnosis (35.9 vs. 37.2 mmol/mol [5.4 vs. 5.6 %], $p = 0.010$) compared to those excluded for any reason. The groups had otherwise similar baseline characteristics ($p > 0.05$) (Supplementary Table 1). The clinical characteristics were similar ($p > 0.05$) between the subjects randomized to metformin vs. insulin in the index pregnancy (Supplementary Table 2).

Descriptive statistics for the study population are given alongside the comparison of study participants who developed or did not develop pre-DM or T2DM during the 9-years follow-up (Table 1). At 9 years 12.6 % (15/119) of the participants had T2DM and 42 % (50/119) had pre-DM or T2DM. Compared to the subjects who remained normoglycemic at 9

Table 1
Study population characteristics.

	Total	n	Pre-DM or T2DM at 9-years follow-up	n	NGT at 9-years follow-up	n	*p*-value
Baseline							
Age (years)	33.0 ± 5.0	119	33.3 ± 5.1	50	32.7 ± 4.9	69	0.51
pBMI	28.7 ± 5.2	119	29.3 ± 5.1	50	28.3 ± 5.2	69	0.25 [a]
Smoking (n)	12 (10.3)	117	6 (12.5)	48	6 (8.7)	69	0.72
Nulliparous (n)	46 (38.7)	119	20 (40.0)	50	26 (37.7)	69	0.95
Early GWG (kg)	6.0 ± 3.8	119	5.1 ± 3.6	50	6.6 ± 3.8	69	**0.034**
GWG (kg)	8.1 ± 4.8	119	7.2 ± 4.4	50	8.8 ± 5.1	69	0.076
Metformin treatment (n)	59 (49.6)	119	21 (42.0)	50	38 (55.1)	69	0.22
Gw at OGTT (wk)	26.9 ± 2.4	119	26.5 ± 2.5	50	27.2 ± 2.3	69	**0.0093** [a]
Gw at randomisation (wk)	30.4 ± 1.7	119	30.2 ± 1.5	50	30.6 ± 1.9	69	0.096 [a]
OGTT fasting glucose (mmol/l)	5.5 ± 0.5	119	5.6 ± 0.5	50	5.4 ± 0.5	69	0.057
OGTT 1-h glucose (mmol/l)	11.2 ± 1.4	119	11.3 ± 1.7	50	11.1 ± 1.1	69	0.23 [a]
OGTT 2-h glucose (mmol/l)	8.1 ± 1.8	117	8.5 ± 1.8	48	7.9 ± 1.8	69	0.079
Fasting C-peptide (nmol/l)	1.05 ± 0.32	115	1.06 ± 0.32	50	1.04 ± 0.32	65	0.77 [a]
HOMA2-IR	2.3 ± 0.8	114	2.4 ± 0.8	50	2.3 ± 0.7	64	0.62 [a]
HbA1c% at GDM diagnosis	5.4 ± 0.3	119	5.5 ± 0.3	50	5.4 ± 0.3	69	0.14
HbA1c at GDM diagnosis (mmol/mmol)	35.9 ± 3.6		36.5 ± 3.4		35.5 ± 3.7		
9 years postpartum							
Age (years)	42.5 ± 5.0	119	42.9 ± 5.1	50	42.2 ± 4.9	69	0.43
BMI (kg/m2)	31.0 ± 5.8	118	32.8 ± 5.6	49	29.8 ± 5.7	69	**0.0069**
Weight (kg)	84.7 ± 16.6	118	89.8 ± 17.8	49	81.1 ± 14.7	69	**0.0042**
ΔWeight from baseline (kg)	4.4 ± 7.7	114	7.5 ± 7.4	47	2.2 ± 7.2	67	**0.00022**
Smoking (n)	15 (12.6)	119	7 (14.0)	50	8 (11.6)	69	0.91
OGTT fasting glucose (mmol/l) [c]	5.7 ± 0.7	112	6.3 ± 0.8	43	5.4 ± 0.4	69	**< 0.00001** [a]
OGTT 2-h glucose (mmol/l) [c]	6.3 ± 2.0	112	7.8 ± 2.4	43	5.4 ± 1.0	69	**< 0.00001** [a]
HbA1c% [c]	5.6 ± 0.4	111	5.9 ± 0.5	42	5.4 ± 0.2	69	**< 0.00001** [a]
HbA1c (mmol/mmol) [c]	38.5 ± 4.4		41.5 ± 4.9		36.7 ± 2.7		
Fasting insulin (mU/l) [c]	13.8 ± 8.6	112	17.2 ± 9.1	43	11.7 ± 7.6	69	**0.00003** [a]
Fasting C-peptide (nmol/l) [c]	0.86 ± 0.33	112	1.03 ± 0.36	43	0.75 ± 0.26	69	**0.00001** [a]
HOMA2-IR [c]	2.0 ± 0.8	112	2.5 ± 0.9	43	1.7 ± 0.6	69	**< 0.00001** [a]
IFG (n) [c]	30 (26.8)	112	30 (69.8)	43	0 (0)	69	NA
IGT (n) [c]	22 (19.6)	112	22 (51.2)	43	0 (0)	69	NA
Elevated HbA1c (n) [c]	20 (18.0)	111	20 (47.6)	42	0 (0)	69	NA
T2DM (n)	15 (12.6)	119	15 (30)	50	0 (0)	69	NA
pre-DM or T2DM (n)	50 (42.0)	119	50 (100)	50	0 (0)	69	NA

