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Mitochondrial function and glucose metabolism in the placenta with gestational diabetes mellitus: role of *miR-143*

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Abstract

A predisposing factor for development of the hyperglycaemic state of gestational diabetes mellitus (GDM) is obesity. We previously showed that increasing maternal obesity is associated with significant reductions in placental mitochondrial respiration. MicroRNA (miR)-143 has been previously shown to regulate the metabolic switch from oxidative phosphorylation to aerobic glycolysis in cancer tissues. We hypothesized that mitochondrial respiration is reduced and aerobic glycolysis is up-regulated via changes in *miR-143* expression in the placenta of women with GDM. Placental tissue was collected at term from women with A1GDM (controlled by diet), A2GDM (controlled by medication) and body mass index (BMI)-matched controls (CTRL). *miR-143* expression was measured by RT-PCR. Expression of mitochondrial complexes, transcription factors peroxisome proliferator-activated receptor- γ co-activator 1 α (PGC1 α) and peroxisome proliferator-activated receptor γ (PPAR γ), components of mammalian target of rapamycin (mTOR) signalling, glucose transporter GLUT1 and glycolytic enzymes [hexokinase-2 (HK-2), phosphofructokinase (PFK) and lactate dehydrogenase (LDH)] were measured by Western blot. Trophoblast respiration was measured by XF24 Analyser. Expression of *miR-143*, mitochondrial complexes, and PPAR γ and PGC1 α , which act downstream of *miR-143*, were significantly decreased in A2GDM placentae compared with A1GDM and CTRL ($P<0.01$). Placental hPL (human placental lactogen) levels, expression of glycolytic enzymes, GLUT1 and mTOR signalling were also significantly increased by more than 2-fold in A2GDM compared with A1GDM and CTRL ($P<0.05$). There was a 50% reduction in mitochondrial respiration in trophoblast cells isolated from A2GDM placentae. Overexpression of *miR-143* was able to increase mitochondrial respiration, increase protein expression of mitochondrial complexes and decrease expression of glycolytic enzymes by 40% compared with A2GDM. Down-regulation of *miR-143* mediates the metabolic switch from oxidative phosphorylation to aerobic glycolysis in placenta of women with A2GDM.

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AUTHOR CONTRIBUTION Sribalasubashini Muralimanoharan, Alina Maloyan and Leslie Myatt prepared the experimental design. Sribalasubashini Muralimanoharan performed all the experiments and conducted data analysis. Alina Maloyan and Leslie Myatt helped in experimental setup and oversaw data analysis. Sribalasubashini Muralimanoharan, Alina Maloyan and Leslie Myatt prepared the manuscript.

Keywords

gestational diabetes; mammalian target of rapamycin (mTOR); *miR-143*; mitochondrial function; placenta

INTRODUCTION

Gestational diabetes mellitus (GDM) is a common metabolic disorder of pregnancy, manifest by impaired maternal glucose tolerance from the late second trimester of pregnancy onwards. The prevalence of GDM has been estimated at ~7% of pregnancies in the USA [1]. Gestational diabetes affects both the mother and the offspring. Elevated blood glucose levels in the mother result in elevated glucose in the fetus, leading to increased fetal insulin secretion. Insulin is a fetal growth factor leading to macrosomia and increased risk of caesarean delivery and birth trauma including vaginal tears, shoulder dystocia and asphyxia [2]. A substantial body of evidence suggests that an abnormal intra-uterine milieu elicited by maternal metabolic disturbances as diverse as malnutrition, placental insufficiency, obesity and diabetes may be able to programme susceptibility of the fetus to develop chronic diseases such as obesity, hypertension, cardiovascular diseases and type 2 diabetes in later life [3]. Studies in the Pima Indian population of the USA showed that the offspring of women with gestational diabetes developed fasting hyperglycaemia, higher rates of abnormal glucose tolerance and obesity, compared with offspring of normoglycemic women [4].

