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The effect of pre-existing maternal obesity and diabetes on placental mitochondrial content and electron transport chain activity

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ABSTRACT

Introduction: Mitochondria dysfunction has been extensively implicated in the progression of these metabolic disorders, their role in placental tissue of diabetic and/or obese pregnant women is yet to be investigated. The aim of this study was to determine the effect of pre-existing type 1 and type 2 diabetes mellitus (DM), and pre-existing maternal obesity on placental mitochondrial function as assessed by mitochondrial content, electron transport chain (ETC) complex activities and oxidative stress..

Methods: Human placenta was obtained at the time of term Caesarean section from (i) non-obese ($n = 19$) and obese ($n = 23$) normal glucose tolerant (NGT) pregnant women; (ii) women with type 1 DM ($n = 14$) and BMI-matched NGT women ($n = 14$); and (iii) women with type 2 DM ($n = 11$) and BMI-matched NGT women ($n = 11$). The following endpoints were assessed: placental mitochondrial content by citrate synthase activity and mitochondrial DNA (mtDNA content); mitochondrial respiratory chain activity (complexes I, II, II & III, III and IV), and mitochondrial ROS (as assessed by mitochondrial hydrogen peroxide (H_2O_2) levels).

Results: When compared to placenta from NGT non-obese women, there was significantly lower mitochondrial DNA (mtDNA) content and electron transport chain complex I activity, and significantly higher mitochondrial H_2O_2 levels in placenta from NGT obese women ($P < 0.05$). Placental tissue from type 1 DM women showed significant reductions in ETC complex I, II & III, and III activity and increased H_2O_2 levels when compared to BMI-matched NGT women ($P < 0.05$). Type 2 DM women only exhibited significantly reduced ETC complex II & III activity when compared to BMI-matched NGT women ($P < 0.05$)..

Discussion and conclusions: Women with pre-existing obesity or diabetes have decreased placental mitochondrial respiratory chain enzyme activities which may have detrimental consequences on placental function and therefore fetal growth and development..

1. Introduction

The incidence of type 1 diabetes mellitus (DM) and type 2 DM in pregnancy has been greatly increasing in various regions across the globe [1,2]. Similarly, the number of pregnancies complicated by obesity has also increased over the past 20 years and is now said to be 'common' in our obstetric population [3–5]. Both these pre-existing conditions carry a number of increased risks for both mother and infant. Short-term risks include a higher risk of developing preeclampsia, thrombosis, sleep apnoea and even pregnancy loss while neonatal outcomes include macrosomia, jaundice, birth defects, stillbirth and perinatal death [4,6–10]. Pre-existing diabetes has negative impacts on embryonic development resulting in fetal malformations [11,12]. In the long-term, both mother and their offspring are at higher risk of developing cardiovascular disease and offspring have a markedly increased risk of obesity in adult life [7,9,10,13–15].

Pregnancy is already well described as a heightened state of oxidative stress [16,17] which is attributed to increased oxygen demand of the mitochondrial rich placenta and a decrease in the bodies scavenging antioxidant power during pregnancy [18]. The production of reactive oxygen species (ROS) and/or decreases in antioxidant defences are major underlying mechanisms of the physiopathology of pregnancies complicated by pre-existing

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maternal diabetes [19] and obesity [20]. ROS is involved in many essential processes, including implantation of the pre-embryo, early embryo development, placental nutrient transport and endothelial and vascular function [19]. However, when ROS levels become too high they are also very damaging during pregnancy and may have detrimental effects on embryo and fetal development and may even result in pregnancy loss [19,21,22].

Small amounts of ROS are generated within mitochondria during normal cellular respiration; the aerobic process of forming energy in the form of ATP. During cellular respiration, electrons flow through the five enzyme complexes of the mitochondrial electron-transport chain whereby a low proportion of molecular oxygen is converted to superoxide. However, in damaged or dysfunctional mitochondria, a deficiency in the electron transport chain results in the over generation of superoxide at the expense of ATP production [23,24]. Superoxide can then be converted to other more reactive species that can damage lipids, proteins, and nucleic acids within the mitochondria, leading to loss of function.

