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Serum lipids and their association with birth weight in metformin and insulin treated patients with gestational diabetes

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ABSTRACT

Aims: To compare the effects of metformin and insulin treatment on maternal serum lipids in patients with gestational diabetes (GDM), and to analyse the associations between individual lipids and birth weight (BW).

Methods: This is a secondary analysis of a randomized trial comparing metformin (n = 110) and insulin (n = 107) treatment of GDM. Fasting serum lipidome was measured at baseline (the time of diagnosis, mean 30 gestational weeks, gw) and at 36 gw using nuclear magnetic resonance spectroscopy.

Results: Total and VLDL triglycerides, and VLDL cholesterol increased from baseline to 36 gw in both treatment groups. The rise in triglycerides was greater in the metformin treated patients ($p < 0.01$). Baseline total and VLDL triglycerides, VLDL cholesterol, and apolipoprotein B to A-1 ratio (apoB/apoA-1) associated positively with BW, more strongly in the metformin group. Among patients in the highest baseline VLDL cholesterol or apoB/apoA-1 quartile, those treated with insulin had lower BWs than those treated with metformin ($p < 0.03$).

Conclusion: Compared to insulin, metformin treatment of GDM led to higher maternal serum concentrations of triglyceride-rich lipoproteins. Especially triglycerides and cholesterol in VLDL were positively associated with BW. Women with high VLDL cholesterol or high apoB/apoA-1 may benefit from insulin treatment over metformin with respect to offspring BW.

Abbreviations: BW, birth weight; FA, fatty acids; GDM, gestational diabetes mellitus; Gw, gestational week; GWG, gestational weight gain; LGA, large for gestational age; LPL, lipoprotein lipase; MUFA, monounsaturated fatty acids; NICU, neonatal intensive care unit; NMR, nuclear magnetic resonance; OGTT, oral glucose tolerance test; PLS, partial least square; PLS-DA, partial least square discriminant analysis; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SGA, small for gestational age; TG, triglycerides

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1. Introduction

Gestational diabetes mellitus (GDM) is a common disorder of pregnancy, posing both the mother and the fetus in the risk of adverse outcomes [1]. In addition to hyperglycemia, alterations in the lipid profile in GDM have been observed [2–4]. Moreover, changes in the maternal lipid profile have been related to fetal growth and adiposity [5–7].

Along insulin, metformin is used as a first line pharmacological treatment of GDM. Compared to insulin it is cheaper and easier to administer. Metformin seems to be superior to insulin also in terms of lower gestational weight gain (GWG), decreased risk of gestational hypertension, neonatal hypoglycemia, and need for neonatal intensive care [8]. On the other hand, metformin crosses the placenta, and it may cause increased growth in the offspring during the first decade of life [9].

The effect of metformin treatment on serum lipidome has been investigated in non-pregnant subjects with type 2 diabetes. In this population metformin decreases LDL cholesterol [10,11] and affects phospholipid species [10–12]. In the largest trial comparing metformin and insulin in GDM (the MiG trial), triglycerides (TG) increased more in metformin treated mothers from baseline (mean 30 gestational weeks, gw) to 36 gw [13]. This has caused concerns about the safety of metformin use, as maternal TG may be associated with high birth weight (BW) [5,13–15].

The main aim of this study was to compare the effects of metformin and insulin treatment on serum lipidome in GDM patients. The second aim was to examine the associations between serum lipids and BW. To our knowledge there are no previous studies comparing the effects of metformin and insulin on detailed components of the serum lipidome in GDM patients.

2. Subjects, materials and methods

This is a secondary analysis of a previous randomized trial comparing metformin and insulin in the treatment of GDM [16]. The study participants were recruited between June 2006 and December 2010 in Turku University Hospital, Turku, Finland, on their first visit for management of GDM. The study was approved by Ethics Committee of the Southwest Hospital District of Finland, The Finnish National Agency of Medicines and the European Union Drug Regulatory Agency (EUDRA), and it is in accordance with the 1964 Helsinki Declaration. The study is registered in Clinicaltrials.gov (NCT01240785, http://clinicaltrials.gov/ct2/show/NCT01240785) and all participants signed an informed consent.

As described earlier in detail [16], women with GDM were randomised to either metformin (n = 110) or insulin (n = 107) by the physician using sealed envelopes. The original trial was powered to prove non-inferiority in BW, as the primary outcome [16]. GDM diagnosis based on 75 g oral glucose tolerance test (OGTT) was determined by Finnish national criteria as described previously [16]. Inclusion criteria were singleton pregnancy and the need for medical treatment of hyperglycemia (during diet therapy fasting plasma glucose $\geq$ 5.5 mmol/L and/or 1 h post-prandial glucose $\geq$ 7.8 mmol/L).

NPH insulin and/or rapid-acting insulin lispro or insulin aspart were used. Metformin was started at 500 mg daily and increased up to 2000 mg depending on the response (median final dose 1500 mg). In the metformin group, if glycemic targets were not reached with metformin, additional insulin was given (n = 23).