As the placenta regulates nutrient composition and supply from mother to fetus and is the source of hormonal signals that affect maternal and fetal metabolism, appropriate development of the placenta is crucial to normal fetal development [5]. The placenta itself consumes nutrients and therefore the considerable metabolic activity of the placenta provides a large and fundamentally important contribution to determining both the quality and quantity of nutrients available to fetus. Thus, consideration of placental metabolism per se is essential to complete understanding of how the placenta regulates nutrient transfer, energy balance, fetal growth and hence fetal programming [6].

Research on trophoblast over the past decades has underlined striking similarities between the proliferative, migratory and invasive properties of trophoblast and those of cancer cells [7]. Metabolic reprogramming is widely observed during cancer development to confer the ability to survive and proliferate on cancer cells. Cancer cells favour the 'Warburg effect', i.e. enhanced glycolysis or aerobic glycolysis to generate energy over mitochondrial oxidative phosphorylation, even when the ambient oxygen supply is sufficient [8]. On the other hand, growth factor signalling mediated by mammalian target of rapamycin (mTOR) drives metabolism of cancer cells by regulating expression of key enzymes in metabolic pathways. Notably, mutations causing hyper-activation of mTOR are common in cancers [9].

miRNA are small non-coding RNAs that regulate gene expression either through translational repression or mRNA degradation [10]. miRNAs regulate cell proliferation, apoptosis, tumorigenesis and many other physiological or pathological processes including glucose metabolism [11]. Human *miR-143*, located on chromosome 5, is involved in

adipogenesis and has been reported to be down-regulated in several cancers, where it inhibits hexokinase-2 (HK-2) and up-regulates aerobic glycolysis [12].

We and others have previously provided evidence for elevated oxidative stress, hypoxia and inflammation in the placenta in various pathological situations [13-14] and their relationship to altered placental function and fetal programming [15]. We have shown that mitochondrial function/oxidative phosphorylation is significantly reduced in the placenta with increasing maternal adiposity [16-17]. Recently, it was shown that mitochondrial protein expression is reduced in skeletal muscle of GDM patients [18].

Maternal obesity and GDM constitute two common, often comorbid pregnancy complications [19]. In the present study, we hypothesized that mitochondrial function and associated metabolic pathways are dysregulated in placentae from pregnancies complicated by gestational diabetes, and that *miR-143* has a role in regulating mitochondrial function in the GDM placenta. We compared trophoblast respiration and mitochondrial function in placentae from women diagnosed with GDM classified as A1GDM (managed with diet to control blood glucose) and A2GDM (needs pharmacological intervention either by glyburide or insulin) with maternal BMI-matched control placentae.

MATERIALS AND METHODS

Oligomycin, FCCP [4-(trifluoromethoxy) phenyl hydrazone], rotenone and antimycin A were obtained from Sigma and dissolved in DMSO as 2.5 mM stock solutions. The human OxPhos antibody cocktail (MS601) was purchased from Abcam. AKT, AKT (Ser⁴⁷³), 4EBP, 4EBP (Thr³⁸⁹), S6K, pS6K (Thr^{37/46}) and peroxisome proliferator-activated receptor- γ co-activator 1 α (PGC1 α), antibodies were purchased from Cell Signaling Technologies, peroxisome proliferator-activated receptor γ (PPAR γ) antibody was purchased from Santa Cruz Biotechnology.

Collection of placental tissue

Placentas were collected from the Labor and Delivery Unit at University Hospital under a protocol approved by the Institutional Review Board of the University of Texas Health Science Center San Antonio, with informed consent from the patients. Placental villous tissue was collected at term after C-section (no labour) from women with gestational diabetes either treated with diet alone (A1GDM) or treated with glyburide or insulin (A2GDM) ($n = 6$ /each group). The control group (CTRL) included placentae collected from healthy women with uncomplicated pregnancies, matched for pre-pregnancy BMI (within 0.5 units difference) and gestational age ($n = 6$). Villous tissue was randomly sampled from five sites in the placenta as described previously [20], flash frozen in liquid nitrogen and stored at -80°C or immediately used for trophoblast culture.

