Title: Remodeling of the Cardiovascular Circulation in Fetuses of Diabetic Mothers: A Fetal Computational Model Analysis

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Abstract

Aims: Myocardial structural and functional abnormalities are known to occur in fetuses of maternal diabetes mellitus (FMDM) and in their offspring. The main aim of this investigation was to explore the cardiovascular circulatory patterns in FMDM using a validated lumped computational model of the cardiovascular system.

Methods: This was a multi-institutional study involving FMDM compared to fetuses of maternal controls (FC). Fetal echocardiographic Doppler data from left and right ventricular outflow tracts, aortic isthmus, middle cerebral and umbilical arteries were fitted into a validated fetal circulation computational model to estimate patient-specific placental and vascular properties. Non-parametric comparisons were made between resistances, compliances and flows in the brain and placenta in FMDM and FC.

Results: Data from 23 FMDM and 31 FC were fitted into the model. In FMDM, compared to FC, placental relative resistance was lower (0.59$\pm$0.50 versus 0.91$\pm$0.41; $p<0.05$) with higher brain relative resistance (2.36$\pm$1.65 versus 1.60$\pm$0.85; $p<0.05$). Middle cerebral artery flow was lower in FMDM than FC (0.12$\pm$0.14 vs. 0.27$\pm$0.21 ml/min; p 0.04) with a lower cerebral-placental flow ratio. Combined stroke volume was lower in FMDM (3.65$\pm$2.05 ml) than FC (4.97$\pm$2.45 ml) (p 0.04).

Conclusions: Blood flow is redistributed in FMDM to the placenta, away from the brain. This alteration may play a role in the postnatal health of these fetuses.

Key words: Fetus; Maternal Diabetes Mellitus; Computational model; Resistance; Compliance

Abbreviations: MDM – Maternal diabetes mellitus; FMDM – Fetuses of mothers with diabetes mellitus; FC – Fetuses of control mothers; EFW – Estimated fetal weight; UA – Umbilical artery; MCA – Middle cerebral artery; LVOT – Left ventricular outflow tract; RVOT – Right ventricular outflow tract; CCO- Combined cardiac output; VTI – Velocity time integral; vol – Volume; SV – stroke volume

Introduction

Significant short and long-term morbidities have been known to occur in the offspring of mothers with diabetes mellitus. There is a five-fold increase in the risk of congenital heart disease in fetuses of mothers with diabetes mellitus (FMDM); they also have a higher incidence of a reversible hypertrophic cardiomyopathy and subclinical myocardial dysfunction.[1-3] Maternal diabetes mellitus (MDM) has been linked to fetal macrosomia, fetal growth restriction (FGR), and fetal and neonatal demise.[4] There also may be other lasting effects in these offspring including a propensity for neurological deficits, obesity, diabetes, hypertension and cardiovascular events later in life.[4-6]

Current knowledge of the underlying mechanism of disease in FMDM suggests a combination of chemical, molecular and epigenetic influences on the fetus and placenta.[4, 7-9] Animal studies have shown that fetuses of hyperglycemic dams have reduced pancreatic β cell mass and reduced expression of insulin like growth factor.[10] Increased villous stromal capillarization and concentration of endogenous nucleoside adenosine (a potent vasodilator and anti-inflammatory agent) are seen in the placentae of FMDM.[11, 12] Rodent experiments have noted lower number of nephrons in the neonatal kidneys of MDM.[4] It is likely that these alterations in the fetal organ systems are associated with circulatory adaptations in FMDM. Computational modeling of the fetal circulation allows assessment of the relevant parameters non-invasively and in their natural environment in human fetuses.

A lumped model of the fetal circulation was created, validated and explored in FGR fetuses (implemented in Simulink, MATLAB 2013b, The MathWorks Inc., Natick, MA).[13, 14] This model provides a good non-invasive approximation of the fetal circulation to study hemodynamic changes induced by abnormal growth conditions. Alterations in fetal hemodynamics (predominantly flows) can be assessed non-invasively in clinical practice by localized Doppler measurements. However, computational models have the advantage of providing a more global view on hemodynamics as well as allowing the quantification of circulatory parameters that are currently not measurable non-invasively, such as pressures and vascular or organ properties like resistance and compliance. We applied this model to FMDM and compared them to normal fetal controls to understand if there were circulatory remodeling patterns in FMDM. This is a pilot study with an exploratory hypothesis that the hemodynamics/blood flow circulation in FMDM and FC may be different.