Venous blood samples were drawn after overnight fasting at the time of randomization (baseline, mean 30 gw) and at 36 gw. Baseline C-peptide and HbA1c at both time points were determined using routine laboratory methods. A targeted metabolome of 66 serum lipid markers was analyzed using a high-throughput nuclear magnetic resonance (NMR) spectroscopy protocol [17]. Serum samples were stored at −70 °C prior to NMR-analyses.

GWG was based on self-reported pre-pregnancy weight and last measured weight at maternity clinic. BW was measured in grams and converted into SD units (i.e. deviation from Finnish general population mean adjusted for gw [18]). As an indicator of large for gestational age (LGA) or small for gestational age (SGA), BW > 90th and < 10th percentiles were included.

2.1. Statistical analyses

The Mann-Whitney *U* test and the paired Wilcox signed rank test were used for univariate comparisons of the metabolites between groups, and within groups between the two time points.

In multivariate analyses the metabolite data at baseline and at 36 gw were scaled and centered and missing values were imputed using k-nearest neighbour method prior to further analyses. Partial least square (PLS) regression and PLS discriminant analyses (PLS-DA) were performed to study the associations between the whole lipidome and BW. Associations between single metabolites and BW were studied with linear or logistic regression analyses. These regression analyses were run unadjusted and adjusted for pre-pregnancy BMI, GWG, and either baseline or 36 gw HbA1c. Regression coefficients for metformin and insulin groups separately at baseline and 36 gw were calculated if the treatment group had a significant interaction ($p < 0.01$) with the association between the independent and outcome variable. 95% confidence intervals for regression estimates were calculated using bootstrapping. Birth weight centiles in patients treated with metformin and insulin were also compared in groups stratified by baseline lipid quartiles using the Mann-Whitney *U* test and ANCOVA.

To control type I error amid multiple testing, $p < 0.01$ was considered statistically significant. All statistical analyses were performed in R statistics software (versions 3.3.2 and 3.6.1, http://cran.r-project.org) and *ropls* R package [19] was used for PLS/PLS-DA analyses.

3. Results

Clinical characteristics of the study population have been previously described in detail by Tertti et al. [16], and are given in Table 1. Briefly, metformin and insulin groups did not differ in maternal age, pre-pregnancy BMI, OGTT fasting glucose, 1 h glucose or 2 h glucose, C-peptide, baseline HbA1c, 36 gw HbA1c, or GWG. Absolute mean (metformin 3610 vs. insulin 3590 g) and adjusted BW (0.16 vs. 0.15 SD), alike incidence of SGA (11.5% vs. 8.4%) or LGA (14.4% vs. 15.9%) were similar between the groups. The rate of labour induction was more frequent in the insulin group (54.2% vs. 38.0%, p = 0.024).

Due to rejection of individual measurements in NMR quality control, there were a few missing values at 36 gw (16 values in 6 mothers), but no missing values at baseline.

3.1. *Changes in the serum lipidome*

Maternal TG rose from baseline to 36 gw in both groups and in all lipoprotein classes (Figs. 1 and 2). Compared to insulin group this increase was significantly (p < 0.01) higher in metformin group in total TG and TG in VLDL and HDL. In lipoprotein subfractions classified by particle size these increases were universally greater in metformin group and the difference between changes was significant in small to very large VLDL, small LDL and, all HDL particles (Fig. 2).

Total cholesterol and free cholesterol increased irrespective of treatment (Figs. 1 and 2). The increase in cholesterol in lipoprotein particles was most prominent in VLDL, while cholesterol in HDL particles decreased (Figs. 1 and 2). In more detailed lipoprotein subclasses cholesterol increased in all VLDL subclasses, except very small VLDL in the metformin group. The increases in medium to extremely large VLDL particles were significantly greater in metformin treated women (Fig. 2). The amount of cholesterol decreased in very large and large HDL in both groups and in medium HDL in the insulin group.

Apolipoprotein B to A-1 ratio increased similarly in both treatment groups, due to the increase in apolipoprotein B (Fig. 2). The mean diameter of HDL particles decreased regardless of treatment, while the diameter of VLDL increased in both groups, but more in the metformin group. The mean diameter of LDL particles tended to decrease only in the metformin group, and the difference in the change between the treatment groups was significant (Fig. 2).

Serum total FA increased in both groups, parallel to an increase in monounsaturated FA (MUFA) and saturated FA (SFA) and a decline in the estimated degree of unsaturation

Table 1 – Clinical characteristics of the study population.