Placental lactogen ELISA

Human placental lactogen (hPL) was measured in placental homogenate using a sandwich ELISA from Genway (GWB-8582D2). Briefly, an aliquot of placental homogenate is incubated in wells coated with monoclonal anti-hPL antibody conjugated with horseradish

peroxidase. The amount of bound peroxidase is proportional to the concentration of hPL in the homogenate.

RNA isolation and RT-PCR

Total RNA including miRNA was isolated from villous tissue and trophoblast cells using miRNAeasy kit (Qiagen). RNA concentration was measured using a Nanodrop 2000. The High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) was used for RT of 1 µg of the total RNA from villous tissue and trophoblast cells. mRNA expression of HK-2, phosphofructokinase (PFK), lactate dehydrogenase (LDH), glucose transporter 1 (GLUT1) PPAR γ and PGC1 α was measured (Primer sequence, listed in Supplementary Table S1). The C_T values were normalized to 18S RNA. For the relative quantification of *miR-143* in placental tissues, 5–10 ng of total RNA was reverse transcribed using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems). *miR-143* expression was determined by using TaqMan assays (Applied Biosystems) and the TaqMan assay custom designed for U18 RNA was used for normalization. Reactions were performed using a Step-One Plus system (Applied Biosystems). U18 miRNA expression was used as the internal control for *miR-143* expression. The changes in the cycle threshold (C_T) values were calculated by the equation $C_T = C_{T \text{ target}} - C_{T \text{ input}}$. The fold differences were calculated as follows: fold difference = $2^{- (C_T)C_T}$.

Western blots

Total proteins for Western blotting were obtained from villous tissue using a standard differential centrifugation protocol and suspended in isolation medium (0.25 M sucrose and 1 mM EDTA, pH 7.4) supplemented with protease and phosphatase inhibitor cocktail (Sigma). Total protein concentrations in the fraction were determined using Bradford's reagent (Bio-Rad Laboratories). Total proteins (25 µg) were separated on 4–20% precast linear gradient gels (Bio-Rad Laboratories), transferred to nitrocellulose membranes and blocked with 5% (w/v) nonfat milk in TBST for 1 h. Membranes were incubated overnight at 4°C with primary antibody diluted in 1% nonfat milk (w/v) in TBST and detected using an appropriate peroxidase conjugated secondary antibody. Products were visualized by ECL chemiluminescence (Millipore). Band intensities were measured using the G-box system (Syngene).

Isolation and culture of primary trophoblasts

Villous cytotrophoblasts (CTs) were isolated from placental tissue of women with A2GDM and BMI matched CTRL using a trypsin/DNAse digestion and percoll gradient purification method as we have previously described [21]. CTs cells were plated in Seahorse XF24 plates, cultured in DMEM containing glucose (17 mM) and allowed to syncytialize over 72 h.

Reporter assay

Primary trophoblast cells isolated from A2GDM placentae were immediately transfected with 1 nM pre *miR-143* or negative control miRNA as described previously [22]. After 24 h cells were washed and transfected with the pGL3+vector containing 3'UTR HK-2 or mutant

3'UTR HK-2 along with *Renilla* normalization control pRL-TK [22]. Luciferase and *Renilla* luminescence was measured 48 h later using the Dual-Glo luciferase kit (E2940 Promega).

Assessment of mitochondrial function

Mitochondrial function of the cultured syncytiotrophoblasts (ST) was measured using a Seahorse XF24 analyser (Seahorse Biosciences) as described [21]. Oxygen consumption rates (OCR) were normalized to total cellular protein (Bradford method). Basal respiration was calculated from four baseline OCR readings. ATP coupled, maximum respiration, spare capacity, proton leak and non-mitochondrial respiration were calculated from OCR readings following the injection of oligomycin (1 μ M), FCCP (1 μ M) and a mixture of rotenone (3 μ M) and antimycin A (1.5 μ M).