Methods

Study Population

This was a cross-sectional multi-institutional case control study of 54 fetuses, 23 FMDM) and 31 fetuses of control mothers (FC). The cases were enrolled from 2013 to 2016; these were compared to normal fetal controls (FC) recruited from 2012 to 2016. Of the 23 FMDM, 18 were recruited at Bronx Lebanon Hospital Center, Bronx, New York (Center 1), the remaining 5 were enrolled at Barcelona Center for Fetal and Neonatal Medicine (Center 2). Of the 31 FC, 9

were enrolled at Center 1 and the remaining at Center 2. All mothers were referred for standard of care clinical indications.[15] Fetuses with arrhythmias, congenital heart disease, known genetic and chromosomal abnormalities, and multiple gestations were excluded. Singleton fetuses of mothers with DM and with structurally normal hearts without hypertrophy were included as cases, if the mothers agreed to participate and signed an informed consent. Cardiac hypertrophy was assessed based on gestational age and previously published nomograms.[16] Singleton fetuses of mothers without DM, with structurally and functionally normal hearts, and with the following additional inclusion criteria were included as FC:

a) Estimated fetal weight within the 10th and 90th percentiles.

b) No history of medical, surgical or obstetric complications.

Gestational age was based on the beginning of the last menstrual period and verified by sonographic measurement of the crown-rump length in early pregnancy. The Institutional Review Boards at both institutions approved the study protocols.

Estimated fetal weight (EFW) was calculated from the biparietal diameter, head and abdominal circumference, and femur length using the Hadlock formula.[17] Umbilical artery (UA) Doppler was evaluated in a free loop of the umbilical cord. Middle cerebral artery (MCA) Doppler was measured in a transverse view of the fetal skull at the level of its origin from the circle of Willis.[18] Aortic isthmus (AoI) flow velocity was recorded either in a sagittal view of the fetal thorax with a clear

visualization of the aortic arch or in a cross section of the fetal thorax at the level of the 3-vessel and trachea view. Pulse wave Doppler velocity waveforms of the left ventricular outflow tract (LVOT) were obtained in the 5-chamber view and of the right ventricular outflow tract (RVOT) were obtained from the short axis of the fetal heart in sagittal section. Doppler tracings were recorded with the sample volume positioned just proximal to the valve in the center of the vessel. The angle of insonation between the vessel and the Doppler beam was kept as close as possible to 0º and always below 30º. Diameters of the aortic and pulmonary artery valves were measured in frozen real-time magnified images during systole by the leading edge-to-edge method.[19]

Lumped Model of Fetal Circulation

Details of the fetal lumped computational model and its validation have been published previously.[13, 14] A brief description is provided here. The electrical equivalent model of the different compartments of the fetal circulation was constructed using two main building blocks of the arterial segments and peripheral vascular beds. The arterial segments were configured to include the local resistance of blood due to blood viscosity that was modeled with a resistor, the arterial compliance was modeled with a capacitor and the blood inertia was modeled with an inductor. The peripheral vascular bed was constructed based on a three-element Windkessel model. The simplified fetal circulation was modeled as a set of 19 arterial segments and 12 vascular beds as described previously.[13] The amount of blood flow that was distributed towards different

fetal areas, including the brain, the placenta and the coronary arteries, was calculated as the percentage of combined cardiac output (CCO). For the purposes of this study, both FMDM and FC Doppler data were fitted to the validated model. Physical dimensions of all arterial segments were calculated relative to the expected value from gestational age of the fetus using previously published equations.[13, 14] Changes in length and diameter of the fetal arterial segments, vascular bed resistances and compliances were scaled as a function of the EFW too, as described in previous publications from this group and reference data.[14, 20] The patient-specific model fitting was done by means of an optimization algorithm in which a set of 13 parameters were estimated automatically by minimizing the difference of model-based and measured flow waveforms in the study cohort. Statistical comparisons were made from the simulation outputs between FMDM and FC to assess differences.

