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Placental structural abnormalities have detrimental hemodynamic consequences in a rat model of maternal hyperglycemia

Lara Lehtoranta [a, b, c, *], Olli Vuolteenaho [b], Jukka Laine [d], Lauri Polari [e], Eeva Ekholm [a], Juha Räsänen [b, f, g]

[a] Department of Obstetrics and Gynecology, University of Turku and Turku University Hospital, Turku, Finland
[b] Institute of Biomedicine, Department of Physiology, University of Oulu, Finland
[c] The Research Centre of Applied and Preventive Cardiovascular Medicine (CAPC), University of Turku, Finland
[d] Department of Pathology, Turku University Hospital, Finland
[e] Department of Cell Biology and Anatomy, University of Turku, and Turku Center for Disease Modeling (TCDM), Finland
[f] Department of Obstetrics and Gynecology, Kuopio University Hospital and University of Eastern Finland, Kuopio, Finland
[g] Department of Obstetrics and Gynecology, Oulu University Hospital, Oulu, Finland

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ABSTRACT

Introduction: Human type 1 diabetic pregnancy is associated with placental structural and hemodynamic abnormalities. We hypothesized that in rat fetuses of hyperglycemic dams, placental and fetal blood flow velocity waveforms demonstrate compromised hemodynamics when compared to control fetuses, and these hemodynamic parameters correlate with placental structural abnormalities at near term gestation.
Methods: Streptozotocin-induced maternal hyperglycemia group comprised 10 dams with 107 fetuses and the control group 20 dams with 219 fetuses. Doppler-ultrasonographic examinations were performed at gestational days 13–14, 16–17, and 19–21. After the last examination, placentas were collected for morphologic, gene expression, and cytokine analysis.
Results: Umbilical artery (UA), descending aorta (DAO), and ductus venosus (DV) pulsatility indices (PI) were significantly higher at each study point in maternal hyperglycemia compared to controls. Placental size, glycogen storages, venous thrombosis formation, and fluid accumulation were increased in maternal hyperglycemia. Epidermal growth factor receptor (*Edgfrb*), platelet derived growth factor receptor beta polypeptide (*Pdgfrb*), and tumor necrosis factor receptor superfamily, member 12α (Tnfrsf12α) expressions were decreased. Interleukin (IL) −2 and −4 concentrations were decreased, and IL-1beta levels were increased in maternal hyperglycemia. UA PIs correlated positively with DV PIV, DAO PI, fluid accumulation, and glycogen storages. UA PIs correlated negatively with IL-4, *Edgfrb*, and *Pdgfrb*.
Discussion: In maternal hyperglycemia, placental and fetal hemodynamics were compromised during the last trimester of pregnancy compared to normoglycemic pregnancies. Placental structural, metabolic, and growth related gene expression, and inflammatory marker abnormalities were associated with hemodynamic compromise.

1. Introduction

Maternal type 1 diabetes is associated with increased placental volume and branching of villous capillaries with disruptions in stromal structure of villi [1], as well as increased placental weight and glycogen content [2]. Furthermore, villous immaturity, fibrinoid necrosis, and vessel chorangiosis are seen [3]. Placental structural and morphologic abnormalities are present even in diabetic pregnancies with optimal glycemic control [4]. Unfortunately, all too often in diabetic pregnancies, maternal glycemic control is far from optimal [5]. Therefore, it is clinically important to understand the physiologic consequences of these placental alterations. Previous experimental studies have shown that streptozotocin (STZ)-induced maternal hyperglycemia was associated with similar placental abnormalities found in human pregnancies [6–8].

In early pregnancy placenta coordinates nutrient transport that depends on maternal nutrient availability, matching fetal growth

* Corresponding author. Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Kiinamyllynkatu 10, FIN-20520 Turku, Finland.
E-mail address: lara.lehtoranta@utu.fi (L. Lehtoranta).

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List of abbreviations

DAO	descending aorta
DV	ductus venosus
ECC	European Community Council
Egfr	epidermal growth factor receptor
Egln3	hypoxia-inducible factor prolyl hydroxylase 3
G-CSF	Granulocyte colony-stimulating factor
GD	gestational day
GM-CSF	Granulocyte macrophage colony-stimulating factor
Gpx3	glutathione peroxidase 3
GRO-KC	Chemokine C-X-C motif ligand 1
H & E	hematoxylin-eosin
Hsl	hormone-sensitive lipase
IFNγ	interferon-γ
IGF	insulin-like growth factor
Igf2bp3	insulin-like growth factor 2 mRNA binding protein 3
IGFBP	insulin-like growth factor binding protein
Igfbp6	insulin-like growth factor binding protein 6
IL	interleukin
Ldha	lactate dehydrogenase A
MCP1	monocyte chemoattractant protein 1
P27kip1	cyclin-dependent kinase inhibitor 1B
PAS	Periodic acid-Schiff
dPAS	Periodic acid-Schiff diastase
Pdgfrb	platelet derived growth factor receptor beta polypeptide
PI	pulsatility index
PIV	pulsatility index for veins
PlGF	placental growth factor
Serca2	sarcoplasmic reticulum Ca^{2+}-ATPase
Slc2a3	facilitated glucose transporter 3
Slc2a4	facilitated glucose transporter 4
STZ	streptozotocin
TNFα	tumor necrosis factor α
UA	umbilical artery
Ucp2	uncoupling protein 2
Ucp3	uncoupling protein 3
US	ultrasonography
Vegfa	vascular endothelial growth factor A