Statistical analysis

Data are reported as mean $\pm$ S.E.M. Comparisons between two groups were performed using a Student's *t* test. One or two-way ANOVA and Turkey's post-hoc test was used to compare data sets with more than two groups. $P < 0.05$ was considered significant. The OCR parameters were analysed by regression and correlation analysis against maternal BMI using Excel and GraphPad (5.0).

RESULTS

Clinical characteristics of the patients involved in the present study are shown in Table 1. The placental weight was significantly higher ($P < 0.04$) in A2GDM (833 ± 53 g) but not in A1GDM (720 ± 76 g, $P < 0.05$) compared with control group (690 ± 41 g). The fetal/placental weight ratio was also significantly lower ($P < 0.03$) in A2GDM (3.45 ± 0.02) but not in A1GDM (4.54 ± 0.03) compared with control group (5.26 ± 0.02). No significant difference was observed in maternal glucose during the diagnostic test for gestational diabetes between women who eventually developed A1 or A2 gestational diabetes.

Dysregulation of placental and mitochondrial function in GDM placentae

hPL production is an indicator of placental function. hPL levels in placentae from A1GDM and A2GDM were significantly higher than BMI matched CTRL (Figure 1A). Previously we have shown that mitochondrial function is compromised in the placentae with increasing maternal adiposity when compared with CTRL placentae. We found mitochondrial electron transfer complex subunits I, II, III and IV were significantly reduced in A2GDM placentae compared with CTRL and to A1GDM. However, complexes III and IV were also significantly reduced in A1GDM compared with CTRL (Figure 1B). Messenger RNA expression of PGC1 α , which regulates mitochondrial biogenesis, and PPAR γ , an important transcription factor regulating mitochondrial fatty acid oxidation, was significantly reduced in placental tissue of A2GDM placentae compared with CTRL but was not different between A1GDM and CTRL (Figure 1C). However at the protein level expression of both PGC1 α and PPAR γ was significantly reduced in A1GDM compared with CTRL and was further significantly reduced in A2GDM compared with A1GDM (Figure 1D).

Glycolytic pathway is activated in GDM placentae

Expression of the glucose transporter GLUT1, of HK-2, the first enzyme of the glycolytic pathway, and PFK and LDH was significantly up-regulated both at mRNA and protein levels in A2GDM compared with CTRL and to A1GDM placental tissue (Figures 2A and 2B). No difference was observed between A1GDM and CTRL.

Expression of *miR-143* in placentae from pregnancies with GDM

miR-143 has been previously reported to up-regulate aerobic glycolysis in several cancer tissues. In the present study, we found a significant 50% reduction of *miR-143* in placental tissue from A2GDM but no such difference was observed in A1GDM compared with the BMI matched CTRL group (Figure 3A). Luciferase reporter activity was significantly down-regulated in trophoblast cells co-transfected with *miR-143* and HK-2 (3'UTR) compared with those co-transfected with *miR-143* and HK-2 (mutant 3'UTR). Thus, the Luciferase reporter assay confirmed that HK-2 is a direct target of *miR-143* (Figure 3B).

Activation of mTOR signalling in GDM placentae

mTOR signalling constitutes a master regulator of protein translation, thereby controlling cell growth and metabolism. The energy status of the cell is signalled to mTORC1 through AMP-activated protein kinase (AMPK), a sensor of intracellular energy status. In response to energy depletion (low ATP/ADP ratio), AMPK is activated and in turn reduces mTORC1 activation. Additionally, AMPK can reduce mTORC1 activity in response to energy depletion by directly phosphorylating Raptor. In the present study, we found significantly reduced phosphorylation of AMPK and elevated mTOR activation. The downstream effects of mTORC1 are mediated by phosphorylation of eukaryotic initiation factor 4E-binding protein 1 (4EBP1) and p70 S6 kinase (S6K). We found significant up-regulation of p70 S6K and 4EBP1 in A2GDM compared with CTRL and A1GDM groups (Figure 4).

