Maternal and Neonatal Circulating Markers of Metabolic and Cardiovascular Risk in the Metformin in Gestational Diabetes (MiG) Trial

Responses to maternal metformin versus insulin treatment

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OBJECTIVE—This study was designed to compare glucose, lipids, and C-reactive protein (CRP) in women with gestational diabetes mellitus treated with metformin or insulin and in cord plasma of their offspring and to examine how these markers relate to infant size at birth.

RESEARCH DESIGN AND METHODS—Women with gestational diabetes mellitus were randomly assigned to metformin or insulin in the Metformin in Gestational Diabetes trial. Fasting maternal plasma glucose, lipids, and CRP were measured at randomization, 36 weeks' gestation, and 6–8 weeks postpartum as well as in cord plasma. Women with available cord blood samples (metformin $n = 236$, insulin $n = 242$) were included.

RESULTS—Maternal plasma triglycerides increased more from randomization to 36 weeks' gestation in women treated with metformin (21.93%) versus insulin (9.69%, $P < 0.001$). Maternal and cord plasma lipids, CRP, and neonatal anthropometry did not differ between treatments. In logistic regression analyses adjusted for confounders, the strongest associations with birth weight >90th centile were maternal triglycerides and measures of glucose control at 36 weeks.

CONCLUSIONS—There were few differences in circulating maternal and neonatal markers of metabolic status and no differences in measures of anthropometry between the offspring of women treated with metformin and the offspring of women treated with insulin. There may be subtle effects of metformin on maternal lipid function, but the findings suggest that treating gestational diabetes mellitus with metformin does not adversely affect lipids or CRP in cord plasma or neonatal anthropometric measures.

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Gestational diabetes mellitus is carbohydrate intolerance first diagnosed during pregnancy (1) and affects up to 18% of pregnancies. The prevalence varies depending on maternal demographics and diagnostic criteria (2). The prevalence of gestational diabetes mellitus is increasing, which is likely driven by the rising population prevalence of overweight and obesity and increasing maternal age at pregnancy (3). Gestational diabetes mellitus increases maternal and infant morbidity and mortality during pregnancy (4). Women with a history of gestational diabetes mellitus are at risk for metabolic syndrome, type 2 diabetes (5), and cardiovascular disease in later life (6). Children born to women with gestational diabetes mellitus have higher rates of type 2 diabetes and obesity (7).

Treating gestational diabetes mellitus improves pregnancy outcomes for both mother and infant (8). Current therapies include modification of diet, increased physical activity, and drug therapy with insulin and oral hypoglycemic agents, including metformin. In addition to improving insulin sensitivity and hyperglycemia, metformin therapy in the setting of type 2 diabetes reduces triglycerides (9), total cholesterol, LDL cholesterol (10), and VLDL cholesterol; increases HDL cholesterol (9); and reduces markers of inflammation and thrombosis (11). Metformin therapy in gestational diabetes mellitus achieves maternal glucose control and pregnancy outcomes similar to insulin therapy (12,13).

In contrast to insulin, metformin crosses the placenta (14) and, therefore, could directly influence fetal metabolism. Our recent follow-up studies in 2-year-old offspring of women enrolled in the Metformin in Gestational Diabetes (MiG) trial showed increased subcutaneous fat measurements with no increase in abdominal adiposity or total fat (15). Further assessments are required to determine whether metformin actually reduces visceral/ectopic fat. Therefore, we hypothesized that metformin would be more effective than insulin in improving markers of insulin sensitivity and cardiovascular risk during pregnancy and postpartum in women with gestational diabetes mellitus and in their newborns.

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RESEARCH DESIGN AND METHODS

Study design and sample collection

The MiG was a prospective, randomized, and multicenter trial in New Zealand and Australia. It compared metformin with insulin treatment in women with gestational diabetes mellitus as previously described (13). The trial enrolled 751 women in total, with 436 from New Zealand and 181 from Adelaide, South Australia. The trial was approved by ethics review boards at each site, and all subjects gave written informed consent. Maternal capillary glucose levels were recorded regularly intrapartum (or in the case of planned cesarean section, just before delivery). Fasting blood samples were collected from each woman after an overnight fast at randomization (20–33 weeks' gestation before the commencement of medication), at 36 weeks' gestation, and at 6–8 weeks postpartum. Cord blood was collected from the placental side of the umbilical cord after it was clamped and divided. All blood was collected in EDTA and plain tubes and sent for processing within 10 min of collection or stored on ice for processing within 90 min. Plasma was then stored at −80°C.

Maternal and neonatal anthropometry

Women had weight and height measured at randomization, at 36 weeks, and at 6 weeks postpartum. BMI was calculated as kg/m^2.

Birth weight was measured at delivery. Birth weight centiles were calculated from customized population–based data adjusted for maternal BMI at randomization, ethnicity and parity, and neonatal sex and gestational age at delivery (www.gestation.net). Neonatal crown–heel length, arm circumference, abdominal circumference, and skinfold thickness (triceps and subscapular) were measured by trained observers within 48 h of birth.

