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Physiological glucose levels associated with gestational diabetes impact the human placental transcriptome in an *ex vivo* model

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ABSTRACT

Introduction: Gestational diabetes mellitus (GDM) increases the risk of pathological fetal growth, including rates of large-for-gestational age (LGA) infants, which in turn increases the risk of offspring later developing cardiometabolic complications. Recent continuous glucose monitoring (CGM) studies have revealed that temporal periods of mild hyperglycaemia are linked to LGA, and too tight glycaemic control can increase periods of maternal hypoglycaemia and increase the risk of delivering small-for-gestational age (SGA) infants. The underlying mechanisms are unclear but likely involve the placenta.

Methods: Ex vivo human placental explants from term uncomplicated pregnancies were cultured in varying glucose concentrations for 48 h to recapitulate *in vivo* maternal glucose profiles. Glucose, osmolality, human chorionic gonadotrophin (hCG) and lactate dehydrogenase (LDH) were measured in conditioned medium, and RNA sequencing performed, followed by functional enrichment analysis (FEA).

Results: Medium changes every 6–18 h in variable (5/5.5 mM), or constant 5 mM or 7 mM glucose were appropriate to model maternal normoglycaemia, periods of mild hypoglycaemia and periods of mild hyperglycaemia, respectively. There were 61 differentially expressed genes (DEGs) in explants cultured in mild hyperglycaemic conditions and 54 DEGs in mild hypoglycaemic conditions. FEA revealed that transcripts altered by mild hyperglycaemia were associated with vascular development and lipid metabolism/homeostasis, whilst those altered by mild hypoglycaemia were associated with cell turnover.

Conclusions: Together this data demonstrates that subtle changes in maternal glucose impact the placenta and may contribute to altered fetal growth. This highlights the importance of employing CGM in pregnancies complicated by GDM and utilising physiological glucose levels in *ex vivo/in vitro* placental studies.

1. Introduction

Gestational diabetes (GDM) is associated with an increased risk of pregnancy complications, including pre-term birth, need for caesarean section, pre-eclampsia and pathological fetal growth, including increased rates of large-for-gestational age (LGA) infants [1,2]. Achieving appropriate glucose control, through dietary advice, blood glucose monitoring, and medication, such as insulin therapy is important for reducing these complications [3,4].

Continuous glucose monitoring (CGM), which provides detailed information on glucose variations and glycaemic excursions across the 24-h day, has demonstrated that glycaemic control achieved in pregnancies complicated by maternal diabetes is in a tighter physiological range than previously anticipated [4,5]. Furthermore, subtle changes (0.5–1.5 mM increases) in glucose profiles over the 24-h day, are linked to complications in pregnancies complicated by both pre-gestational and GDM,

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including altered fetal growth [4–9]. For example, when CGM was conducted between 30 and 32 weeks' gestation in GDM pregnancies, higher nocturnal glucose was observed over a 6-h period in mothers that went on to deliver LGA infants (6.0 ± 1.0 mM) compared to mothers that delivered appropriate for gestational age (AGA) infants (5.5 ± 0.8 mM) [4]. Moreover, too 'tight' control of glucose has been associated with periods of maternal hypoglycaemia (below ~3.5 mM) [4,5,9,10], infants being born small-for-gestational age (SGA) [11,12], and adverse neonatal outcomes, including birth trauma and neonatal hypoglycaemia [13].

The mechanisms linking these subtle changes in maternal glucose to altered fetal growth remain to be established, although they are likely linked to changes in the placenta as placental size and vascularisation are associated with the level of glycaemic control [12,14,15]. Furthermore, previous *in vitro* and *ex vivo* studies have demonstrated that hyperglycaemia impacts the placenta, including trophoblast function, proliferation, apoptosis, invasion [16–20], placental metabolism [20–22] and endothelial dysfunction [23,24]. However, many of these studies have primarily utilised supraphysiological concentrations of glucose (>20 mM) which do not recapitulate *in vivo* glucose levels or fluctuations that occur in pregnant women with diabetes. Better models that enable the effect of physiologically relevant glucose profiles on the placenta are required. Therefore, the aim of this study was to establish a model to recapitulate *in vivo* CGM glucose profiles and to determine the impact on the placenta. Specifically, we modelled temporal periods of mild hyperglycaemia and mild hypoglycaemia in *ex vivo* human placental villous explants and demonstrated that even subtle changes in glucose were sufficient to exert placental transcriptomic changes in pathways associated with placental development and function.

2. Methods

Development of an *ex vivo* human placental model to study the impact of physiological changes in maternal glucose levels in pregnancies complicated by GDM.