Overexpression of *miR-143* reduces aerobic glycolysis and rescues mitochondrial complexes in trophoblast cells

When *miR-143* was overexpressed in trophoblast cells (72 h) from A2GDM placentae a significant reduction in HK-2, PFK and LDH expression was seen compared with controls (Figures 5A and 5B). On the other hand mitochondrial complexes I, II and III were significantly up-regulated when *miR-143* was overexpressed in A2GDM trophoblast cells (Figures 5C and 5D). No changes were observed in complexes IV and V in trophoblast cells where *miR-143* was overexpressed. Mitochondrial oxygen consumption was measured using the mitostress test to determine basal, ATP-coupled and maximal respiration and the reserve (or spare) capacity of trophoblast using the Seahorse XF24 [21]. We found no difference in the rates of trophoblast syncytialization with GDM. However, we found significant down-regulation of basal, ATP coupled, maximum respiration and spare capacity in the ST from A2GDM compared with BMI matched CTRL (Figures 5E and 5F). Transient overexpression of pre-*miR-143* in the trophoblast was able to rescue the basal and maximum mitochondrial respiration to a level close to the BMI matched CTRL (Figure 5G).

DISCUSSION

The placenta has multiple roles: providing the immune interface between the mother and the fetal allograft, serving to transport nutrients and waste products between mother and fetus and being a source of many peptide and steroid hormones that influence fetal, placental and maternal metabolism and development [5]. The placenta is thought to have a critical role in the pathogenesis of GDM, as GDM-associated complications resolve following delivery of the placenta [23]. Although there was a tendency towards increased fetal weight in the A1 and A2GDM groups compared with BMI matched controls the differences were not significant. However the placental weight was higher and the fetal/placental weight ratio significantly lower in the A2GDM group suggesting that placental hypertrophy occurs to maintain fetal weight and hence the placenta is less efficient compared with other groups. The Apgar score was also not significantly different between the groups (results not shown). So fetal size is maintained by placental hypertrophy in the face of reduced placental respiration.

hPL is a major stimulus for adaptation of the maternal endocrine pancreas during gestation, thereby increasing β -cell mass-insulin production and hence helping the mother to compensate for hyperglycaemia during pregnancy [24]. Concentrations of hPL increase progressively throughout gestation and are secreted directly into both fetal and maternal circulations [25]. Increased levels of hPL in the maternal circulation have been reported, thought to be due to increased placental size [26], although our data suggest increased production per unit of tissue. Maternal hyperglycaemia impacts the production of various placental proteins and hormones including hPL, with dysregulation potentially adversely impacting fetal growth and postnatal metabolic function [25].

In healthy humans and rats, at least one-third of glucose entering the placenta is metabolized by oxidative phosphorylation through mitochondria to cover the high metabolic rate of the trophoblast [27]. In the present study, we observed significant reduction in expression of mitochondrial complexes *in vivo* and respiration *in vitro* in the A2GDM placentae. We have previously shown reduction in the expression of mitochondrial respiratory complexes and activity in placentae from pregnancies complicated by preeclampsia and maternal adiposity as well as reduced respiration in the isolated trophoblast cells of placentae from obese mothers [28-29]. Hastie et al. [17] have shown that placentae of women with pre-pregnancy obesity or pre-gestational diabetes have decreased expression of mitochondrial respiratory chain enzymes, which may have detrimental consequences on placental function. Recently a pilot study by Qiu et al. [30] found reduced mitochondrial DNA copy number and oxidative DNA damage in placental tissue from GDM women. The A2GDM subjects were being treated with either glyburide or insulin and it is conceivable that the reduction in respiration is due to the drug, however very little if any glyburide crosses the placenta [31] as the placenta actively effluxes glyburide [32], and it is bound tightly to plasma proteins making it unlikely that there is cellular uptake and effect in the placenta.