Statistical Analysis

Descriptive data were expressed as mean $\pm$ standard deviation. Kolmogorov-Smirnov test were conducted in all variables to test for normality. Two-tailed t-test comparisons were made for normally distributed data and Mann-Whitney U test was used for non-parametric data comparisons. All tests of statistical significance were two-sided and a p value ≤ 0.05 was considered significant. Linear regression analysis was performed in FMDM and FC groups for some key parameters to determine the effects of gestation age on the variables (Table 4). Also, liner regression including all the data (FMDM and FC) and an interaction

158 term between GA and case group was performed to evaluate the relationship
159 between gestational age and some key model parameters. All statistical analyses
160 were performed using SPSS version 9.4.
161
162 **Results**
163 Data from 23 FMDM and 31 FC were used to create a personalized fetal
164 circulation computational model. The baseline characteristics in the two groups
165 are detailed in table 1. The median gestational age (weeks) was similar in FMDM
166 and FC. Overall, in the FMDM, 17 mothers were controlled on insulin (10 mothers
167 had type 2 DM, 4 had type 1 DM, 3 had gestational DM), 4 on oral medications
168 (all mothers had gestational diabetes) and 2 were controlled on diet alone (2 had
169 gestational DM). Mean maternal BMI in FMDM group was significantly higher
170 than the FC group. Two mothers in the FMDM group had additional co-
171 morbidities of chronic hypertension, three mothers had pre-pregnancy
172 hypothyroidism and one mother had genetic prothrombin deficiency. None of the
173 mothers in the FC group had additional co-morbidities. The estimated fetal
174 weights (grams) were similar between the two groups. All fetuses were born full
175 term (>37 weeks gestation), except for one born premature at 31 weeks
176 gestation in the FMDM group and one in the FC group at 30 weeks 6 days. Birth
177 weights were also similar in both groups.
178 Table 2 shows the results of the hemodynamic parameters that were measured
179 and modeled from the echocardiographic data. There were significant differences

in the baseline parameters for the velocity time integrals (VTI) of the left and right ventricular outflow tracts (LV_VTI: VTI of left ventricular outflow tract Doppler, RV_VTI: VTI of right ventricular outflow tract Doppler) measured from the recorded fetal Doppler echocardiograms between the two groups. Right ventricular stroke volume (RVSV) and total stroke volume were lower in FMDM; the differences in left ventricular stroke volume (LVSV), RVSV and SV between FMDM and FC became more apparent beyond 22 weeks (Figure 1A, 1B and 1C).

The results of the fitted organ and vessel parameters from the fetal cardiovascular lumped model have been presented in Table 3. There was redistribution of blood flow away from the brain toward the placenta in FMDM. There was an increase in model-based brain resistance (Rbrain/Rbrain0) (FMDM 2.36 $\pm$ 1.66, FC 1.60 $\pm$ 0.85, p 0.03) (Table 3) with associated lower MCA blood volume (MCA_vol/SV) (Figure 2A, Table 2) in FMDM compared to FC. Aortic isthmus blood volume (AoI_vol/SV) (Figure 2B) was higher in FMDM (Table 2). Model-based placental resistance (Rplac/Rplac0) was significantly lower in FMDM compared to FC (0.59$\pm$0.5 vs. 0.91$\pm$0.41; $p<0.05$) (Table 3) with associated increased UA blood volume (UA_vol/SV) (Table 2). Thus, compositely there the cerebral placental blood volume ratio (MCA_vol/UA_vol) was lower in FMDM group (Table 2) (0.23$\pm$0.20 vs. 0.46$\pm$0.34; p 0.05).