rate to nutrient supply [9]. In human diabetic pregnancies, glucose transfer across the placenta is increased leading to fetal hyperinsulinemia [9]. Increased serum levels of placental growth factor (PlGF), insulin-like growth factor (IGF) −1, IGF-2, IGF binding protein 3 (IGFBP3), and leptin, and decreased serum levels of IGFBP1 have been reported in newborns of diabetic mothers [10].

In human type 1 diabetic pregnancies, placental hemodynamic studies by Doppler ultrasonography have revealed increased umbilical artery (UA) pulsatility index (PI) values that typically reflect the number of placental tertiary villous arterioles [11]. In fetal circulation, ductus venosus (DV) has an important regulatory role in the oxygenated venous blood return from the placenta and the pulsatility of the DV blood flow velocity waveform can reflect fetal cardiac compromise [12]. In human diabetic pregnancies, DV PI for vein (PIV) values are increased compared to controls, even after excluding cases with signs of placental insufficiency. In addition, there is a significant correlation between maternal glycemic control and fetal DV PI values suggesting that intrauterine exposure to hyperglycemia affects fetal heart [13]. Furthermore, fetal descending aortic (DAO) volume blood flow is lower in diabetic pregnancies than in control pregnancies [14].

Based on previous clinical studies on human type 1 diabetic pregnancies, we used a well-established rat model of STZ-induced pregestational maternal hyperglycemia to test our hypothesis that in fetuses of hyperglycemic dams, placental and fetal blood flow velocity waveforms demonstrate compromised hemodynamics when compared to control fetuses during the last trimester of pregnancy. Furthermore, we hypothesized that these hemodynamic parameters correlate with placental structural abnormalities at near term gestation.

2. Materials and methods

The rats were purchased from the University of Turku Central Animal Laboratory and housed in pathogen-free conditions with a 14:10-h light-dark cycle and free access to nutrients. The study protocol was approved by the University of Turku Laboratory Animal Care and Use Committee (permission 1664/06). The animal care conformed to the ECC Directions 86/609/EC and followed the council's principles of laboratory animal care.

In female Sprague Dawley rats, hyperglycemia was induced with an intraperitoneal injection of 35 mg/kg STZ prior to mating [15]. Ten rats with a glucose level exceeding 15 mmol/l comprised the maternal hyperglycemia group (Table 1). Blood glucose levels were obtained 2 days after the STZ-injection (Elite glucometer, Bayer, Leverkusen, Germany). All samples were gathered from the tail vein before noon. Pregestational glucose samples were collected from conscious animals, whereas samples during pregnancy were collected under anesthesia prior to US examination. Twenty healthy female Sprague Dawley rats served as controls. All female rats were caged overnight with a male. Positive vaginal smear designated gestational day (GD) 1. The control group consisted of 219 fetuses and the maternal hyperglycemia group of 107 fetuses. The number of fetuses varied between 9 and 12 for hyperglycemic and 10–16 for control dams.

2.1. Ultrasonography

Ultrasonography was performed on GD 13–14, 16–17, and 19–21 under isoflurane-induced anesthesia (3–4%) in an oxygen-air mixture, and maintained with 1.5–2% isoflurane. Ultrasonography (US) was performed using Acuson Sequoia 512 equipment (Mountain View, CA) with a 15L8W linear array probe (frequency 14.0 MHz, dynamic range contrast 74 dB, edge 0, color frame rate 28/sec, space-time temporal resolution 68%, wall motion filter set 3). The high-pass filter was set at its minimum [16]. The fetuses were localized in each uterine horn starting from the top. From the fetal sagittal view, DAO and DV were located with color Doppler and blood flow velocity waveforms were obtained by pulsed Doppler

Table 1
Maternal glucose concentrations (mmol/l) in the control and maternal hyperglycemia groups.

Variable	n	Control	n	Hyperglycemia
Pregestation	2	5.1 ± 0.2	10	25.0 ± 5.3**
GD 13-14	20	6.2 ± 1.2	9	29.6 ± 4.6**
GD 16-17	20	4.4 ± 0.8	9	29.3 ± 3.3**
GD 19-21	20	4.0 ± 0.8	10	29.6 ± 4.5**

Data are presented as mean ± SD. **p < 0.0001. n, number of mothers. GD, gestational day.