Data given as mean ± SD or n (%) for the whole study population (Total; participants included in any univariate or multivariate analysis) and for the participants with known glucose metabolism status at 9-years follow-up (pre-diabetes [pre-DM] or type 2 diabetes [T2DM] vs. normal glucose tolerance [NGT]). P-values are given for the t-test, the Mann-Whitney U test (a), the χ^2 test, or the Fisher's exact test (b), for comparison between the pre-DM or type 2 diabetes T2DM, and NGT groups. P-values below 0.05 are bolded. At 9-years follow-up the participants with previously diagnosed and treated T2DM are excluded from the analysis of glucose, HbA1c, C-peptide, Insulin, and HOMA2-IR (c). Elevated HbA1c was defined as above 42 mmol/mol. (p)BMI: (pre-pregnancy) body mass index, GDM: gestational diabetes, Gw: gestational weeks, GWG: gestational weight gain, HbA1c: glycated hemoglobin, HOMA2-IR: homeostasis model assessment of insulin resistance 2, IFG: impaired fasting glucose (≥6.1 mmol/l), IGT: impaired glucose tolerance (OGTT 2-h glucose ≥7.8 mmol/l), OGTT: oral glucose tolerance test, wk.: weeks, NA: not applicable.

years those who developed disorders of glucose metabolism had GDM diagnosed earlier (26.5 vs. 27.2 weeks, $p = 0.0093$) and lower weight gain preceding initiation of pharmacologic treatment (early GWG; 5.1 vs. 6.6 kg, $p = 0.034$). The subjects with disorders of glucose metabolism at 9 years had higher fasting insulin, C-peptide, HOMA2-IR, BMI, and weight, and 3-fold higher weight gain from baseline to 9-years follow-up compared to those with normal glucose tolerance (Table 1).

3.1. Univariate associations

Associations between baseline metabolites and continuous outcome variables at 9 years (OGTT fasting and 2-h glucose, HbA1c, HOMA2-IR,