Hormone and metabolite analyses

Maternal plasma glucose and triglycerides were measured in women at each of the three time points as well as in cord plasma. Maternal glucose and glycated hemoglobin (HbA_{1c}) have previously been reported (13). In addition, maternal HDL cholesterol, LDL cholesterol, and total cholesterol were measured in both the Adelaide and New Zealand cohorts from the MiG trial. Maternal C-reactive protein (CRP) and C-peptide and cord plasma HDL cholesterol, LDL cholesterol, CRP, and C-peptide were measured in the Adelaide cohort, and cord leptin was measured in the New Zealand cohort.

Plasma C-peptide concentrations were measured using a commercially available radioimmunoassay (RIA) kit (Human C-Peptide RIA Kit [HCP-20K]; Millipore Corporation, Billerica, MA). Plasma glucose, cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and CRP were measured in duplicate by colorimetric enzymatic analysis on a Hitachi 912 automated metabolic analyzer, using commercially available kits (GLU, CHOL, LDL-C plus, HDL-C plus, TG, and CRPL3, respectively; Roche Diagnostics GmbH, Mannheim, Germany). HbA_{1c} was measured in local laboratories by methods yielding results that were consistent with those of the Diabetes Control and Complications Trial.

Plasma leptin was measured with a commercially available RIA kit (Human Leptin RIA Kit [HL81K]; Millipore Corporation).

Statistical analysis

Effects of treatment were analyzed on an intention-to-treat basis using SPSS version 20 (IBM SPSS Statistics [2011]; IBM, Chicago, IL). Continuous variables are presented as mean (95% CI) and were analyzed using Student *t* test for independent samples or paired *t* test as appropriate. When not normally distributed, data were logarithmically transformed for analysis (with the addition of 1 to the raw data before transformation, if required); geometric means and 95% CIs are reported. Categorical variables are presented as frequencies and percentages and analyzed using the χ^2 test. Maternal outcomes were analyzed for effects of treatment, time (pretreatment at randomization, 36 weeks' gestation, and 6–8 weeks postpartum), and treatment × time interactions using a repeated-measures ANOVA. Where treatment was a significant factor in the repeated-measures ANOVA, to remove any variability because of heterogeneity among women in baseline measures, the difference between treatment groups was analyzed as the percentage change from randomization. Relationships between nontransformed maternal measures and infant outcomes were analyzed by Spearman rank correlation test.

Logistic regression analyses were performed to examine the relationships between maternal and cord blood variables and customized birth weight >90th and <10th centiles. For the regression analyses, ethnicity was coded as Caucasian and other, and parity was coded as nulliparous or multiparous. The regression analyses were also performed with ethnicity in five categories as follows: Caucasian, Indian, Polynesian, Chinese/other Asian, and other. This made no substantive difference to the presented results but resulted in instability in the CIs because of small numbers in some categories. The univariate analyses are presented in Supplementary Tables 1 and 2. These univariate analyses were then adjusted for maternal age, ethnicity, parity, maternal smoking during pregnancy, infant sex, time from study randomization to delivery, and maternal BMI at randomization and are presented as adjusted univariate analyses.

RESULTS

Randomization characteristics

Of the 733 women recruited to the MiG trial, cord plasma samples were available from 236 in the metformin arm and 242 in the insulin arm. Women in both arms were of similar age (metformin, 33.1 years [95% CI 32.4–33.8]; insulin, 32.5 [31.9–33.2], $P = 0.2$) and BMI (metformin, 35.2 kg/m^2 [95% CI 34.2–36.2]; insulin, 34.6 [33.7–35.5], $P = 0.4$) at randomization. The ethnic composition of the two groups was similar ($P = 0.55$), with an overall 49.6% of participants identifying as Caucasian, 21.3% as Polynesian, 14.9% as Indian, 11.3% as Asian, and 2.9% as other ethnicity. There were no maternal demographic differences between the subset of women in this study and those in the main trial population (13). Infants included in this study were born later (270.2 vs. 266.4 days, $P < 0.001$), and preeclampsia was a less-common pregnancy complication (4.8 vs. 10.4%, $P = 0.01$) than in the main trial population (13).

Maternal measures

Circulating hormones and metabolites at randomization were similar in the metformin- and insulin-treated groups (Table 1) as was the mean number of days from randomization to the blood tests at 36 weeks (metformin, 41.1 days [SD 23.3]; insulin, 41.1 [22.8], $P = 0.97$). Women randomized to insulin gained more weight from randomization to 36 weeks (1.9 kg [SD 3.4]) than women randomized to metformin (0.4 [2.9], $P < 0.001$). Maternal HbA_{1c} at randomization (metformin, 5.71% [SD 1.12]; insulin,