Model-based diameters of the cerebral arteries were significantly smaller compared to controls whereas aortic diameters were higher and umbilical arteries

remained unchanged (Table 3, figure 3). No vessels or organ compliances were significantly altered. Pressures estimates by the model were not different at any location. No significant differences in the variables were noted when data was reevaluated after exclusion of FDM with maternal DM control on diet alone or after excluding FDM with maternal hypertension. No significant correlation was found between Rplac/Rplac0 (R^2 = 1.3, p= 0.95) or Rbrain/Rbrain0 (R^2 = 0.0234, p= 0.49) to HgbA1c levels. When evaluating the relationship between GA and some of the model parameters, Rplac/Rplac0 was noted to slightly increase with increasing GA (R^2 = 0.221, p = 0.006) and no significant change was noted in Rbrain/Rbrain0 with GA (R^2 = 0.125, p = 0.08). However, when considering the non-normalized values of both placenta and brain resistances (Rplac, Rbrain) the same results were observed (see supplementary figure). Moreover, when performing the linear regression analysis in their normal values (Rplac0, Rbrain0), no differences between control and FDMD groups were found (see supplementary figure), which suggest that differences in model-based parameters were not due to differences in GA between groups.

Discussion

The present investigation assessed circulatory remodeling in FMDM as compared to FC using a validated lumped model of the fetal circulation. Our key findings from the model are that placental resistance decreases in FMDM, (while compliance remains similar), and that cerebral resistance increases

concomitantly. Consequently, there is a redistribution of blood flow predominantly towards the placenta, and diminished blood flow to the brain with concurrently with decreased SV.

Morphological changes such as vascular anomalies, increased placental thickness and weight have commonly been seen in placentae of women with DM.[7, 21] A higher release of cytokines, such Tumor Necrosis Factor-α, an upregulation of inflammation related genes, increased concentration of vasodilator endogenous nucleoside adenosine and increased vascular endothelial growth factor involvement have been noted in these placentae.[7, 11, 12] Increased size, vascularization and vasodilatation in FMDM placentae support the decrease in placental resistance noted in our study and the resulting alterations in uterine artery flow. Interestingly, no change in placental compliance was found suggesting the absence of fibrosis of tissue damage altering vessel and tissue elasticity.

In this study, we have shown that blood flow to the brain in FMDM is altered with higher brain resistance, lower MCA flow and lower relative cerebral placental blood volume. It is likely that these changes contribute to the functional and developmental neurological abnormalities in FMDM that are seen in postnatal life. Electroencephalograms performed on neonates of MDM have been described to have features suggestive of abnormal development of brain function that correlate to maternal diabetes control.[22] Abnormal visual evoked

potentials, lower cognitive scores and lower gross and fine motor achievements as well as higher attention deficits are seen in children born to MDM.[6, 23]

We noted decreased SV in FMDM compared to FC. In a previous publication, we have noted a subclinical decrease in myocardial deformation in FMDM that further supports this finding.[3] There have been limited publications that evaluate CO in MDM. Previous fetal MCA and UA Doppler studies have not been able to demonstrate any changes in FMDM likely due to their limited and focused evaluations.[24, 25]

The EFW in FMDM were comparable to FC. It is speculative if other circulatory abnormalities may be seen in FMDM who are large for gestational age or have evidence of intra-uterine growth retardation. The circulatory abnormalities in IUGR have been well characterized. A fascinating observation in this report was that, from 22 weeks GA, there seemed to a different trend in the change in circulatory parameters with GA between FMDM and FC (Figures 2-4). It is unknown if these alterations are a continuum of ongoing processes from the first trimester or if this GA represents a critical tipping point when the changes become irreversible.

The observed decrease in stroke volume (and to a lesser extent cardiac output) is either related to myocardial dysfunction or to decreased demand from the peripheral organs. Given that there is no evidence of pressure overload and that the enlarged placenta, with increased flow, likely increases oxygenation and nutrition, a decreased demand is most likely. Interestingly, this seems to go

together with a trend of a blunted decrease in organ resistances/diameters (in our model, the brain and coronaries, from literature, possibly the kidneys as well), ultimately resulting in the decreased organ flow as clearly illustrated in the brain in FMDM and potentially predisposing them to post-natal problems when oxygenation and nutrition normalizes.