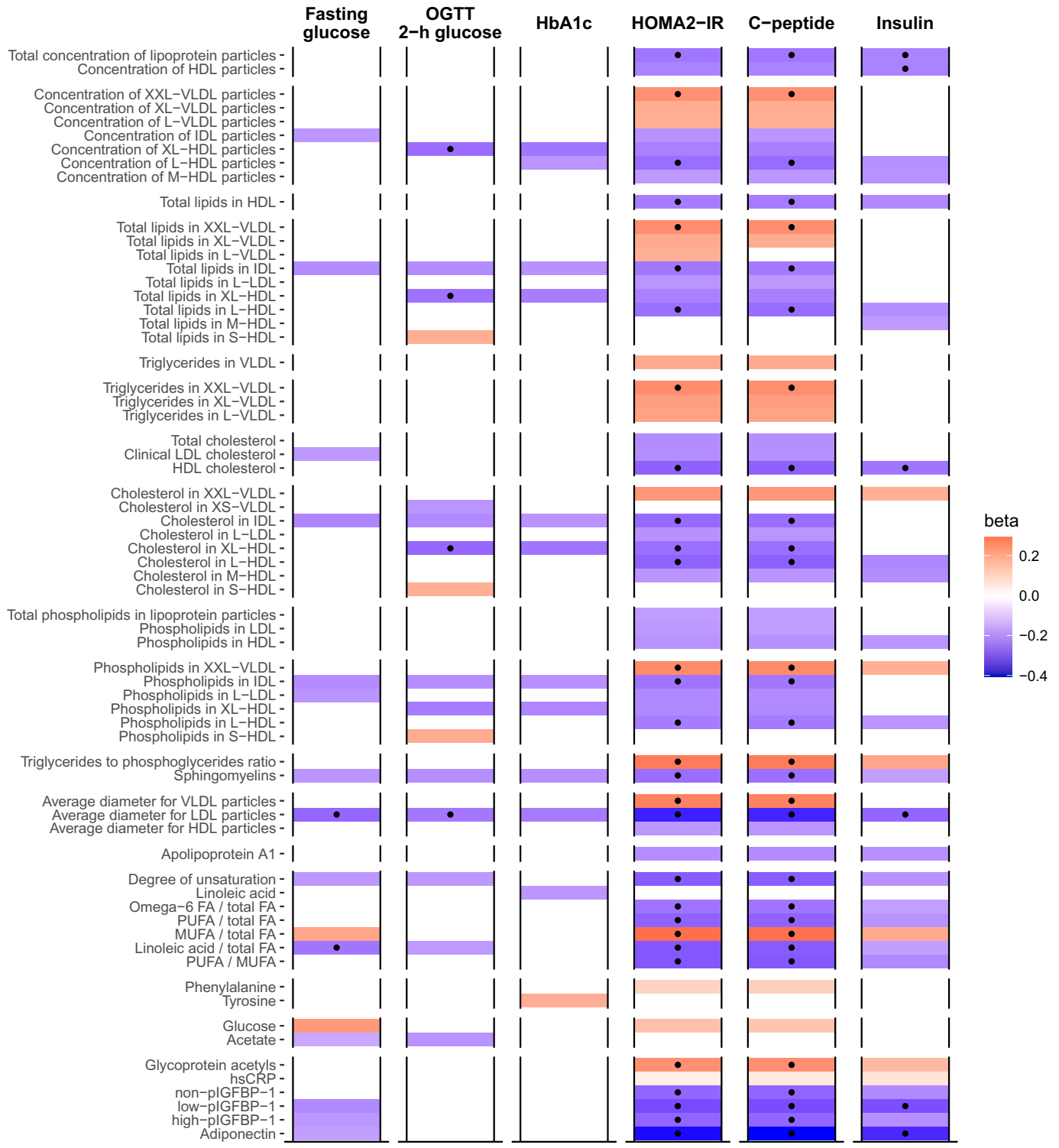

Fig. 2. Associations between biomarkers in pregnancy and glucose metabolism at 9 years postpartum.
Regression coefficient values (beta) are given for adjusted models. Associations with a borderline-significant ($p < 0.05$) at any time point (unadjusted analyses included) are presented and $p < 0.01$ are denoted with a dot (●). HbA1c: glycated hemoglobin, HOMA2-IR: homeostasis model assessment of insulin resistance 2, VLDL: very-low-density lipoprotein, LDL: low-density lipoprotein, IDL: intermediate-density lipoprotein, HDL: high-density lipoprotein. FA: fatty acids, MUFA: monounsaturated FA, PUFA: polyunsaturated FA, non/low/high-pIGFBP-1: non/low/highly-phosphorylated insulin-like growth factor-binding protein-1.

fasting C-peptide, and fasting insulin) are represented in Fig. 2. The adjusted and unadjusted associations are reported in detail in Supplementary Table 3.