Table 1—*Maternal outcomes*

	Randomization		36 weeks' gestation		6–8 weeks postpartum		Significance (*P*)		
	Metformin	Insulin	Metformin	Insulin	Metformin	Insulin	Treatment	Time	Treatment by time
Glucose metabolism									
Glucose (mmol/L)	5.18 (5.06–5.31) [215]	5.11 (5.00–5.23) [194]	4.43 (4.34–4.52) [196]	4.29 (4.20–4.38) [210]	4.94 (4.84–5.05) [170]	5.04 (4.93–5.14) [187]	0.07	<0.0001	0.02
C-peptide (μg/L)	1.80 (1.57–2.04) [65]	1.57 (1.39–1.76) [66]	1.65 (1.45–1.86) [55]	1.46 (1.22–1.61) [52]	1.29 (1.11–1.49) [50]	1.35 (1.20–1.51) [48]	0.27	<0.0001	0.18
Markers of inflammation and cardiovascular risk									
Triglycerides (mmol/L)	2.48 (2.36–2.60) [213]	2.37 (2.26–2.48) [188]	2.88 (2.73–3.03) [188]	2.63 (2.52–2.75) [205]	1.32 (1.19–1.46) [135]	1.26 (1.15–1.37) [144]	0.03	<0.0001	0.38
Cholesterol (mmol/L)	6.03 (5.81–6.27) [152]	5.89 (5.61–6.17) [139]	6.25 (5.97–6.53) [127]	6.11 (5.91–6.31) [141]	5.43 (4.95–5.95) [98]	5.49 (5.31–5.69) [124]	0.80	<0.0001	0.64
LDL cholesterol (mmol/L)	3.08 (2.88–3.29) [152]	3.01 (2.81–3.23) [139]	3.03 (2.79–3.29) [127]	3.08 (2.91–3.26) [141]	3.42 (3.19–3.67) [98]	3.27 (3.10–3.45) [124]	0.99	0.15	0.45
HDL cholesterol (mmol/L)	1.60 (1.54–1.67) [154]	1.68 (1.61–1.74) [143]	1.64 (1.56–1.71) [130]	1.66 (1.59–1.73) [141]	1.35 (1.28–1.43) [101]	1.39 (1.33–1.45) [127]	0.15	<0.0001	0.54
CRP (mg/L)	5.53 (4.40–6.95) [70]	5.14 (4.22–6.27) [66]	5.69 (4.47–7.24) [60]	6.25 (4.90–7.97) [63]	3.53 (2.59–4.81) [49]	4.21 (3.23–5.50) [57]	0.47	<0.0001	0.17

Data are mean (95% CI) [*n*] unless otherwise indicated. Circulating markers of metabolic and cardiovascular disease risk and inflammation were measured in plasma collected at randomization, at 36 weeks' gestation, and at 6–8 weeks postpartum from women with gestational diabetes mellitus who were randomized to either insulin or metformin therapy.

5.66 [1.12], $P = 0.49$) and at 36 weeks (metformin, 5.60 [1.10]; insulin, 5.60 [1.12], $P = 0.92$) did not differ between groups. Time from randomization to delivery did not differ between groups (metformin, 57.5 days [SD 24.3]; insulin, 58.8 [22.5], $P = 0.53$).

All maternal variables except LDL cholesterol changed with time (Table 1). Maternal plasma triglycerides were similar in women from both treatment groups at randomization and 6 weeks postpartum. Maternal plasma triglycerides were higher at 36 weeks than at randomization ($P = 0.01$) and had a greater percentage increase from randomization in women treated with metformin (21.9%) than in those treated with insulin (9.7%) at 36 weeks ($P < 0.001$). Maternal plasma glucose declined equally from randomization to 36 weeks in both groups (metformin, −13.4%; insulin, −15.5%, $P = 0.19$) and then rose from 36 to 6 weeks postpartum in both groups. Effects of treatment on maternal plasma glucose varied with time: Women in the metformin group had a greater percentage fall in plasma glucose from randomization to 6 weeks postpartum (metformin, −2.88%; insulin, −0.68%, $P = 0.04$). There were no other differences in the pattern of change in maternal variables between treatment groups. Overall, maternal plasma C-peptide fell between randomization and 36 weeks ($P = 0.005$), decreasing further postpartum ($P = 0.014$). Maternal HDL cholesterol and CRP remained unchanged from randomization to 36 weeks ($P = 0.083$ and 0.37, respectively) but fell postpartum ($P < 0.001$ and 0.001, respectively). Maternal total plasma cholesterol rose from randomization to 36 weeks ($P = 0.009$) and then fell postpartum ($P = 0.002$).

Neonatal measures

There were no differences in birth weight and neonatal body composition between infants whose mothers were allocated to metformin and those whose mothers were allocated to insulin (Table 2). Infants of women allocated to metformin were born an average of 1 day earlier ($P = 0.03$). Cord plasma C-peptide and other cord plasma markers did not differ between treatment groups (Table 2).