The peroxisome proliferator-activated receptors (PPARs) are a family of transcription factors that regulate energy balance by promoting either energy deposition or energy dissipation [33]. PGC1 α is a co-activator of PPAR γ that induces the expression of genes that promote

mitochondrial biogenesis and fatty acid oxidation [34]. Down-regulation of both PGC1 α and PPAR γ contributes to decreasing mitochondrial function in A2GDM placentae. Our data are consistent with the recent study in high fat-fed rats showing reduced mRNA levels of genes associated with mitochondrial function, including PGC1 α in the placenta as well in the liver and skeletal muscle of the offspring [34].

GLUT1 is a ubiquitous isoform of the facilitated-diffusion glucose transporter family, expressed in almost all tissues including ST of placenta [35]. GLUT1 expression and activity appear to be inversely related to extracellular glucose concentration, however, within the physiological range, GLUT1 expression is relatively refractory to glucose concentration [36]. We found significantly elevated GLUT1 expression in A2GDM placentae compared with A1GDM and CTRL, which is consistent with reports that GLUT1 transporter expression is increased in the basal membrane in diabetic pregnancies [37]. We reasoned that increased availability and consumption of glucose in GDM placentae might be reflected by an increased expression or activity of rate-limiting enzymes in the glycolytic pathway. Consistently we found significantly higher expression of HK-2, PFK (rate-limiting enzymes) and LDH (marker for aerobic glycolysis) in A2GDM placentae. It appears then that mitochondrial function is compromised and glycolysis is up-regulated in A2GDM placentae.

miRNAs are expressed in placenta and alterations in expression have been described in association with exposure to xenobiotics and cigarette smoking or with adverse pregnancy outcomes including PE and growth restriction [10]. Placenta-specific miRNAs may contribute to the pathology of GDM. *miR-143* represents one of the best-characterized anti-oncomiRs [38], is located at a fragile site, often inhibited in cancers, and its expression is frequently down-regulated in cancer cell lines and in primary tumours such as colon and gastric cancers, and B-cell lymphoma [12]. Accordingly, *miR-143* overexpression inhibits cell proliferation in several cancer cell lines, suggesting that the loss of *miR-143* observed in human cancers maintains and supports tumour growth [39]. HK-2 is a well-studied target of *miR-143* with their expression being inversely proportional to each other. Interestingly, similar to our results in A2GDM placentae, *miR-143* expression was inversely associated with HK-2 expression in human lung cancer samples [40]. Peschiaroli et al. [11] have reported that *miR-143* inhibits HK-2 expression via a conserved *miR-143* recognition motif located in the 3'UTR of HK-2 mRNA in head and neck squamous cell carcinoma. Consistently, we found *miR-143* overexpression in isolated trophoblast cells could inhibit luciferase activity in the 3'UTR of HK-2 expressing cells compared with mutant 3'UTR of HK-2. To the best of our knowledge, this is the first report describing the role of *miR-143* in glucose metabolism in placenta.

AMPK is the primary cellular energy sensor and is phosphorylated at Thr¹⁷² in response to increased AMP/ATP ratio as associated with energy deprivation [41]. Down-regulation of pAMPK in A2GDM placentae might be due to the excessive nutrient availability/hyperglycaemia observed in the placentae. We also observed significantly higher levels of AKT (Ser⁴⁷³) phosphorylation in A2GDM placentae. The observed changes in AMPK and AKT signalling may therefore activate mTOR. mTOR is a serine/threonine kinase and represents another important nutrient-sensing pathway in mammalian cells, controlling cell growth, proliferation and metabolism in response to nutrient availability (hyperglycaemia)

and growth factor signalling [42]. Activation of mTOR is known to inhibit *miR-143* expression in cancer tissue [43]. mTOR has been suggested to have a very significant role in determining the energy shift away from mitochondrial oxidative phosphorylation to aerobic glycolysis [44]. In contrast reports also suggest that in rat skeletal muscle exercise-induced mTOR activation induces mitochondrial biogenesis and oxidative capacity [43]. However, activated mTOR and the shift towards aerobic glycolysis in the A2GDM placenta need to be investigated further in detail.