3.1.1. Lipoproteins

Total lipoprotein particle concentration was inversely related to HOMA2-IR, C-peptide, and insulin.

TG, phospholipids and total lipids in extremely large (XXL) very-low-density lipoprotein (VLDL), XXL-VLDL particle concentration, and VLDL-size were positively related to HOMA2-IR and C-peptide. Low-density lipoprotein (LDL)-size had a strong inverse association with HOMA2-IR ($p < 0.0001$), C-peptide ($p < 0.0001$), and insulin ($p < 0.01$). Moreover, LDL-size was inversely related to fasting glucose and OGTT 2-h glucose.

Cholesterol, phospholipids, and total lipids in intermediate-density lipoprotein (IDL) were inversely related to HOMA2-IR and C-peptide.

HDL cholesterol and particle concentration were inversely predictive of insulin, and HDL total lipids and HDL cholesterol predicted HOMA2-IR and C-peptide. Cholesterol and total lipids in very large (XL) HDL, and XL-HDL particle concentration were inversely related to OGTT 2-h glucose. XL-HDL cholesterol was also inversely related to HOMA2-IR and C-peptide. In large HDL, particle concentration, total lipids, phospholipids, and especially cholesterol, were inversely related to HOMA2-IR and C-peptide.

3.1.2. Fatty acids and phospholipids

Proportion of linoleic acid (LA) of total FA was inversely related to fasting glucose, HOMA2-IR, and C-peptide. Similarly, the omega-6-to-total-FA ratio was inversely related to HOMA2-IR and C-peptide.

FA unsaturation, polyunsaturated FA (PUFA)-to-total-FA and monounsaturated FA (MUFA)-to-PUFA ratios were inversely, and the MUFA-to-total-FA ratio positively related to HOMA2-IR and C-peptide.

TG-to-phosphoglycerides ratio was positively and sphingomyelins inversely associated with HOMA2-IR and C-peptide at.

3.1.3. Adiponectin, IGFBP-1, and inflammatory markers

Adiponectin was the strongest predictor of HOMA2-IR, C-peptide, and insulin with strong inverse associations ($p < 0.0001$). High-pIGFBP-1 had strong inverse associations with HOMA2-IR ($p < 0.0001$), C-peptide ($p < 0.0001$), and insulin ($p < 0.001$) in the unadjusted models, but after adjusting for confounding factors this association was significant only in HOMA2-IR and C-peptide ($p < 0.01$) (Supplementary Table 3). In contrast, the inverse associations between low-pIGFBP-1 and HOMA2-IR, C-peptide, and insulin and between non-pIGFBP-1 and HOMA2-IR, and C-peptide were essentially unaffected by the adjustments. GlycA was associated only with HOMA2-IR and C-peptide. hsCRP and IL-6 were not significantly related to any outcome variables.

Table 2
Multivariate prediction models.

Predictors	Beta-coefficient	p-value	Model ROC-AUC
Model 1 (Clinical)			0.65 [0.55; 0.75]
OGTT fasting glucose	0.33 [−0.05; 0.75]	0.10	
OGTT 2-h glucose	0.34 [−0.05; 0.76]	0.092	
Early GWG	−0.41 [−0.82; −0.02]	0.044	
Model 2 (Clinical + NMR)			0.75 [0.65; 0.84]
IDL cholesterol	−0.70 [−1.19; −0.26]	0.0028	
OGTT fasting glucose	0.39 [−0.02; 0.84]	0.074	
OGTT 2-h glucose	0.38 [−0.03; 0.81]	0.071	
Early GWG	−0.35 [−0.79; 0.06]	0.10	

Standardized regression beta-estimates [95 % confidence intervals] for baseline predictors of pre-diabetes and/or type 2 diabetes at 9-years follow-up. GWG: gestational weight gain, IDL: intermediate-density lipoprotein, OGTT: oral glucose tolerance test, ROC-AUC: area under receiver operating characteristics curve.