Relationships of maternal and cord plasma measures with birth weight and customized birth weight

Randomization measures. Birth weight and customized birth weight centile (data not shown) correlated positively with maternal BMI and HbA_{1c} (Fig. 1*A* and *B*). Birth weight but not customized birth weight centile was positively correlated with maternal plasma glucose (Fig. 1*C*). Customized birth weight centile was positively correlated with maternal plasma triglycerides (overall, $r = 0.17$, $P = 0.001$, $n = 401$; metformin, $r = 0.14$, $P = 0.05$, $n = 213$; insulin, $r = 0.20$, $P = 0.005$, $n = 188$), as was neonatal abdominal circumference (overall, $r = 0.17$, $P = 0.002$, $n = 333$; metformin, $r = 0.1$, $P = 0.19$, $n = 176$; insulin, $r = 0.25$, $P = 0.002$, $n = 178$), but birth weight was not correlated with maternal triglycerides before treatment. Overall, neither birth weight nor customized birth weight centile was correlated with randomization measures of maternal cholesterol, HDL cholesterol, LDL cholesterol, C-peptide, or CRP.

The adjusted odds of infants' customized birth centile at >90th are presented in Table 3. In women randomized to metformin treatment, there was an association between C-peptide and infant birth centile >90th. In women randomized to insulin treatment, there was an association between plasma triglycerides and infant birth centile >90th. For infants <10th centile (Supplementary Table 3), there was a nonsignificant trend in relationship with C-peptide for women randomized to metformin (odds ratio [OR] 0.22 [95% CI 0.04–1.20], $n = 64$, $P = 0.08$) and triglycerides in women randomized to insulin (0.56 [0.29–1.09], $n = 187$, $P = 0.09$).

Thirty-six weeks' gestational measures. In both treatment groups, birth weight and customized birth weight centile were positively correlated with maternal plasma glucose and C-peptide (Fig. 1*D*).

Table 2—*Neonatal clinical and biochemical outcomes stratified by maternal treatment allocation*

	Metformin	Insulin	*P*
Clinical parameters			
Female sex	108 (45.8)	118 (48.8)	0.51
Gestational age at delivery (days)	269.4 (268.4–270.4) [236]	270.9 (270.0–271.8) [242]	0.03
Weight (g)	3,401 (3,333–3,470) [236]	3,428 (3,361–3,494) [242]	0.59
Placental weight (g)	653 (628–678) [162]	670 (645–696) [149]	0.33
Customized birth weight centile	53.9 (50.0–57.8) [236]	53.0 (49.1–56.9) [242]	0.76
Customized birth weight >90th centile	41 (17.4)	35 (14.5)	0.38
Customized birth weight <10th centile	23 (9.7)	31 (12.8)	0.29
Crown–heel (cm)	50.4 (50.3–50.7) [234]	50.5 (50.2–50.8) [241]	0.60
Arm circumference (cm)	11.3 (11.1–11.4) [194]	11.3 (11.1–11.5) [201]	0.89
Abdominal circumference (cm)	33.0 (32.6–33.3) [194]	32.6 (32.2–33.0) [201]	0.35
Triceps skinfold thickness (cm)	5.1 (4.9–5.3) [194]	5.0 (4.8–5.2) [194]	0.39
Subscapular skinfold thickness (cm)	5.1 (4.9–5.3) [193]	5.1 (4.9–5.3) [194]	0.99
Subscapular-to-triceps skinfold thickness ratio	1.0 (1.0–1.1) [193]	1.1 (1.0–1.1) [194]	0.32
Biochemical markers in cord plasma			
Glucose (mmol/L)	4.38 (4.15–4.63) [218]	4.46 (4.28–4.65) [233]	0.60
Triglycerides (mmol/L)	0.40 (0.37–0.42) [220]	0.40 (0.38–0.43) [233]	0.70
C-peptide (μg/L)	0.72 (0.60–0.84) [31]	0.70 (0.58–0.83) [30]	0.82
CRP (mg/L)	0.13 (0.11–0.14) [72]	0.14 (0.12–0.16) [70]	0.19
Leptin (μg/L)	12.04 (9.39–15.43) [44]	13.64 (10.70–17.38) [43]	0.47
HDL cholesterol (mmol/L)	0.69 (0.63–0.76) [72]	0.71 (0.65–0.78) [71]	0.66
LDL cholesterol (mmol/L)	0.50 (0.45–0.55) [72]	0.53 (0.48–0.58) [71]	0.43

Data are *n* (%) and mean (95% CI) [*n*].

Customized birth weight centile, but not birth weight, was positively correlated with maternal plasma triglycerides (r = 0.14, P = 0.009, n = 356), as was neonatal abdominal circumference (r = 0.14, P = 0.02, n = 295). Birth weight and customized birth weight centile were negatively correlated with maternal HDL cholesterol in insulin-treated women but not in metformin-treated women (Fig. 1*E*). There was no correlation between birth weight overall and maternal plasma triglycerides at 36 weeks. Birth weight centile was positively correlated with maternal plasma triglycerides at 36 weeks overall (r = 0.14, P = 0.009, n = 356), as was neonatal abdominal circumference (r = 0.14, P = 0.02, n = 295). Birth weight and customized birth weight centile were not correlated with maternal total cholesterol, LDL cholesterol, or CRP at 36 weeks.