	Metformin	Insulin	p-value
n	*100–108*	*95–107*	
Patient characteristics			
Age (years)	31.8 ± 5.0	32.0 ± 5.5	0.82
Smoking	9 (8.7)	17 (16.0)	0.16
Primipara	42 (38.9)	49 (45.8)	0.38
Pre-pregnancy BMI (kg/m^2)	29.5 ± 5.9	28.9 ± 4.7	0.42
Glucose metabolism			
HbA1c% at OGTT	5.5 ± 0.34	5.5 ± 0.34	0.51
HbA1c at OGTT (mmol/mol)	36.4 ± 3.7	36.7 ± 3.7	
HbA1c% at 36 gw	5.7 ± 0.33	5.7 ± 0.36	0.85
HbA1c at 36 gw (mmol/mol)	38.5 ± 3.6	38.6 ± 3.9	
OGTT fasting (mmol/L)	5.5 ± 0.55	5.6 ± 0.42	0.49
OGTT 1 h (mmol/L)	11.2 ± 1.5	11.2 ± 1.2	0.68#
OGTT 2 h (mmol/L)	8.3 ± 1.8	7.9 ± 1.8	0.081
C-peptide at baseline (nmol/L)	1.1 ± 0.33	1.1 ± 0.29	0.90#
Pregnancy outcomes			
Gestational hypertension	2 (1.9)	4 (3.7)	0.45§
Preeclampsia	5 (4.6)	10 (9.3)	0.19§
Operative vaginal delivery	9 (8.3)	8 (7.5)	0.99
Cesarean delivery	15 (13.9)	18 (16.8)	0.68
Induction of labor	41 (38.0)	58 (54.2)	0.024
Gestational weight gain (kg)	8.0 ± 5.2	7.8 ± 5.3	0.77
Gestational age at delivery (weeks)	39.2 ± 1.4	39.4 ± 1.6	0.43
Neonatal outcomes			
Birth weight (g)	3610 ± 490	3590 ± 450	0.78
Birth weight (SD)	0.16 ± 1.1	0.15 ± 0.96	0.92
Birth weight < 10th percentile	12 (11.5)	9 (8.4)	0.60
Birth weight > 90th percentile	15 (14.4)	17 (15.9)	0.92
Admission to NICU	33 (30.8)	39 (36.5)	0.47
Newborn I.V. glucose	25 (23.4)	25 (23.6)	1

Data is shown as mean ± SD or n (%). The p-value is given for the *t*-test or the Mann-Whitney U (indicated with #) and for categorical data for the χ^2-test or Fisher's exact test (indicated with §). OGTT = oral glucose tolerance test, Gw = gestational weeks, SD = standard deviation, NICU = neonatal intensive care unit, I.V. = intravenous.

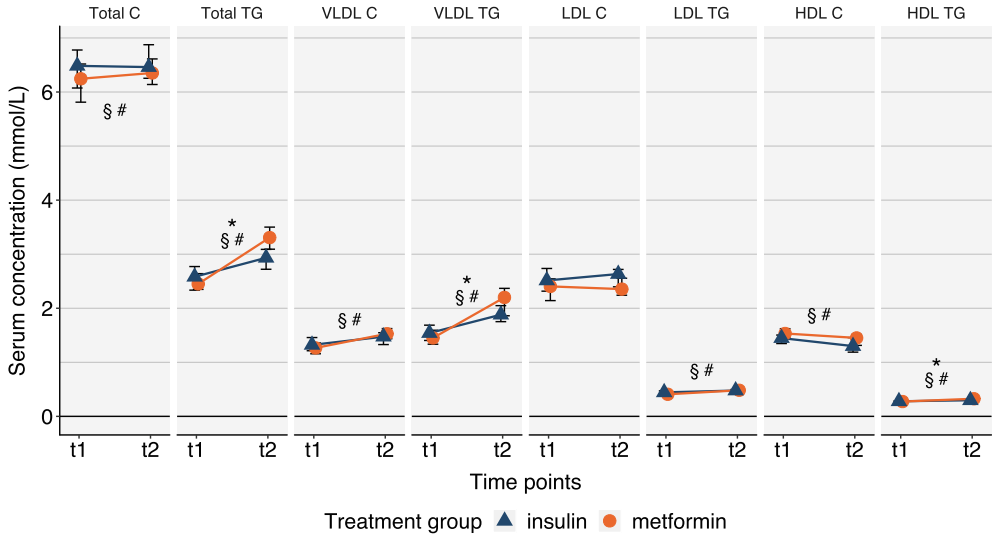

Fig. 1 – Changes in lipids from baseline to 36 gestational weeks. Median values (±95% CI) of maternal serum cholesterol (C) and triglycerides (TG) in total and in lipoproteins measured at baseline (t1) and at 36 gestational weeks (t2) are depicted in line graph. Statistical significance (p-value < 0.01) is denoted for changes within group (# metformin, § insulin) and for differences in median changes between groups (*). Metformin n = 99, insulin n = 91.

(Fig. 2). The increases in total FA, SFA, and MUFA were greater in the metformin group.

3.2. *Associations between the lipidome at baseline and birth weight*

The associations between maternal serum lipids and BW were initially analysed with both treatment groups combined. Separate regression coefficients for metformin and insulin groups were calculated if there was a significant interaction between treatment group and the association between BW and the independent variable. All metabolites with significant univariate (β-estimate $p < 0.01$) association with BW in combined analysis are shown in Fig. 3. A full list of regression results are available as Supplementary tables 1–2. In the estimation of the role of the whole lipidome on BW, the Q2 value of the PLS model (i.e. the amount of variation in outcome variable the model explained in internal cross-validation) for BW was 4.66%.

At baseline total TG and TG in all VLDL, IDL, and LDL subclasses and in small HDL was associated positively with BW (Fig. 3 and Supplementary Table 1). Cholesterol in small to extremely large VLDL was associated positively and cholesterol in medium HDL inversely with BW. VLDL and remnant cholesterol, apolipoprotein B, apolipoprotein B to apolipoprotein A-1 ratio, total FA, SFA, and MUFA were associated positively with BW. Adjustment for pre-pregnancy BMI, GWG, or baseline HbA1c had only minimal effects on these associations (Supplementary Table 1).