The present fetal circulation model does not account for changes that may occur because of other alterations in fetal milieu such as chemical and inflammatory markers and genetic influences in FMDM. However, since we used patient specific data to build the model and its boundary conditions (GA, EFW, heart rate, Doppler velocities and valve radius) to estimate the specific hemodynamic parameters variation for each individual fetus, we believe this provides a reasonable estimate of the circulatory adaptations in FMDM. Limitations of the model have been discussed in a previous publication.[14] The changes described in this study may not be applicable to all trimesters of pregnancy in FMDM. Additionally, most mothers in the FDM group were well controlled. It is likely that some changes in the FDM were blunted because of the adequate glucose control in the mothers; it is speculative that the results may vary in the setting of inadequate maternal diabetes control. Despite these significant limitations, the novel application of these emerging methods suggests the potential for future applications in prospective studies.

This study provides a comprehensive evaluation of the circulatory remodeling in FMDM using patient specific computational modeling. Increased cerebral resistance and decreased placental resistance contribute to the reversal of CPR that is unique to FMDM. The prognostic impact of these findings is unclear at the present time, however, we believe this study is utilitarian to future investigations.

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A.K designed the study, collected data and wrote the manuscript. PGC designed the study, analyzed the data and edited the manuscript. ABK, JML, KB, BVL, MCL, OG researched data and aided in data collection. EG contributed to discussion. FC collected data and reviewed/edited manuscript. BB reviewed data, reviewed/edited manuscript.

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Figure legends:

Figure 1: Regression plots illustrating left ventricular stroke volume (LVSV)(1A), right ventricular stroke volume (RVSV) (1B) and total stroke volume (SV) (1C) as a function of gestation age (GA)

Figure 2: Regression plots illustrating middle cerebral artery blood volume (MCA_vol)(2A) and aortic isthmus blood volume (AoI_vol) (2B) as a function of gestation age (GA)

Figure 3: Regression plots illustrating the modelled vessel diameters (relative to the expected value for gestational age (GA) from literature) and their changes with GA: A: aortic diameter (Ao_diam); B: cerebral artery diameter (cA_diam); C: umbilical artery diameter (uA_diam)

425 **Table 1:** Baseline maternal, fetal and neonatal characteristics in diabetic and
426 control groups

	Diabetic group (n=23)	Control group (n=31)	P value
Gestational age **at time of fetal echocardiogram** (weeks)	26.8±3.8	28.1±4.1	0.24
Hemoglobin A1c	6.06±0.8%	-	-
Maternal BMI	32.5±7.4	23.8+4.02	<0.001
Estimated fetal weights (grams)	1164±683	1371±688	0.58
Birth weights (grams)	3411±554	3240±556	0.27

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Table 2: Comparisons of (modeled and measured) flow related parameters between fetuses of mothers with diabetic mellitus (FMDM) and fetal controls (FC)

	Variable	FMDM (n=23)	FC (n=31)	p value
Heart	**LV_VTI (cm)**	1.57 ± 0.96	2.19 ± 1.23	0.058§
	RV_VTI (cm)	2.08 ± 1.14	2.78 ± 1.35	0.069§
	LV_SV (ml)	1.57 ± 0.96	2.19 ± 1.23	0.058§
	RV_SV (ml)	2.08 ± 1.14	2.78 ± 1.35	0.05*
	SV (ml)	3.65 ± 2.05	4.97 ± 2.45	0.04*
	RCO (ml/min)	303 ± 165	391 ± 183	0.07
	LCO (ml/min)	228 ± 138	307 ± 160	0.076§
	CCO (ml/min)	531 ± 295	698 ± 326	0.06
	HR	147 ± 9	142 ± 10	0.05*
Brain	**MCA_VTI (cm)**	6.44 ± 2.04	7.09 ± 2.17	0.48
	MCA_vol (ml)‡	0.12 ± 0.14	0.27 ± 0.21	0.01*§
	MCA_vol/SV (%)‡	5.79 ± 3.94	9.08 ± 5.06	0.01*§
Aorta	**AoI_VTI (cm)**	11.6 ± 2.07	11.29 ± 2.62	0.82
	AoI_vol (ml)‡	0.81 ± 0.48	0.78 ± 0.44	0.82
	AoI_vol/SV (%)‡	23.07 ± 8.93	16.8 ± 5.83	< 0.01*§
Placenta	**UA_VTI (cm)**	10.57 ± 2.53	11.38 ± 3.71	0.86
	UA_vol (ml)‡	0.64 ± 0.56	0.56 ± 0.27	0.54
	UA_vol/SV (%)‡	34.1 ± 15.94	25.32 ± 11.7	0.01*§
	MCA_vol/UA_vol‡	0.23 ± 0.20	0.46 ± 0.34	0.01*§