In the women of both treatment groups, there was an association between HbA_{1c} and infant birth centile >90th and plasma triglycerides and infant birth centile >90th (Table 3). In addition, for women randomized to insulin, there was an association between fasting glucose and infant birth centile >90th. The negative relationship between infant size and maternal HDL was not seen for infants in the >90th centile but remained a trend in infants in the <10th centile, with those infants born to women taking insulin trending to a greater risk of being <10th centile with increasing maternal HDL (insulin, OR 4.38 [95% CI 0.97–19.92], n = 141, P = 0.06; metformin, 0.72 [0.19–2.81], n = 130, P = 0.64) (Supplementary Table 3).

Cord plasma measures. Birth weight was positively correlated with cord plasma leptin and C-peptide (Fig. 1*F* and *G*), as was customized birth weight centile (data not shown). Birth weight and customized birth weight centile were negatively correlated with cord plasma glucose and triglycerides (Fig. 1*H* and *I*). Birth weight and customized birth weight centile were not correlated with cord plasma LDL cholesterol, HDL cholesterol, or CRP. Cord plasma triglycerides were not correlated with maternal plasma triglycerides at randomization (r = 0.025, P = 0.63, n = 381) or at 36 weeks' gestation (r = −0.018, P = 0.75, n = 346). There was no difference in mean maternal capillary glucose measurements in the intrapartum period (metformin, 5.31 [SD 1.02]; insulin, 5.18 [0.85], P = 0.14).

In women randomized to metformin, there was an association between cord plasma triglycerides and customized birth centile at >90th (Table 3). This was not present for women randomized to insulin. For infants in the <10th centile, there was a significant relationship with cord plasma triglycerides for both groups (metformin, OR 27.61 [95% CI 2.97–256.98], n = 219, P = 0.004; insulin, 8.53 [1.55–46.84], n = 231, P = 0.01).

CONCLUSIONS—In the current study, we have described changes in plasma glucose, lipids, and CRP in women with gestational diabetes mellitus randomized to either metformin or insulin treatment. In general, measures of glucose and CRP improved on treatment, and triglycerides increased similarly to the changes seen in normal pregnancy. However, contrary to our hypothesis, we found that the increase in maternal plasma triglycerides from randomization to 36 weeks was greater in women treated with metformin than in those treated with insulin. Despite these differences in maternal triglycerides, there were no differences in cord plasma triglycerides, neonatal metabolic markers, or anthropometric measurements between the treatment groups. We examined how these markers correlated with birth weight and customized birth weight centile and showed that maternal BMI at randomization was the strongest predictor of birth weight. Customized birth centile and abdominal circumference, but not birth weight, were positively correlated with maternal triglycerides at both randomization and 36 weeks' gestation. Furthermore, we found that the relationship between maternal HDL cholesterol and infant birth weight differed between treatments.