In PLS-DA analyses, lipidome was weakly associated with SGA ($Q^2 = 1.36\%$) but not at all with LGA ($Q^2 < 0$). In the logistic regression analyses only cholesterol in medium HDL had a significant positive association with SGA risk. This association was not affected by adjusting for pre-pregnancy BMI, GWG, or baseline HbA1c.

Cholesterol in total VLDL and in small VLDL, remnant cholesterol, apolipoprotein B, and apolipoprotein B to A-1 ratio were significantly positively associated with BW in the metformin but not in the insulin group. Linoleic acid, omega-6 FA, and PUFA were associated positively with BW only in the metformin group (Fig. 3 and Supplementary Table 1). There were no interactions between the treatment groups and the associations between these metabolites and SGA.

We next analyzed whether selected baseline lipid variables that were strongly related to BW (Fig. 3) are associated with BW differently during insulin or metformin treatment in patient groups stratified by baseline lipid levels. We formed four quartiles of total triglycerides, VLDL triglycerides, VLDL cholesterol, and apolipoprotein B to A-1 ratio. We observed that among women in the highest quartile of VLDL cholesterol and apolipoprotein B to A-1 ratio the BW centile was significantly lower in those treated with insulin compared to those treated with metformin (Table 2). A similar tendency was found in total and VLDL triglycerides. Among women in the three lower lipid quartiles there was no difference in BW percentiles between insulin and metformin treated women ($p > 0.3$).

3.3. *Associations between the lipidome at 36 gestational weeks and Birth weight*

At 36 gw, the serum lipidome was weakly associated with BW ($Q^2 = 1.48\%$) in PLS analysis. Compared to baseline, there were fewer significant individual lipid associations to BW. Cholesterol in small and very small VLDL, and TG in IDL, LDL and medium to large LDL subclasses were positively associated with BW (Fig. 4 and Supplementary Table 2). Adjusted for either pre-pregnancy BMI or HbA1c at 36 gw, cholesterol in LDL, free cholesterol, TG in LDL, linoleic acid, and omega-6