LV_VTI: Velocity time integral (VTI) of left ventricular outflow tract Doppler, RV_VTI: VTI of right ventricular outflow tract Doppler, LV_SV: Left ventricular stroke volume, RV_SV: Right ventricular stroke volume, SV: Stroke volume, RCO: Right ventricular cardiac output, LCO: Left Ventricular cardiac output, CCO: Combined left and right ventricular cardiac output, HR: Heart rate MCA_VTI: VTI of middle cerebral artery Doppler, MCA_vol: Middle cerebral artery blood volume/heartbeat, AoI_VTI: VTI of aortic isthmus Doppler, AoI_vol: Aortic isthmus blood volume/heartbeat, UA_VTI: VTI of umbilical arterial Doppler, UA_vol: Umbilical Artery blood volume/heartbeat , ‡ Modeled variables**,**

*Significant $p \leq 0.05$, § not-normally distributed.

Table 3: Comparisons of the modeled vessel diameters, organ resistances and compliances between fetuses of mothers with diabetic mellitus (FMDM) and fetal controls (FC)

Variable	FMDM (n=23)	FC (n=31)	p value
Rplac/Rplac0	0.59 ± 0.5	0.91 ± 0.41	<0.01*§
Cplac/Cplac0	1.54±0.78	2.12±1.35	0.07
Rbrain/Rbrain0	2.36 ± 1.66	1.60 ± 0.85	0.03*
Cbrain/Cbrain0	0.43±0.46	0.40±0.25	0.74
RcorA/RcorA0	1.64 ± 0.60	1.65 ± 1.10	0.09§
D_Aorta/D_Aorta0	1.19 ± 0.25	1.07 ± 0.14	0.05*§
C_Aorta/C_Aorta0	2.49±0.90	2.69±1.12	0.48
D_cerA/D_cerA0	0.73 ± 0.25	1.00 ± 0.33	< 0.01*§
C_cerA/C_cerA0	1.04±1.26	0.75±0.67	0.28
D_UA/D_UA0	1.18 ± 0.32	1.10 ± 0.19	0.35
C_UA/CUA0	1.87±1.14	1.80±0.84	0.80

Rplac: Placental resistance, Cplac: Placental compliance, Rbrain: Brain resistance, Cbrain: Brain compliance, RcorA: Coronary arteries resistance, D_Aorta: Aorta diameter, C_Aorta: Aorta compliance, D_cerA: Cerebral arteries diameter, C_cerA: Cerebral arteries compliance, D_UA: Umbilical arteries diameter, C_UA: Umbilical arteries compliance, * Significant $p \leq 0.05$, § not-normally distributed.

Table 4: Linear Regression Analysis of Fetuses of Mothers with Diabetes Mellitus (FMDM) and Fetal Controls (FC)

Variable	R^2 FMDM (n=23)	R^2 FC (n=31)
Rplac/Rplac0	0.055	0.188
Rbrain/Rbrain0	0.024	0.095
RcorA/RcorA0	0.099	0.528
LV_SV	0.615	0.753
RV_SV	0.707	0.783
MCA_vol	0.395	0.596
AoI_vol	0.412	0.467
UA_vol	0.314	0.422
CCO	0.685	0.849

Rplac: Placental resistance, Rbrain: Brain resistance, RcorA: Coronary arteries resistance, LV_SV: Left ventricular stroke volume cardiac output, RV_SV: Right ventricular stroke volume, MCA_vol: Middle cerebral artery blood volume/heartbeat, AoI_vol: Aortic isthmus blood volume/heartbeat, UA_vol: Uterine Artery blood volume/heartbeat, CCO: Combined left and right ventricular cardiac output.