There is ample evidence showing that mitochondrial respiratory function and enzymatic activity of mitochondrial complexes are lower in diabetes [25–32] and obesity [33]. Altered mitochondrial function is also evident in pregnancies complicated by pre-eclampsia [34], exposed to high pollutant areas [35], cigarette smoke [36] and hypoxia [37]. There are, however, no studies on the effects of pre-existing maternal diabetes or obesity on placental mitochondrial function. We hypothesise that pregnancies complicated by pre-existing maternal diabetes and obesity will be associated with decreased activity of the electron transport chain complexes and increased mitochondrial ROS. Thus, the purpose of this study was to investigate the effect of pre-existing maternal diabetes type 1 and type 2 DM and obesity on placental mitochondrial content (as assessed by citrate synthase activity and mitochondrial DNA (mtDNA content)); mitochondrial respiratory chain activity (complexes I, II, II & III, III and IV), and mitochondrial ROS (as assessed by mitochondrial hydrogen peroxide (H_2O_2) levels).

2. Materials and methods

2.1. Tissue collection

Approval for this study was obtained from the Mercy Hospital for Women's Research and Ethics Committee and written informed consent was obtained from all participating subjects. Women were invited to provide samples on the day of admission for surgery. All tissues were obtained at the time of term Caesarean section in the absence of labour to ensure there were no effects of labour on placental mitochondrial function. Indications for Caesarean section included repeat Caesarean section or breech presentation. Women fulfilling any of following criteria were excluded; vascular/renal complication, multiple gestations, asthma, smokers, preeclampsia, chorioamnionitis, placental abruption, acute fetal distress and women with any other adverse underlying medical conditions. In addition, women diagnosed with GDM in this pregnancy were excluded.

Human placenta was obtained from a total of 42 normal glucose tolerant (NGT) pregnant women and classified as non-obese or obese based on their pre-pregnancy BMI. Placental samples were collected from non-obese (BMI $\leq$29.9 kg/m^2) and obese (BMI $\geq$30 kg/m^2) subjects. The relevant clinical details of the subjects are detailed in Table 1A. Placental samples were also obtained from women with pre-existing type 1 or type 2 DM. For these patients, the NGT controls were matched for BMI. Diagnosis of pre-existing diabetic women was made before the onset of pregnancy. The relevant clinical details for the type 1 and type 2 DM patients and their respective controls are detailed in Table 1B and 1C, respectively.

Placenta was obtained within 15 min of delivery. Placental lobules (cotyledons) were obtained from various locations of the placenta; the basal plate and chorionic surface were removed from the cotyledon, and villous tissue was obtained. The placenta samples were thoroughly washed in ice-cold PBS, and snap frozen in liquid nitrogen and immediately stored at −80 °C.

2.2. Isolation of intact mitochondria

Isolation of intact mitochondria was performed as previously described [32,38]. Briefly; placental tissue was homogenised in ice-cold Zheng buffer (210 mM mannitol, 70 mM sucrose, 5 mM HEPES and 1 mM EGTA, pH 7.2). The homogenate was centrifuged at 600 g for 10 min at 4 °C and resulting supernatant transferred to a new ice-cold microfuge tube. This was freeze thawed via 1 min freezing in a dry ice methanol bath followed by a 4 min thawing in room temperature water. Freeze thawing was repeated three times. A BCA protein assay (Pierce, Rockford, USA) was then performed following manufacturer's instructions.

Table 1A
Characteristics of the NGT non-obese and NGT obese group.