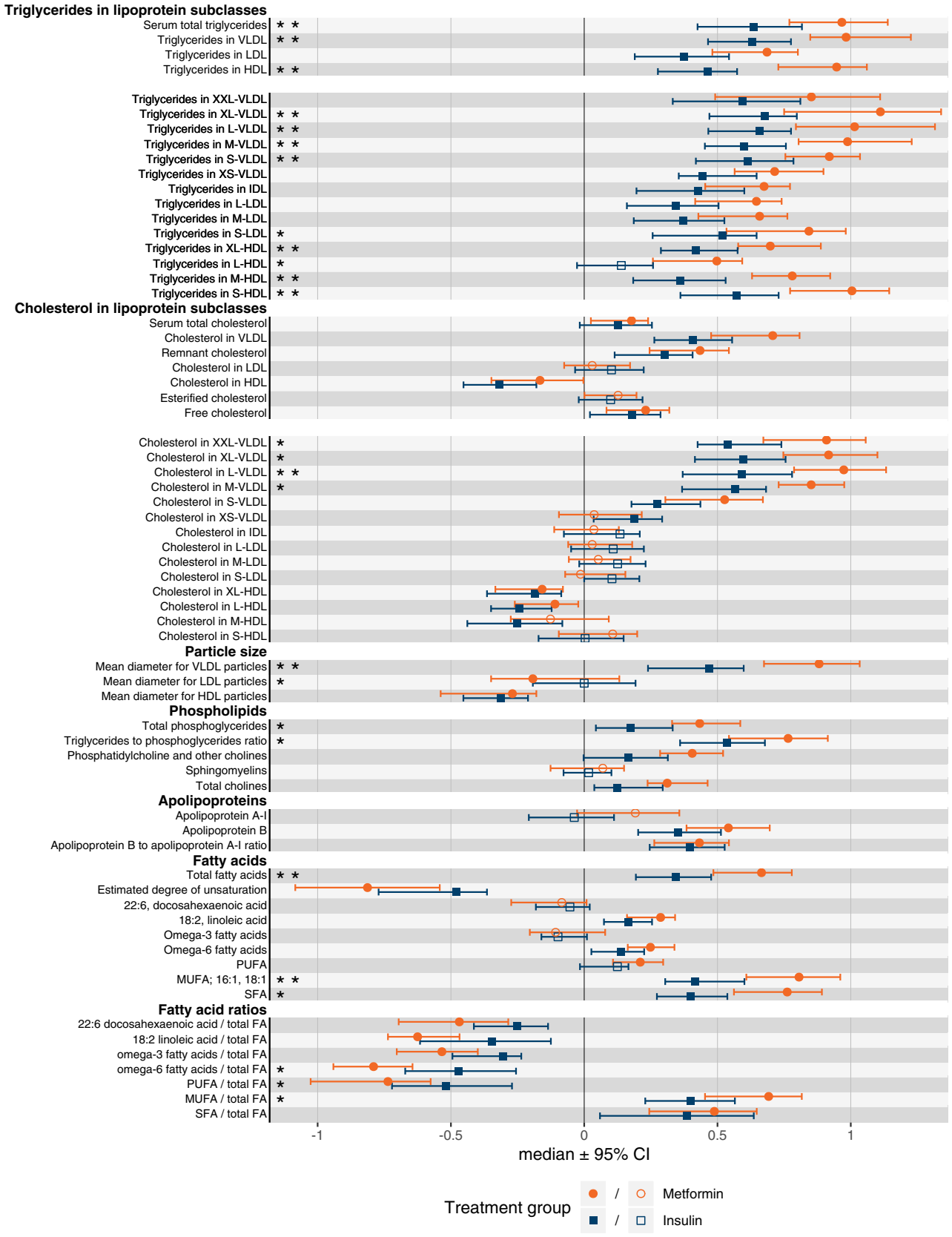

Fig. 2 – Change in detailed serum lipid concentrations from baseline to 36 gestational weeks in patients treated with metformin or insulin. Median change scaled by baseline SD ± 95% CI, closed squares / circles denote significant ($p < 0.01$) and open squares / circles non-significant change from baseline within group, $^{*}p < 0.01$ for difference in change between groups, $^{**}p < 0.001$ for difference in change between groups. Metformin n = 95–99 (circles), insulin n = 89–91 (squares).

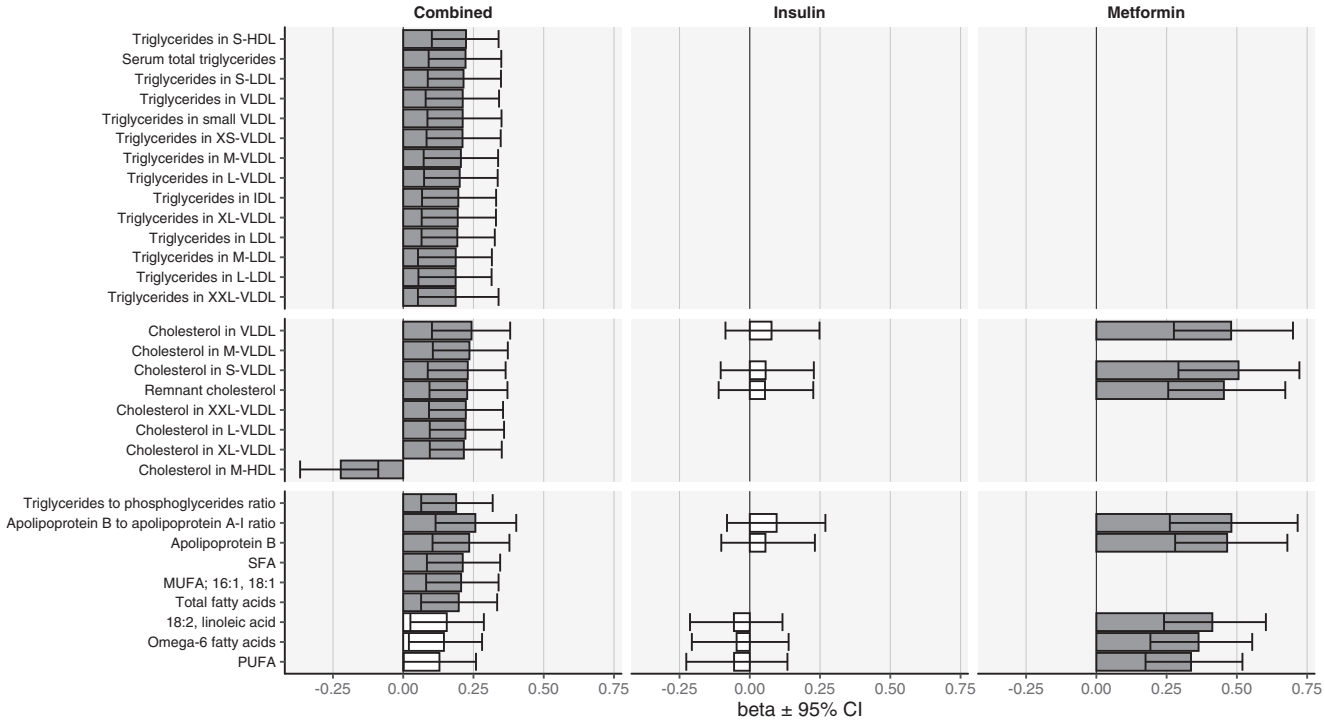

Fig. 3 – Associations between lipids at baseline and birth weight. Unadjusted regression β-estimates with 95% confidence intervals (CI) for significant (p < 0.01) associations between serum lipids at baseline and birth weight when analysing both groups combined. β-estimates for the groups individually are given if there was a significant interaction between treatment groups and the association between independent and outcome variables. Gray bars denote significant (p < 0.01) association. Birth weight was calculated as deviation from Finnish general population mean adjusted for gestation length. n = 204 (metformin 104, insulin 100).

FA were also positively related to BW (Supplementary Table 2). When adjusted for GWG, only cholesterol in small and very small VLDL, TG in IDL and in LDL were positively associated with BW.