2.1. Human placental villous explant culture

Placentae (n = 9) from uncomplicated, singleton term deliveries (38–39 + 4 weeks' gestation) were collected within 30 min following elective caesarean section delivery at the Leeds Teaching Hospital NHS trust (REC reference: 18/LO/0067; IRAS project ID: 234385). All participants gave written informed consent and human tissue processing, data curation and analysis, was conducted in accordance with Declaration of Helsinki guidelines and the Human Tissue Act. Maternal demographic and pregnancy outcome information were recorded. Birthweight centiles were calculated using the Gestation Related Optimal Weight (GROW) calculator [25], which considered maternal BMI, parity, birthweight, ethnicity, fetal sex, and gestational age, where available. All samples used were from AGA pregnancies (BWC >10 and < 90).

The fetal membranes and umbilical cord were removed, and 5 cm^2 full thickness placental tissue samples were taken from the centre, middle and edge regions of the placenta, at random. Maternal blood was removed by washing in PBS until clear. For placental villous explants, the basal and chorionic plates were removed, and tissue was dissected into 2 mm^3 fragments. Three fragments, one centre, middle and edge piece (total weight ~10–30 mg), were placed in each Netwell™ (3477, Costar) in 12-well plates, containing 2 mL DMEM-F12 medium (L0091-500, Biowest), supplemented with 10 % fetal bovine serum (10270–106, Gibco), 1 % penicillin, streptomycin, and glutamine (10378–016, Gibco) and 9.9 μL/mL sterile filtered D-glucose (G8644, Sigma-Aldrich) to generate a final medium glucose concentration of 5.5 mM. Explants were cultured overnight in medium containing 5.5 mM glucose before transferring into different conditions for glucose fluctuation experiments.

2.2. Glucose fluctuations

DMEM-F12 without glucose was supplemented with 9 μL/mL, 9.9 μL/mL and 12.6 μL/mL sterile filtered D-glucose (G8644, Sigma-Aldrich) to generate concentrations of 5, 5.5- and 7-mM glucose, respectively. Placental explants were cultured in either constant 5 mM or 7 mM glucose, replenished at 0, 18, 24 and 42 h, or fluctuating 5- and 5.5 mM glucose every 18 and 6 h, respectively (5.5 mM at 0 and 24 h, 5 mM at 18 and 42 h). Explants were cultured for a total of 48 h to assess the acute impact of glucose fluctuations. Conditioned medium was collected prior to each medium refreshment and stored at −80 °C. At the end of the culture period, explants were snap frozen in liquid nitrogen and stored at −80 °C.

2.3. Assessment of glucose levels and osmolality in conditioned medium

Glucose concentrations in the conditioned medium were assessed using the GlucCell® Glucose Monitoring System (CLS-1322-02, GPE Scientific). Medium samples from each time point were warmed to 37 °C and 3 μL was loaded onto GlucCell® test strips (CLS-1324-01, GPE Scientific) in triplicate. Glucose levels were recorded as mM. To ensure that any changes observed in tissue was attributed to glucose and not osmolality, osmolality was assessed using a Single-Sample Micro Osmometer (Model 3320, Advanced Instruments) with measurements recorded in mOsm/kg.

2.4. Lactate dehydrogenase (LDH) assay

Levels of LDH released from placental explants into medium was determined using a cytotoxicity detection kit (11644793001, Roche). Levels of LDH in the conditioned medium were extrapolated from a standard curve, which was generated using LDH from rabbit muscle (10127876001, Roche, Switzerland) diluted in DMEM-F12, containing no glucose or FBS, at a concentration range of 0.0078125–0.5 U/mL. The enzymatic reaction was stopped by addition of 50 μL 1M HCl (35328, Honeywell, Fluka). Absorbances at 492 nm and 690 nm were measured using a Powerwave™ HT microplate reader with Gen5 Microplate Reader software (BioTek). Final absorbance values were calculated as the absorbance at 690-492 nm.

2.5. hCG ELISA

Levels of human chorionic gonadotrophin (hCG) secretion from placental explants into medium was assessed using a hCG ELISA (EIA-1469, DRG Diagnostics), following manufacturer's instructions. Levels of hCG in conditioned medium were extrapolated from a standard curve, with a concentration range of 5–1000 mIU/mL. Absorbances at 450 nm were measured using a Powerwave™ HT microplate reader with Gen5 Microplate Reader software (BioTek).

2.6. RNA extraction

Snap frozen placental villous explants (~10–30 mg) were homogenised in 500 μL of lysis/binding buffer using 5 mm metal balls (69989, Qiagen) and a Tissue Lyser II (Qiagen) set to a frequency of 27/s for 2 min. Total RNA was extracted using the mirVana™ miRNA isolation kit (AM1561, Invitrogen, UK) following manufacturer's instructions. All centrifugation steps were performed at 10,000 RCF, except for elution which was performed at maximum speed in 100 μL of DNase/RNase free water. The concentration of RNA (ng/μL) and quality was recorded using the Nanodrop-1000 (ThermoFisher Scientific).