3.2. Prediction models

Univariate associations between clinical and serum biomarker predictors and disorders of glucose metabolism are given in detail in Supplementary Table 4. After initial selection based on univariate associations and filtering of highly intercorrelated metabolites early GWG, OGTT fasting and 2-h glucose, IDL cholesterol, IDL particle concentration, clinical LDL cholesterol and phospholipids in small LDL were fed into the stepwise regression model. Consequently, OGTT fasting and 2-h glucose, and lower early GWG were the predictors included in the "Clinical" multivariate model, and OGTT fasting glucose, 2-h glucose, (lower) early GWG, and (lower) IDL cholesterol were included in the second ("Clinical + NMR") multivariate model (Table 2). The Clinical + NMR model had higher ROC-AUC compared to the Clinical model (0.75 vs. 0.65, $p = 0.020$) (Fig. 3).

4. Discussion

4.1. Main results

We found several components of maternal serum metabolome at the time of GDM diagnosis to be associated with long-term glucose metabolism. Compared to clinical variables, the addition of serum IDL cholesterol yielded a better prediction of future disorders of glucose metabolism.

Pregnancy is characterized by a physiologic increase in insulin resistance that is further augmented in GDM. Lipoprotein metabolism is fundamentally controlled by insulin and consequently affected by insulin resistance. Accordingly, we found several features of maternal metabolome to reflect future glycemia and especially insulin resistance. Yet, in agreement with a recent study by Liu et al. (2021)[8] significant associations between metabolites in pregnancy and follow-up fasting glucose were scarce. In our study the associations with HOMA2-IR and C-peptide that reflect glucose homeostasis during a wider timeframe, were stronger than with OGTT fasting or 2-h glucose that may be more prone to day-to-day variation. Also, associations to HOMA2-IR and C-peptide were stronger compared to fasting insulin, which might reflect the physiologic pulsatile insulin secretion compared to more stable C-peptide.

Particularly, XL-HDL lipids and LDL-size were associated with OGTT 2-h glucose, and adiponectin, IGFBP-1 phosphoisoforms, FA unsaturation, proportions of MUFA, PUFA and LA, XXL-VLDL lipids, LDL-size, and L-HDL lipids were associated with HOMA2-IR and C-peptide.

Our data demonstrate that prediction of long-term morbidity already during pregnancy is possible, and the accuracy can be improved by implementation of serum metabolomics analysis. Furthermore, as serum lipids, including increased proportion of MUFA and decreased PUFA and degree of FA saturation, were related to future insulin resistance, lifestyle interventions aiming to ameliorate these metabolic alterations could yield beneficial effects in high-risk individuals. Previous trials from 2019 and 2021 have shown that lifestyle interventions[36] and fish-oil supplementation[37] in pregnant women has been related to decrease in VLDL TG[36] and overall improved FA profile,[37] which might translate into improved maternal health in long term.

4.2. Amino acids

In the large HAPO follow-up study from 2021[8] there was a clear positive association between several amino acids, leucine/isoleucine, valine, and phenylalanine, in pregnancy and long-term glucose metabolism. However, we found none of the amino acids to be significantly related to the long-term outcomes. The different findings could be related to differences in study populations: all the subjects in our study had GDM requiring antihyperglycemic medication, whereas in the HAPO population none of the subjects had GDM diagnosis.[8]