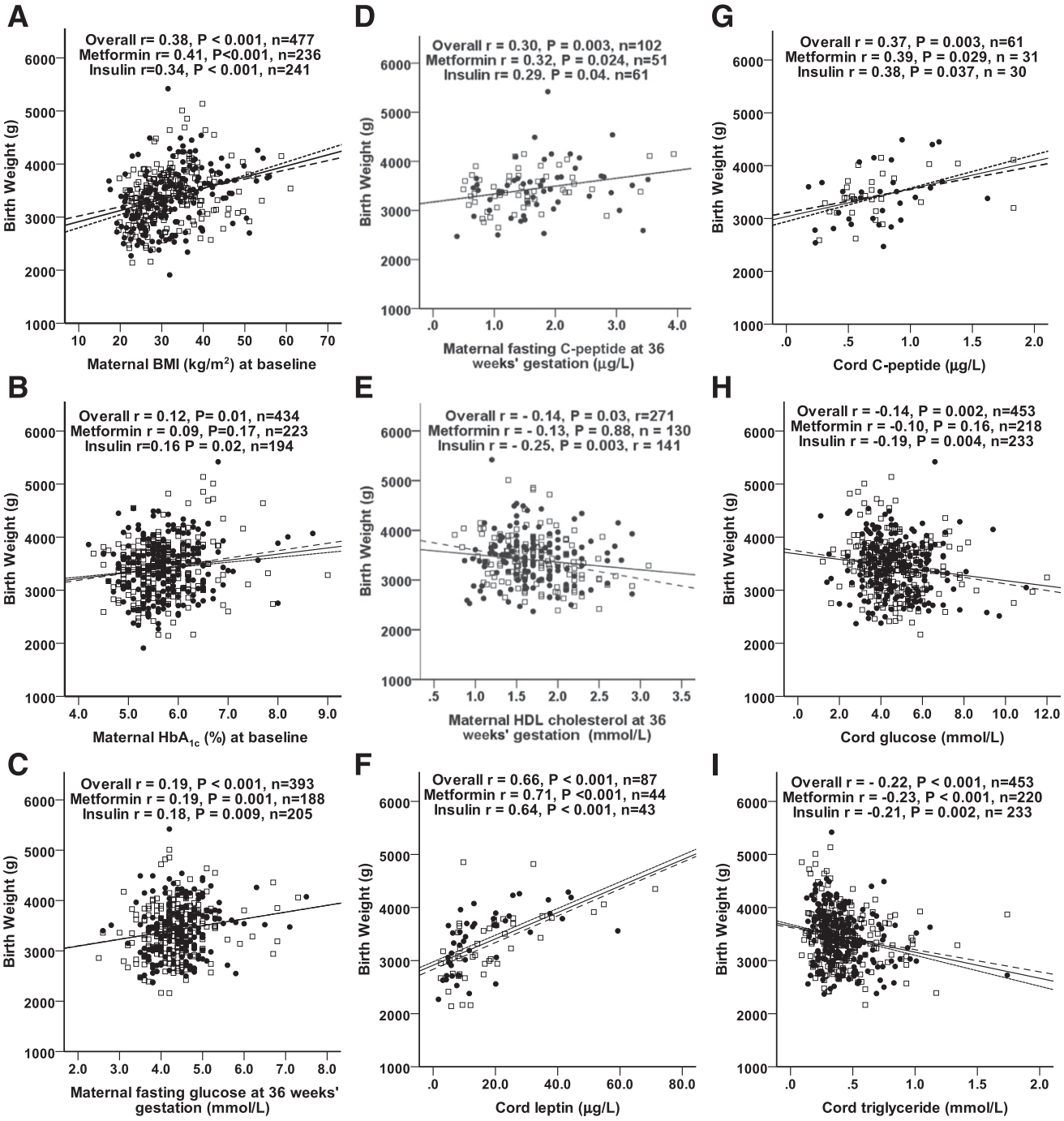

Figure 1—*Correlations between maternal variables and between cord plasma variables and birth weight. Graphs represent correlations between birth weight and maternal BMI at baseline (kg/m^2)* (A)*, maternal HbA$_{1c}$ (%) at baseline* (B)*, maternal fasting glucose at 36 weeks' gestation (mmol/L)* (C)*, maternal fasting C-peptide at 36 weeks' gestation* (D)*, maternal HDL cholesterol at 36 weeks' gestation (mmol/L)* (E)*, cord leptin (µg/L)* (F)*, cord C-peptide (µg/L)* (G)*, cord glucose (mmol/L)* (H)*, and cord triglyceride (mmol/L)* (I)*. Subjects with gestational diabetes mellitus were randomized to either insulin or metformin therapy. Relationships between variables were tested using two-sided Spearman rank correlation. Regression lines are shown only where significance was P < 0.1. □, data for insulin-treated women; ●, data for metformin-treated women. Regressions are shown with solid lines for overall relationships, with dashed lines for the insulin-treated group, and with dotted lines for the metformin-treated group.*

When adjusted for maternal age, ethnicity, parity, maternal smoking, infant sex, time from randomization to delivery, and maternal BMI, the variables that were significantly associated with birth weight >90th centile were markers of insulin resistance, maternal glucose control, and triglycerides. Likewise, the variable associated with birth weight <10th centile after adjustment was triglycerides.

The maternal plasma triglyceride values reported here in both treatment groups are similar to those seen in normal pregnancy (16,17) and in women with gestational diabetes mellitus (18), with a

Table 3—*Univariate logistic regression for 90th centile, with maternal variables at randomization and at 36 weeks' gestation, and for cord plasma variables*

Variable	Metformin	*P*	Insulin	*P*
Maternal plasma variables at randomization				
C-peptide (μg/L)	3.41 (1.20–9.70) [64]	0.02	0.23 (0.04–1.37) [61]	0.11
Triglycerides (mmol/L)	1.22 (0.82–1.83) [213]	0.33	1.88 (1.11–3.19) [187]	0.02
HbA_{1c} (%)	1.31 (0.70–2.44) [223]	0.40	1.85 (0.94–3.64) [210]	0.07
Fasting glucose (mmol/L)	1.23 (0.83–1.85) [215]	0.31	1.08 (0.58–2.01) [193]	0.81
HDL cholesterol (mmol/L)	1.22 (0.32–4.56) [154]	0.77	0.36 (0.08–1.72) [143]	0.20
LDL cholesterol (mmol/L)	0.78 (0.46–1.33) [151]	0.36	0.97 (0.60–1.56) [141]	0.88
Cholesterol (mmol/L)	0.78 (0.49–1.23) [152]	0.29	1.09 (0.75–1.58) [139]	0.66
Maternal plasma variables at 36 weeks' gestation				
HbA_{1c} (%)	2.96 (1.25–7.00) [195]	0.01	2.30 (1.16–4.58) [209]	0.02
Triglycerides (mmol/L)	1.49 (1.02–2.16) [167]	0.04	1.75 (1.05–2.92) [187]	0.03
Fasting glucose (mmol/L)	1.43 (0.78–2.61) [187]	0.25	2.02 (1.13–3.61) [204]	0.02
HDL cholesterol (mmol/L)	2.54 (0.54–11.99) [51]	0.24	13.31 (0.47–376.43) [51]	0.13
Cholesterol (mmol/L)	0.78 (0.23–2.65) [130]	0.68	0.89 (0.24–3.29) [141]	0.86
LDL cholesterol (mmol/L)	0.72 (0.46–1.12) [127]	0.15	1.07 (0.70–1.62) [141]	0.77
C-peptide (μg/L)	2.54 (0.54–11.99) [51]	0.24	13.31 (0.47–376.43) [51]	0.13
Cord plasma variables				
Triglycerides (mmol/L)	0.08 (0.01–1.02) [219]	0.05	1.10 (0.14–8.97) [231]	0.93
Glucose (mmol/L)	0.98 (0.74–1.30) [217]	0.89	0.83 (0.60–1.13) [231]	0.23
LDL cholesterol (mmol/L)	0.37 (0.02–8.44) [72]	0.53	4.79 (0.26–87.75) [71]	0.29
HDL cholesterol (mmol/L)	0.80 (0.06–9.94) [72]	0.86	2.77 (0.13–59.36) [71]	0.51