Interestingly, the *miR-143* promoter has binding sites for PPAR γ . Further studies on the regulation of *miR-143* by PPAR γ will reveal the relevance of *miR-143* to mitochondrial function in A2GDM placenta. However, transient overexpression of pre *miR-143* in trophoblast cells isolated from A2GDM placenta was able to rescue respiration compared with CTRL cells, reduce the glycolytic enzyme and improve mitochondrial complexes. These data collectively suggest that mitochondrial function is compromised in A2GDM placenta compared with A1 and BMI matched CTRL.

The placenta is a programming agent of adult health and disease [45]. Adaptations of placental phenotype in response to maternal diet and metabolic status alter fetal nutrient supply. It is therefore essential to understand how these factors can impact development and predispose individuals to metabolic disorders in the long term [45]. Hyperglycaemia during GDM results in changes in placental function, particularly with respect to the up-take, transfer and/or utilization of glucose [46]. Therefore, it is important when assessing placental handling of glucose to consider not only transport, but also placental utilization of glucose. In summary, we found significantly elevated hPL, reduced mitochondrial function, increased glycolysis, reduced *miR-143* and mTOR activation in A2GDM placenta but not in A1GDM placenta. Importantly *miR-143* overexpression was able to partially rescue mitochondrial function and reduce glycolytic enzymes in the trophoblast cells from A2GDM placenta. We have provided evidence for the role of *miR-143* in mediating the switch to aerobic glycolysis in term A2GDM placenta. Previously we have shown sexual dimorphism in the role of *miR-210* in the placental function with increasing maternal adiposity [29]. However due to small sample size we were not able to determine the fetal sex-dependent differences in *miR-143* in the placenta with GDM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

4EBP eukaryotic translation initiation factor 4E-binding protein

A1GDM	GDM controlled by diet and exercise
A2GDM	GDM treated with medication
BMI	body mass index
AMPK	5'AMP-activated protein kinase
CT	cytotrophoblasts
CTRL	non-diabetic control
FCCP	<i>p</i> -trifluoromethoxy carbonyl cyanide phenyl hydrazone
GDM	gestational diabetes mellitus
GLUT1	glucose transporter 1
HK-2	hexokinase-2
hPL	human placental lactogen
LDH	lactate dehydrogenase
mTOR	mammalian target of rapamycin
OCR	oxygen consumption rates
PFK	phosphofructokinase
PGC1α	peroxisome proliferator-activated receptor- γ co-activator 1 α
PPARγ	peroxisome proliferator-activated receptor γ
pS6K	ribosomal protein S6 kinase and AKT-serine/threonine-specific protein kinase
ST	syncytiotrophoblasts

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CLINICAL PERSPECTIVES

- Placental efficiency is reduced in women with A2GDM compared with BMI-matched A1GDM and non-diabetic controls. A2GDM placentae show mitochondrial dysfunction and an up-regulation of glycolysis.
- Down-regulation of *miR-143* might be at least partially responsible for mitochondrial dysfunction in A2GDM placentae.
- Metabolic dysfunction in the A2GDM placenta may underlie altered placental nutrient transport, fetal growth and development and hence programming of the offspring for disease in later life.

Figure 1. Dysregulation of mitochondrial function in GDM placentae

(A) hPL levels measured in placental tissue homogenates. (B) Representative Western blots and quantification of mitochondrial complex protein expression in placental tissue. (C) mRNA expression of PGC1 α and PPAR γ in placental villous tissue relative to 18S RNA. (D) Representative Western blot and quantification of PGC1 α and PPAR γ expression relative to actin. Values are mean $\pm$ S.E.M., $n = 6$ in each group. * $P < 0.05$ compared with CTRL and ^a $P < 0.05$ compared with A1GDM.