TG in IDL and cholesterol in small VLDL were positively associated with BW in the metformin but not in the insulin group. TG in small LDL and in very small VLDL, remnant cholesterol and total VLDL cholesterol, apolipoprotein B, apolipoprotein B to apolipoprotein A-1 ratio, total FA, linoleic acid and omega-6 FA were positively associated with BW only in the metformin group (Fig. 4 and Supplementary Table 2).

Serum lipidome at 36 gw was very weakly associated with SGA (Q2 = 0.60%) but not at all with LGA ($Q^2 < 0$) in PLS-DA. In logistic regression analyses none of the metabolites were significantly associated with SGA risk.

4. Discussion

In GDM patients treated with metformin or insulin, serum TG concentrations, particularly in LDL and VLDL lipoproteins, increased during the last trimester, and this increase was greater in the metformin treated women. We found that apolipoprotein B to A-1 ratio, and TG and cholesterol in VLDL had the most evident positive relationship with BW. TG in LDL and FA were also positively related to BW, and these associations were stronger in the metformin group.

The overall changes observed in the lipidome during the last trimester (after mean 30 gw) are in agreement with previous studies [20–23]. During a normal and GDM pregnancy maternal serum VLDL lipid concentrations rise [20–23]. VLDL secretion from the liver is increased probably due to increased energy intake and elevated estrogen, while the clearance of VLDL is decreased by the suppression of lipoprotein lipase (LPL) activity in adipose tissue. The activity of cholesterol ester transfer protein also increases in pregnancy resulting in TG enrichment of HDL particles [23,24].

In this study, the increase in serum TG was uniform across various lipoprotein particles, and greater in the metformin group compared to the insulin group. These changes were also reflected in greater VLDL and smaller LDL particle sizes in the metformin group. A comparable increase in TG has been previously described in the MiG trial [13], and our results extend the observation of the increase in total TG to individual TG-containing particles. However, when metformin treatment was compared to placebo outside pregnancy [25], or in obese pregnant patients without diabetes [26], it did not affect TG concentrations. Based on our data it is possible that metformin does not affect normal physiologic elevation of TG in pregnancy [20], while insulin inhibits this increase as insulin promotes storage of FA as TG in adipocytes by activating LPL and inhibits the release of FA from adipose tissue by downregulating hormone-sensitive lipase. This could therefore explain the smaller increase of serum TG in the insulin group.

The patients in the metformin group showed a significant increase in VLDL TG and VLDL cholesterol compared to insulin group. In agreement with our data, two other randomized

Table 2 – Birth weight of children in metformin and insulin treated GDM patients stratified by baseline maternal serum lipid quartiles.

	Q1	Q2	Q3	Q4
Total triglycerides				
range (mmol/L)	0.91–2.05	2.06–2.52	2.53–3.02	3.03–5.13
metformin	0.38 [0.31; 0.58] (29)	0.46 [0.31; 0.66] (29)	0.69 [0.54; 0.79] (26)	0.74 [0.58; 0.84] (20)
insulin	0.42 [0.31; 0.62] (24)	0.60 [0.34; 0.76] (22)	0.67 [0.58; 0.88] (24)	0.54 [0.34; 0.69] (30)
p	0.50	0.31	0.96	0.061
VLDL cholesterol				
range (mmol/L)	0.55–1.06	1.07–1.32	1.33–1.58	1.59–2.63
metformin	0.38 [0.24; 0.56] (29)	0.48 [0.34; 0.58] (26)	0.6 [0.46; 0.79] (32)	0.79 [0.66; 0.86] (17)
insulin	0.42 [0.29; 0.65] (23)	0.54 [0.34; 0.71] (26)	0.71 [0.58; 0.9] (18)	0.54 [0.34; 0.71] (33)
p	0.36	0.52	0.37	**0.014**[#]
VLDL triglycerides				
range (mmol/L)	0.41–1.17	1.18–1.52	1.53–1.93	1.94–3.86
metformin	0.42 [0.31; 0.66] (29)	0.48 [0.34; 0.62] (26)	0.66 [0.46; 0.76] (27)	0.76 [0.58; 0.84] (22)
insulin	0.42 [0.29; 0.54] (23)	0.62 [0.44; 0.76] (26)	0.67 [0.46; 0.88] (22)	0.54 [0.42; 0.73] (29)
p	0.80	0.30	0.64	0.085
ApoB to apoA1 ratio				
range (ratio)	0.36–0.67	0.68–0.79	0.80–0.90	0.91–1.34
metformin	0.34 [0.18; 0.42] (31)	0.54 [0.46; 0.71] (26)	0.62 [0.42; 0.76] (29)	0.76 [0.60; 0.96] (18)
insulin	0.42 [0.27; 0.66] (22)	0.58 [0.48; 0.76] (23)	0.64 [0.46; 0.76] (22)	0.66 [0.38; 0.76] (33)
p	0.32	0.76	0.92	**0.026**[#]

Data is reported as median birth weight percentile [95% confidence interval] (n) in quartiles (Q1-Q4) of each lipid (total triglycerides, VLDL cholesterol, VLDL triglycerides, apolipoprotein B to A-1 ratio). p-value is given for Mann-Whitney *U* test comparing birth weights between treatment groups in each lipid quartile.