Data are mean (95% CI) [*n*]. All adjusted for maternal age, ethnicity, parity, maternal smoking, infant sex, time from randomization to delivery, and maternal BMI at randomization; cord leptin and cord C-peptide were not analyzed because of small sample size.

rise in maternal plasma triglycerides in the last trimester. The difference in maternal plasma triglycerides between metformin-treated and insulin-treated women was an unexpected finding. Previous studies in type 2 diabetes outside pregnancy have suggested that metformin improves lipid profiles, with a decline in triglycerides and LDL cholesterol and an elevation in HDL cholesterol (10), whereas insulin decreases lipolysis in adipose tissue, thereby reducing free fatty acids and hepatic synthesis of triglycerides (19). Thus, the current findings from women with gestational diabetes mellitus are in stark contrast to findings in nonpregnancy studies. There are several possible explanations for this difference. First, metformin may stimulate an increase in maternal plasma triglycerides, but the levels reported in the current study are not elevated compared with normal pregnant women, and this explanation would imply a unique effect of metformin on plasma lipids in gestational diabetes mellitus. Second, during pregnancy, insulin therapy suppresses the normal rise of maternal lipids to a greater extent than metformin. Finally, women treated with metformin may have modified their diet further than those allocated to insulin treatment in order to avoid supplementary insulin treatment. Further restrictions to carbohydrate intake could have led to increased free fatty acid release from maternal adipose stores and increased hepatic triglyceride production. This explanation would also fit with reduced maternal weight gain in metformin-treated women.

Maternal triglycerides have been reported to be positively correlated with cord blood triglycerides in women with well-controlled gestational diabetes (20). This relationship was not found in the current study, but it has been reported in women with poorly controlled type 1 diabetes (21) or in obese women with macrosomic infants (22). The increase in maternal plasma triglycerides seen in late pregnancy, exaggerated in gestational diabetes mellitus, is accompanied by an increased lipoprotein density, with triglyceride enrichment of lipoproteins. The placenta expresses hydrolases and lipases as well as fatty acid binding proteins, allowing the breakdown of maternal lipoproteins and hydrolysis of maternal triglycerides. The released fatty acids are transported through the placenta, increasing fetal fatty acid supply for fetal hepatic triglyceride (and lipoprotein) synthesis (23). Placental expression of lipases and lipid transfer has been shown to be altered in the setting of maternal diabetes (24) and maternal obesity (25). The discordance in relationship between maternal and cord triglycerides described in the literature and in the current study may be indicative of underlying differences in placental processing of lipids in the various maternal pathological states.

In this study, maternal BMI at randomization and maternal plasma C-peptide at 36 weeks' gestation showed the strongest positive association with infant birth weight, confirming data from previous studies (26,27). In addition, in the metformin-treated women, randomization C-peptide was associated with the greatest risk of being born >90th centile while in insulin-treated women, randomization C-peptide was nonsignificant and randomization triglycerides conferred the greatest risk. Maternal C-peptide in mid-gestation has previously been reported to be associated with infants being born large for gestational age (28). Maternal C-peptide declined over the course of the study in both treatment arms. There was no significant difference in maternal C-peptide level or in measures of glucose control between treatment arms. The reason for the difference in relationship between maternal C-peptide and risk of being born >90th centile between the treatment arms in this study is unclear.