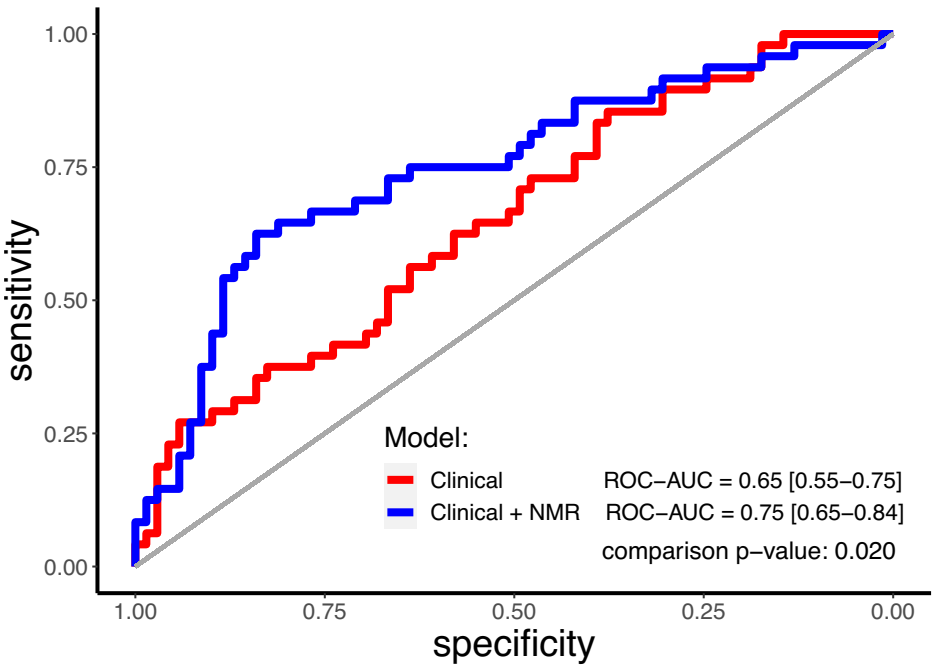

Fig. 3. Prediction models.
The clinical model included oral glucose tolerance test (OGTT) fasting and 2-h glucose at the time of gestational diabetes diagnosis in the index pregnancy and early gestational weight gain (early GWG). The Clinical + NMR model included OGTT fasting and 2-h glucose, early GWG, and cholesterol in intermediate-density lipoprotein. Area under receiver operating characteristics curve (ROC-AUC) values are given with 95 % confidence intervals, and the *p*-value is given for comparison between the ROC-AUC values using the deLong method.

4.3. Fatty acids and lipoproteins

FA unsaturation, and proportions of PUFA and omega-6 FA of total FA were inversely related to outcome variables whereas the MUFA-to-total-FA ratio showed positive associations. The associations between different FA classes and cardiovascular health in general population are well known,[38] and if not causally related to disorders of glucose metabolism, reflect more unfavorable metabolic health. Although both dietary MUFA and PUFA have a positive effect on glucose metabolism[39] we found a positive association between the serum MUFA-to-total-FA ratio and long-term insulin resistance. This discrepancy could be related to the fact that dietary and circulating MUFA are only partially correlated.[40] Notably, the LA-to-total-FA ratio showed consistent inverse associations with the outcome variables. This is in agreement with two studies from 2017 and 2023 using samples collected during pregnancy[7,9] and large-scale data published 2019 of over 11,000 individuals from general population demonstrating an inverse association between LA and T2DM risk.[32] Taken together, based on our data the serum FA in pregnancy seem to have similar long-term associations to glucose metabolism as described outside pregnancy.[32]

We observed an inverse association between sphingomyelins and insulin resistance at 9 years postpartum, whereas previous studies spanning from 2013 to 2016 and using different analysis methods and postpartum samples have described varying associations between different sphingomyelin species and future T2DM.[3,4,41] A more recent (2019) study utilizing the same NMR-protocol as the present study found negligible associations between sphingomyelins and long-term glucose metabolism in general population.[32]

Total lipoprotein particle concentration was inversely related to HOMA2-IR, C-peptide, and insulin. As HDL is the most abundant lipoprotein, this association likely reflects the amount of HDL particles. In accordance XL-HDL concentration was inversely related to OGTT 2-h glucose and L-HDL concentration to HOMA2-IR and C-peptide.

Total lipids, TG, and phospholipids in XXL-VLDL, and XXL-VLDL particle concentration, as well as TG-to-phospholipids ratio, a surrogate of lipoprotein TG content, were positively related to insulin resistance at 9 years postpartum. The associations between insulin resistance and TG in smaller lipoprotein particles, along total VLDL TG, were, however, not significant. Insulin resistance leads to increased production of VLDL which subsequently undergoes gradual TG depletion by lipoprotein lipase, which's activity is attenuated in insulin resistance. Accordingly, in GDM alike outside pregnancy VLDL size[16,42] and VLDL TG[16,43] are positively related to insulin resistance and seem to predict persistent insulin resistance postpartum.[9] Also in general, TG in VLDL and LDL particles predict long-term glycemia.[32]