Figure 2. Expression of glucose transporter GLUT1 and glycolytic enzymes in placentae with GDM

(A) mRNA expression of GLUT1, HK-2, PFK and LDH relative to 18S RNA. (B) Representative immunoblots and quantification of GLUT1, HK-2, PFK and LDH expression. Actin was used as the loading control. Values are mean $\pm$ S.E.M. $n = 6$ in each group. * $P < 0.05$ compared with CTRL, ^a $P < 0.05$ compared with A1GDM group. (C) Glycolysis pathway.

Figure 3. Expression of *miR-143* and its target gene *HK-2* in placentae with GDM

(A) *miR-143* expression relative to U18 in placentas from CTRL, A1GDM and A2GDM.

Expression in A1 and A2GDM were normalized to values in CTRL cells. (B) Validation of HK-2 as *miR-143* target gene. Luciferase reporter assay in trophoblast cells overexpressing 3'UTR HK-2 or mutant HK-2 with or without *miR-143* overexpression. Values are mean $\pm$ S.E.M. $n = 6$ in each group. * $P < 0.05$ compared with CTRL.

Figure 4. Activation of mTOR signalling in GDM placentae

Representative Western blots and quantification of phospho (Thr¹⁷²) and total AMPK; phospho (Ser⁴⁷³) and total AKT; phospho (Thr³⁸⁹) and total S6K; phospho (Thr^{37/46}) and total 4EBP. Actin was used as the loading control. $n = 6$ in each group. * $P < 0.05$ compared with CTRL, ^a $P < 0.05$ compared with A1GDM.

Figure 5. *miR-143* overexpression reduces expression of glycolytic enzymes and rescues mitochondrial function in trophoblast cells of A2GDM

(A) Representative immunoblots and (B) quantification of glycolytic enzyme expression in trophoblast cells of CTRL and of A2GDM with and without overexpression of *miR-143*, $n = 4$. (C) Representative immunoblots and (D) quantification of mitochondrial complex expression in trophoblast cells of CTRL and of A2GDM with or without overexpression of *miR-143*. $n = 3$ for complex I and $n = 4$ for complexes II–V. Values are mean $\pm$ S.E.M. * $P < 0.05$ compared with CTRL, ^a $P < 0.05$ compared with A2GDM. Mitochondrial respiratory parameters were measured in ST of (E) CTRL and (F) A2GDM pregnancies with or without *miR-143*. OCR were measured using a Seahorse Bioscience XF24 extracellular flux analyser as described previously [21]. (G) OCR values for each respiration parameter in A2GDM with or without *miR-143* were normalized to values in CTRL cells. $n = 4$ placentas in each group. Values are mean $\pm$ S.E.M. * $P < 0.05$ compared with CTRL, ^a $P < 0.05$ compared with A2GDM.

Table 1

Clinical characteristics of study patients

Group	BMI (kg/m ²)	Age (years)	Gest age (weeks)	Birth weight (g)	Placental weight (g)	Fetal/placental weight ratio	OGTT 0 h (glucose mg/dl)	OGTT 1 h (glucose mg/dl)	OGTT 2 h (glucose mg/dl)	OGTT 3 h (glucose mg/dl)
CTRL	29.3±0.2	26±2	38.7±0.5	3203±354	690±41	5.26±0.02	—	—	—	—
A1GDM	27.8±1.5	32±1	39.1±0.5	3511±116	720±76	4.54±0.03	95.6±3.1	185.3±4.8	160.8±10.5	142.3±7.6
A2GDM	30.5±2.2	35±1 *	38.2±0.3	3399±243	833±53 *	3.45±0.02 *	95.7±1.1	188.7±11.4	180.0±8.7	141.5±13.5

BMI, maternal age and gestational (Gest) age of the control, A1 and A2GDM groups are shown. Placental efficiency is shown as fetal/placental weight ratio. Blood glucose levels were indicated as measured during the oral glucose tolerance test (OGTT). Values are mean ± S.E.M., $n = 6$ in each group.

* $P < 0.05$ compared with CTRL.