The relationship between maternal triglycerides and neonatal anthropometry appears complex. We found a positive correlation between maternal plasma triglycerides and customized birth centile as

well as with neonatal abdominal circumference but not birth weight at both randomization and 36 weeks' gestation. We also found that with adjustment for potential confounders, maternal triglycerides at 36 weeks remained associated with increased odds of birth weight >90th centile. Birth weight has been reported to be positively correlated with maternal triglycerides in normal pregnancy as well as in pregnancy complicated by gestational diabetes mellitus and impaired glucose tolerance (29–31). A positive correlation between maternal plasma triglycerides and neonatal abdominal circumference but not birth weight has previously been reported in women with treated gestational diabetes mellitus (20). Together with our findings, this suggests that in treated gestational diabetes mellitus, maternal plasma triglycerides may determine neonatal adiposity to a greater extent than neonatal birth weight.

We found that maternal HDL cholesterol was negatively correlated with birth weight only in those women treated with insulin. Previous studies in normal pregnancy (29) and gestational diabetes mellitus (32) have found a similar negative relationship between maternal HDL cholesterol and birth weight, which is exaggerated in overweight or obese women (33). A suggested possible explanation for this negative correlation is an alteration in the role of HDL cholesterol in reverse cholesterol transport in extraembryonic fetal tissues, thereby altering the fetal sterol balance (33).

In metformin-treated women, there was a lack of correlation between maternal HDL cholesterol and birth weight. Because both maternal and cord HDL cholesterol were unaltered by treatment in the current study, the lack of correlation is unlikely to be related to a direct effect of metformin on HDL cholesterol levels. There are several possible explanations for the lack of relationship between maternal HDL cholesterol and birth weight in women allocated to metformin. First, reduced weight gain in women taking metformin was possibly confounded by differing dietary composition, particularly if women on metformin had a greater restriction in carbohydrate intake. However, there was no difference in HDL cholesterol levels between treatment groups, which might be expected with a direct weight effect or a dietary effect (34). Second, metformin may influence HDL cholesterol subfraction distribution, with a subsequent impact on HDL cholesterol function. In support of this hypothesis, metformin has been shown to increase HDL2 in patients with type 2 diabetes (35). Third, metformin alters the function of HDL cholesterol in reverse cholesterol transport. In vitro, metformin reverses the negative effect of glycation of HDL cholesterol on cellular efflux of cholesterol (36), but this has not been demonstrated in vivo. However, if the relationship between HDL cholesterol and birth weight is mediated by the role of HDL cholesterol in reverse cholesterol transport and if the reported improvement in cholesterol efflux because of metformin were occurring in vivo here, then the relationship between HDL cholesterol and birth weight should be exaggerated rather than weakened. Finally, metformin could have a direct effect on the maternal and placental inflammatory milieu. Metformin has been shown to reduce oxidative stress in the setting of type 2 diabetes (37). If metformin were to reduce oxidative stress in gestational diabetes mellitus, then placental weight or birth weight might be expected to be higher in the metformin group because of reduced placental vascular disease. However, these phenomena were not observed in the current study.

In agreement with the current findings, cord leptin and C-peptide have been reported to be positively correlated with birth weight and cord triglycerides to be negatively correlated with birth weight (38). The positive associations may reflect the fat mass of the infant, and the negative association with triglycerides might reflect low lipoprotein lipase activity in the adipose tissue of lean infants (20).

In the current study, there was a negative correlation between cord plasma glucose and birth weight. This relationship has not been previously reported (20) and could be attributed to differences in maternal glycemic control between the current study and earlier studies (20). We suggest that the lower cord glucose is in keeping with a higher cord C-peptide in heavier neonates. Both neonatal macrosomia and higher neonatal ponderal indices have been linked to increased rates of neonatal hypoglycemia (39). An elevated cord C-peptide is expected in the setting of exposure of the fetus to increased maternal glucose, which stimulates fetal pancreatic hypertrophy and insulin production (40). This is supported by the positive correlation between birth weight and maternal glucose and C-peptide at 36 weeks' gestation in the current study.

This study shows that treatment of women with gestational diabetes with metformin or insulin results in similar maternal and cord plasma markers of metabolic status and no differences in measures of neonatal anthropometry. The higher maternal triglycerides in metformin-treated women than in insulin-treated women are of unclear clinical significance. It is noteworthy that cord triglycerides were not increased by metformin treatment. The lack of a relationship between maternal HDL cholesterol and birth weight in women treated with metformin contrasted with the expected negative relationship as seen in women treated with insulin and suggests a potential influence of metformin on HDL cholesterol or placental function. There may be subtle effects of metformin on maternal lipid function, but the findings suggest that treating gestational diabetes mellitus with metformin does not adversely affect either lipids or CRP in cord plasma or cause any change in neonatal anthropometric measures.

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H.L.B. performed the statistical analyses; contributed to discussion; and wrote, reviewed, and edited the manuscript. K.L.G., W.M.H., and J.A.R. researched data, contributed to discussion, and reviewed and edited the manuscript. C.M.H., M.J.D., S.C., and J.A.O. researched data and reviewed and edited the manuscript. H.D.M. contributed to discussion and reviewed and edited the manuscript. L.K.C. and M.D.N. contributed to discussion and wrote, reviewed, and edited the manuscript. J.A.R. 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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