IDL cholesterol and phospholipids were inversely related to HOMA2-IR and C-peptide. IDL particles are formed from VLDL particles after TG is removed by lipoprotein lipase. Therefore, as lipoprotein lipase is stimulated by insulin, higher concentration of IDL lipids may reflect better insulin action.[43]

Interestingly, we found LDL-size to be consistently inversely related to long-term outcome variables. Accordingly, incident T2DM is associated with an atherogenic decrease in LDL particle size[44] due to hypertriglyceridemia. Although pregnancy itself and GDM are associated with increased LDL TG,[15,45] LDL-size was increased rather than decreased in GDM.[15] Other changes in LDL composition may therefore underlie the inverse association between LDL-size and long-term glycemia.

Previously outside pregnancy VLDL-size was shown to be positively and HDL-size inversely related to long-term measures of glycemia, insulin resistance, and T2DM.[32] While we found positive significant association between VLDL-size and insulin resistance, the associations between HDL-size and long-term glycemic variables and insulin resistance were non-significant. During pregnancy HDL-size changes more drastically than VLDL-size or LDL-size,[45] and therefore, the pregnancy-induced increase in HDL-size could have attenuated the observed association between HDL-size and long-term outcomes.

Insulin was inversely related to HDL particle concentration and insulin, HOMA2-IR, and C-peptide were inversely related to HDL cholesterol, which increases in normal pregnancy[45] but is lower in GDM.[15] Alike shown in our study, outside pregnancy HDL cholesterol was inversely related to future HOMA-IR, but also to OGTT 2-h glucose, albeit the association was weaker.[32] Additionally, we found a pattern of inverse associations between XL-HDL cholesterol and OGTT 2-h glucose. Further, XL-HDL cholesterol, L-HDL cholesterol, and L-HDL phospholipids associated directly with HOMA2-IR and C-peptide, in agreement with previous literature from non-pregnant population.[32]

4.4. Inflammation, IGFBP-1, and adiponectin

In pregnancy IGFBP-1 is inversely related to C-peptide,[46] HOMA-

IR,[23] and BMI.[46] In the present data the three IGFBP-1 phosphoisoforms did not improve the prediction of disorders of glucose metabolism but were associated with HOMA2-IR and C-peptide, and low-pIGFBP-1 associated also with insulin at 9 years postpartum. In a previous study published in 2016 IGFBP-1 and IGFBP-2 twelve weeks postpartum were lower in the women with GDM who later developed T2DM compared to those who remained euglycemic, although the marginal difference regarding IGFBP-1 did not reach statistical significance.[11] Different phosphoisoforms were not measured in that study.

Adiponectin in late second trimester has been inversely associated with HOMA-IR and fasting glycemia at three months postpartum.[10] We found adiponectin to be the strongest predictor (with inverse association) of HOMA2-IR, C-peptide, and insulin, but not glucose or HbA1c, at 9 years postpartum.

Despite inflammatory markers such as hsCRP have been related to persistence of postpartum hyperglycemia,[12] GlycA was the only inflammatory marker we found to be positively related to HOMA2-IR and C-peptide at 9 years postpartum. These associations were attenuated after adjusting for confounding factors and may thus better reflect the prevailing risk factors[46] than provide additional information regarding long-term metabolic health.

4.5. Prediction analyses

Previous studies from 2015 to 2020 on predicting development of pre-DM and/or T2DM have mainly used samples collected 6–12 weeks after the pregnancy,[4,5,41] while only three studies (from 2017, 2021, and 2023) have analyzed maternal serum or plasma metabolome at mid-pregnancy.[7–9]

In our data, compared to clinical variables alone, addition of IDL cholesterol yielded a better prediction model (ROC-AUC 0.75 vs. 0.65, $p = 0.020$). Notwithstanding the different methodology (mass-spectrometry vs. NMR), two of the previous studies found 3-hydroxybutyrate to predict T2DM and/or pre-DM[7,8] whereas we found no associations between any of the outcome variables and 3-hydroxybutyrate.