[#] The differences between metformin and insulin groups in Q4 were significant ($0.01 < p < 0.05$) also after adjustment (ANCOVA) separately for pre-pregnancy BMI, maternal gestational weight gain and baseline HbA1c.

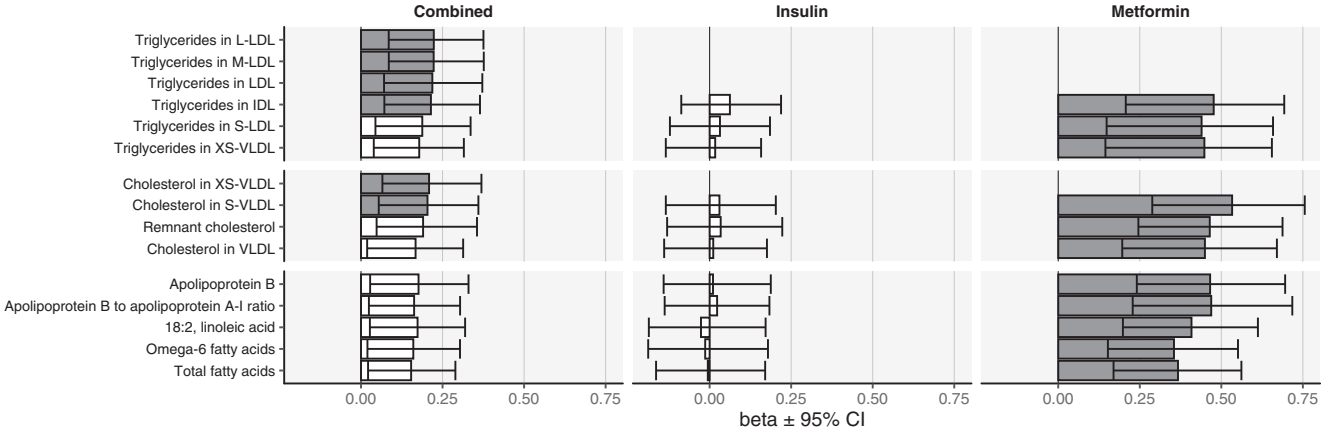

Fig. 4 – Associations between lipids at 36 gestational weeks and birth weight. Unadjusted regression β-estimates with 95% confidence intervals (CI) for significant ($p < 0.01$) associations between serum lipids at 36 gestational weeks and birth weight when analysing both groups combined. β-estimates for the groups individually are given if there was a significant interaction between treatment groups and the association between independent and outcome variables. Gray bars denote significant ($p < 0.01$) association. Birth weight was calculated as deviation from Finnish general population mean adjusted for gestation length. n = 194 (metformin 96, insulin 98).

trials described no differences in total [13,27], HDL [13,27] or LDL [13] cholesterol between metformin and insulin treatments of GDM. VLDL lipids however were not examined in these studies [13,27]. On the other hand, in non-pregnant subjects metformin was associated with a decline in LDL cholesterol [10] and in VLDL and LDL particle concentrations [12]. The difference in elevation of VLDL lipids between metformin and insulin treatments in our study may be attributable to insulin mediated inhibition of lipolysis and reduction in free FA – the substrates for TG synthesis in the liver. Barrett et al. [13] hypothesized that the patients who received metformin in the MiG trial, might have been more likely to restrict the amount of carbohydrate intake while the amount of dietary fat increased. Similarly, changes in the maternal diet

could explain the observed difference in VLDL lipids in our study. However, neither the MiG trial nor our study had dietary data available. Moreover, neither of the two studies had a placebo group, thus the independent effects of metformin and insulin remain hard to define.

It is well established that FA concentrations increase and their degree of unsaturation decrease during pregnancy [20,21]. In our study the increase in SFA and MUFA concentrations were greater during metformin treatment compared to insulin. However, in non-pregnant subjects metformin treatment did not affect SFA, MUFA, PUFA or the degree of FA unsaturation [25].

Maternal dyslipidemia worsens towards the end of pregnancy, but is ameliorated within 6–8 weeks postpartum [13]. Hence, it seems unlikely that this shift in the lipidome after 30 gw towards a more atherogenic profile especially in the metformin group would have a major long-term impact on maternal cardiovascular health.

In previous studies the associations between maternal lipids and BW have varied according to study population [5,7,13–15,28–30]. In normal pregnancies without GDM maternal TG was not associated with BW [30]. In undernourished, normoglycemic Indian pregnant women, both TG and total cholesterol were positively related to increased BW [28], whereas in Caucasian women with normal OGTT, TG was associated with BW independently of OGTT values [15], and in overweight/obese pregnant women TG was positively and HDL cholesterol inversely related to BW [29]. Even though maternal TG is rather constantly associated with new-born size [5,13–15], a large Mendelian randomization analysis did not support causal association [31].