	NGT non-obese (n = 19)	NGT obese (n = 23)
Maternal age (years)	34.7 ± 4.5	32.2 ± 3.8
Maternal BMI at 12 weeks (kg/m^2)	22.4 (21.2, 23.3)	39.0 (36.1, 45.3)**
Maternal BMI at delivery (kg/m^2)	28.1 (26.3, 29.8)	42.8 (40.8, 46.8)**
Pregnancy fasting OGTT (mmol/l)	4.5 ± 0.3	4.8 ± 0.3
Pregnancy 2 h OGTT (mmol/l)	5.8 ± 1.4	5.6 ± 1.1
Gestational age at birth (weeks)	39.2 ± 0.3	38.9 ± 0.2
Fetal gender	10 Male; 9 Female	11 Male; 12 Female
Neonate birthweight (g)	3469 ± 350	3725 ± 456*
Ponderal index	2.7 ± 0.3	2.8 ± 0.3
Placental weight	574 ± 119	704 ± 171*
Parity	2.0 ± 0.7	2.1 ± 0.7
Gravida	2.7 ± 1.3	2.7 ± 1.3

*P < 0.05 vs. NGT non-obese (Student's t-test).
**P < 0.05 vs. NGT non-obese (Mann–Whitney test).
Data represents the mean ± SD or median (interquartile ranges).

Table 1B
Characteristics of the patients in the type 1 DM study.

	NGT (n = 14)	Type 1 DM (n = 14)
Maternal Age (years)	32.4 ± 5.5	29.9 ± 4.1
Maternal BMI at 12 weeks (kg/m^2)	26.6 ± 7.7	26.9 ± 6.4
Maternal BMI at delivery (kg/m^2)	32.3 ± 7.7	32.1 ± 7.7
HbA1c (%)	Not done	7.2 ± 1.0
Gestational age at birth (weeks)	38.7 ± 0.9	37.2 ± 1.9
Fetal Gender	8 Male; 6 Female	8 Male; 6 Female
Neonate birthweight (g)	3130 (3040, 3321)	3999 (3633,4393)**
Ponderal index	2.6 (2.4,2.8)	2.8 (2.6,3.4)**
Placental weight (g)	562 ± 123	716 ± 171*
Parity	2.1 ± 0.9	1.8 ± 0.6
Gravida	2.6 ± 0.7	2.0 ± 0.8

*P < 0.05 vs. NGT (Student's t-test).
**P < 0.05 vs. NGT (Mann–Whitney test).
Data represents the mean ± SD or median (interquartile ranges).

Table 1C
Characteristics of the patients in the type 2 DM study.

	NGT (n = 11)	Type 2 DM (n = 11)
Maternal Age (years)	33.1 ± 4.2	33.8 ± 4.7
Maternal BMI at 12 weeks (kg/m^2)	36.4 ± 7.3	34.1 ± 7.6
Maternal BMI at delivery (kg/m^2)	39.7 ± 5.5	37.3 ± 8.2
HbA1c (%)	Not done	6.1 ± 0.9
Gestational age at birth (weeks)	38.6 ± 0.8	38.0 ± 1.0
Fetal Gender	7 Male; 4 Female	6 Male; 5 Female
Neonate birth weight (g)	3564 ± 327	3449 ± 551
Ponderal Index	2.7 ± 0.3	2.5 ± 0.3
Placental weight (g)	609 ± 123	625 ± 118
Parity	2.4 ± 0.7	2.2 ± 1.0
Gravida	2.5 ± 0.8	2.5 ± 1.1

Data represents the mean ± SD.

2.3. Isolation of disrupted mitochondria

Isolation of disrupted mitochondria was performed as previously described [32,38]. Placental tissue was homogenised in ice-cold mitochondrial isolation buffer (10 mM HEPES pH 7.5, 200 mM mannitol, 70 mM sucrose, 1.0 mM EGTA, 2.0 mg/ml of fatty acid free BSA). The homogenate was centrifuged at 800 g for 10 min (all centrifugation was at 4 °C). The pellet was discarded, supernatant collected and centrifuged at 8000 g for 15 min. The resulting supernatant was discarded and the pellet re-suspended in the ice-cold mitochondria isolation buffer and centrifuged at 15,000 g for 15 min. The supernatant was discarded and pellet re-suspended in 75 μl