Liu et al. (2021)[8] and Muhli et al. (2023)[9] found fasting metabolome combined with clinical variables to predict future disorders of glucose metabolism in pregnant women without diagnosed GDM[8] or in a mixed population of predominantly normoglycemic women.[9] The ROC-AUC-value of the larger study with longer (10–14 years) follow-up was similar (0.71) to ours (0.75), while in the other study with 2 years follow-up the ROC-AUC was slightly lower: 0.67.[9] These results are relatively similar, despite the differences in populations and methodology. In our data all the patients had GDM (and required pharmacological treatment) compared to the 14.9 % who met the IADPSG GDM-criteria in the HAPO follow-up study, and therefore despite the shorter follow-up time the incidence of disorders of glucose metabolism at follow-up was higher in our data (42.0 % vs. 26.4 %). Moreover, the subjects in our study, all having GDM diagnosis, may have been aware of their increased risk of T2DM, which could have lowered the predictive performance. The recent study by Muhli et al. used the same NMR methodology, but targeted different population.[9] Only 30 % of the women had GDM diagnosis and most of them (78 %) were managed without pharmacological treatment. At the 2-years follow-up 22.5 % had prediabetes, which was diagnosed with only on the basis of fasting glucose whereas we used 2 h OGTT.[9] Finally, the size of our study was considerably smaller compared to the HAPO follow-up study,[8] and to avoid overfitting, limited application of more complex prediction models.

Contemporary studies (from 2016 and 2020) involving *postpartum* metabolomics have yielded considerably higher ROC-AUC-values (0.83–0.88),[4,5] likely by avoiding confounding factors related to pregnancy. However, our results strengthen the evidence that prediction of future disorders of glucose metabolism is feasible using metabolomics already during pregnancy.[7–9] In future studies the collection of serum samples at a given gestational week, or using relative values standardized by gestational age, could possibly reduce physiologic variation caused by pregnancy. Also, it is reasonable to expect more accurate prediction models to be built based on larger datasets.

4.6. Strengths and limitations

Our study has several strengths; homogenous study population, prospective design, validated NMR protocol, and assessment of glucose metabolism by OGTT and HbA1c at follow-up visit (to detect cases of pre-DM and previously undetected T2DM). Rather than mass-spectrometry used in most of the previous studies,[3–5,8] we applied NMR. Compared to mass-spectrometry NMR has high consistency, may by highly automated, and has high throughput,[31] making it a compelling and likely more cost-effective option for population screening. There are still some limitations. As a secondary analysis, we could not affect the number of patients, and some patients were lost to follow-up. These subjects tended to be younger with slightly higher HbA1c at baseline. Given that the metabolome in pregnancy is most clearly related to future 2-h glucose and measures on insulin resistance[8] and that these variables did not differ between the participants vs. non-participants of the follow-up study, the selection at follow-up has unlikely caused considerable bias. Nevertheless, the number of participants limited our ability to develop more complex prediction models, and more large-scale data is needed to substantiate our results. Additionally, although the validated targeted NMR protocol has good reproducibility it precludes the possibility to discover new biomarkers.

5. Conclusions

Certain lipid and lipoprotein components of metabolome seem to be predictive of glucose homeostasis and disorders of glucose metabolism nearly a decade after the index pregnancy. Determination of serum lipids might yield better prediction of the risk of future glucose metabolism disorders in GDM patients requiring pharmacological treatment, over the use of clinical variables alone, already at the time of GDM diagnosis.

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CRediT authorship contribution statement

Mikael Huhtala: Conceptualization, Formal analysis, Visualization, Writing – Original Draft. Tapani Rönnemaa: Conceptualization, Resources, Writing – Review & Editing. Elisa Paavilainen: Resources, Writing – Review & Editing. Harri Niinikoski: Resources, Writing – Review & Editing. Outi Pellonperä: Resources, Writing – Review & Editing. Juuso Juhila: Resources, Writing – Review & Editing. Kristiina Tertti: Conceptualization, Resources, Writing – Review & Editing. All authors have approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Ethics approval

The study was approved by the ethics committee of the Hospital District of Southwest Finland (ETMK 31/2015) on April 27, 2015 and the study participants signed an informed consent.

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