To cross the placenta, FA need to be hydrolysed from TG and phospholipids by lipases, including endothelial lipase, LPL, hormone sensitive lipase and adipose triglyceride lipase, expressed in the placenta [32,33]. In GDM the expression of placental lipases is altered [33,34]. In parallel with these changes, an altered expression of gene clusters associated with cholesterol and TG biosynthesis in placental cells in GDM has been reported [34].

The discrepancy between the studies reporting correlations between maternal lipids and BW may result from the difference in lipid transfer and metabolism at the level of placenta in mothers varying in their glycemic status and treatment. The studies have consisted mostly of women without GDM [5,15,30] or with GDM not requiring antihyperglycemic medication [5], or women with GDM of whom approximately 30% were treated with insulin [14,30]. In the MiG trial maternal HDL cholesterol at 36 gw was inversely correlated with BW in insulin but not in metformin treated GDM patients [13], whereas we did not observe any correlation between HDL cholesterol at 36 wk and BW. The MiG study participants had higher pre-pregnancy BMI and higher GWG from randomization to 36 gw in the insulin group, possibly explaining the differences between the two studies. Furthermore, the association between HDL cholesterol and BW seems to be dependent of maternal obesity [29].

In the MiG trial maternal serum TG at baseline and at 36 gw was positively correlated with BW percentiles in both metformin and insulin treated GDM patients, but no correlation between LDL cholesterol and BW was found [13]. In our study the association between total TG and BW was attenuated at 36 gw, while LDL cholesterol and apolipoprotein B to A-1 ratio were related to BW only in metformin treated patients. These differences between our and the MiG trial data may be explained by differences in the ethnic background of the patients and/or differences in dietary composition.

Although there were no differences in BW between the treatment groups, the BW distributions were different between the groups when stratified according to maternal lipid (VLDL cholesterol, apolipoprotein B to A-1 ratio) quartiles. Among patients in the highest quartiles at baseline those treated with metformin delivered heavier neonates compared to those treated with insulin. Thus metformin and insulin may affect fetal growth on different mechanisms, although the overall differences in BW observed were subtle.

We hypothesize that insulin could, to a greater extent than metformin, ameliorate the altered lipid metabolism and transfer in placenta. This could explain the differences in associations of lipids and BW between the treatment groups. And hence, the insulin treatment may be more beneficial in those patients that are hyperlipidemic to control the excessive fetal growth. In favour of our hypothesis, the women who had BW associated lipid values in the highest quartiles, had lower BW centiles if they were assigned for insulin, compared to metformin treatment. However this finding needs to be confirmed in a larger study. On the other hand the women who are hyperlipidemic, tend also to be more obese and insulin resistant, and could therefore benefit from metformin treatment, as metformin reduces insulin resistance [35] and GWG [8].

We have demonstrated, in agreement with the MiG trial [13], that compared to insulin metformin treatment of GDM has different effects on maternal serum lipids, and that some of maternal lipids at different stages of pregnancy are related to fetal growth. Still, no differences in BW between metformin and insulin treated women have been reported [8]. One explanation for this might be that compared to metformin, the patients treated with insulin have greater variability in serum glucose, including glucose peaks leading to temporary fetal hyperglycemia favouring fetal growth. This could explain the absence of differences in BW between insulin and metformin treated patients, despite the lower concentrations of serum TG rich lipoproteins in insulin treated women.

The associations between lipidome and BW were attenuated at 36 gw, possibly due to dietary or pharmacological interventions. At baseline PUFA, including linoleic acid, were not associated with BW as they were at 36 gw when adjusted for pre-pregnancy BMI or HbA1c. Accordingly, placental transfer and lipid metabolism may change after 30 gw, and the relative importance of PUFA may increase near term [36]. Importantly, none of the lipids in our study was associated with the LGA risk.

4.1. *Strengths and limitations of the study*

In this study we present novel data of changes in maternal lipidome in response to pharmacological treatment of GDM.

NMR spectroscopy provides a fast and reliable way of measuring concentrations of lipid variables, including detailed classification of lipoprotein subclasses. As both of the groups received pharmacological treatment, and including a placebo group was considered unethical, we cannot definitely state to which extent these changes in the lipidome are accountable to either treatment. Moreover, our study population consists of homogenous (99% Caucasian) patients in good glycemic control and therefore, the results should be cautiously generalized to all GDM populations.

4.2. *Conclusions*

Maternal serum lipids increased during the last trimester in metformin and insulin treated GDM patients. The observed increase in total TG, TG in most VLDL subclasses, cholesterol in most VLDL subclasses, and total FA were greater in the metformin treated patients. Several maternal lipid measures, including VLDL TG, VLDL cholesterol and apolipoprotein B to A-1 ratio, were associated with BW. These associations were stronger in the metformin group, although there was no difference in BW between the treatment groups. However, based on our results women with high VLDL cholesterol or high apolipoprotein B to A-1 ratio at the time of GDM diagnosis may benefit from insulin treatment over metformin with respect to offspring BW. This finding however needs to be confirmed in a larger study.

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.

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Author Contributions

K.T. provided clinical data on the metformin and insulin treated patients and serum samples of all patients from the previous study (ref. [16]) M.H. analysed the data and wrote the first draft of the manuscript. K.T. and T.R. designed the present study and revised the manuscript. All authors have approved the final version of the manuscript.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2020.108456.

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