2.7. RNA sequencing

RNA sequencing was performed by Novogene (Cambridge, UK). Library preparation was conducted using the Novogene next generation

sequencing (NGS) RNA Library Prep Set (PT042). The cDNA library was then loaded into Illumina sequencers for mRNA-sequencing (Illumina Novaseq 6000, S4 flow cell, PE150 sequencing). Raw reads of FASTQ format were processed through fastp to remove reads containing adapter sequences, poly-N and low-quality reads [26]. A Q30 score, which represents an error rate of 1 in 1000 base pairs, was calculated and used as a cut-off so that all downstream analyses were based on clean data with high quality. Reads were then mapped to a reference genome (Homo Sapiens, GRCh38/hg38), downloaded from NCBI [27] and aligned using STAR [28]. featureCounts was used to count the read numbers mapped to each gene [29].

Only genes that had at least 10 read counts in total across all samples examined were retained. DESeq2 was used to perform the differential expression analysis on raw read counts [30]. Due to inherent variability between placental samples from different patients (Supplementary Figure 1), all differential gene expression analyses were designed to incorporate the patient variable into the design formula and to enable matched sample analysis [30,31]. Genes were considered differentially expressed (DEGs) when Log_2 Fold Change (Log_2FC) > 1 or < -1 and $p < 0.01$ (Wald Test in DESeq2 [30]). Further gene symbol, type and description were determined using BioMart (https://www.ensembl.org/biomart/martview/) [32].

2.8. Functional enrichment analysis

Functional predictions of DEGs were performed using WebGestalt (http://www.webgestalt.org/) for Over Representation Analysis (ORA) [33], which produced a list of enriched gene ontology (GO) annotations (biological processes, cellular components, and molecular functions), where redundant terms were excluded. A p value of <0.05 was used as threshold. Enrichment ratios, which is the number of observed genes divided by the number of expected genes in each GO/pathway category, were also reported. REVIGO was used to generate plots (http://revigo.irb.hr/) [90]. Ingenuity Pathway core analysis was also performed (IPA; Qiagen, https://digitalinsights.qiagen.com/IPA) [34], which included canonical pathway and disease and function analysis. For identified canonical pathways and diseases and functions $p < 0.05$ (-log (p value) > 1.3) was used as a threshold.

2.9. Data visualisation and statistical analysis

All data analysis, statistical analysis and data visualisation were performed using R (v4.04) and R Studio (v1.3.959), including the R packages ggplot2 [35], ggbreak [36], and EnhancedVolcano [37]. Distribution of data was assessed using quantile-quantile (QQ) plots and Shapiro-Wilk tests. For normally distributed data, a one-way analysis of variance (ANOVA) was performed followed by a Tukey's post-hoc test. For non-parametric data, a Kruskall-Wallis test was used with a Dunn's post-hoc test. The impact of glucose fluctuations on osmolality overtime was assessed using a two-way ANOVA on log transformed data (non-parametric) with a Tukey's post-hoc test. A p value < 0.05 was considered indicative of statistical significance.

3. Results

3.1. An ex-vivo human placental explant model for assessment of physiological changes in maternal glucose levels in pregnancies complicated by GDM

Standard placental explant culture conditions have supra-physiological concentrations of glucose (up to 27 mM depending on basal medium used). Moreover, given that CGM studies have shown glucose concentrations fluctuate over the 24-h day, even in normoglycaemic/non-diabetic pregnancies [8], we assessed whether it was possible to model *in vivo* CGM glucose profiles using human placental explants (n = 9; Table 1).

Table 1
Maternal and fetal demographic information for placental explant samples used for glucose, osmolality, LDH and hCG measurements (n = 9). Data is presented as the mean ± standard error of the mean (SEM). Abbreviations: BMI – body mass index. All explants were from elective caesarean section deliveries and appropriately grown for gestational age (AGA) pregnancies. [a]n = 8.

	Mean ± SEM
Maternal age (years)	35 ± 2.76
BMI at booking (kg/m^2)	25.76 ± 0.89[a]
Ethnicity	White British = 5 Pakistani = 1 Asian/Persian = 1 Mixed White Black Caribbean = 1 Unknown = 1
Gestational age (days)	273.33 ± 1.23
Parity	P0 = 3 P1 = 2 P2 = 3 P3 = 1
Birthweight (g)	3247.11 ± 106.29
Placental weight (g)	465.96 ± 27.78
Fetal Sex	Males = 7 Females = 2
Birthweight Centile	37.51 ± 8.55

In explants where input culture medium concentrations fluctuated between 5- and 5.5 mM glucose every 18 and 6 h, respectively, medium glucose levels remained stable throughout culture, within the range of 5.5–4.38 ± 0.27 mM, over the 48-h period (Fig. 1A). Medium osmolality remained stable across the culture period. Patterns of hCG (placental hormone production; Fig. 1C) and LDH (to indicate tissue necrosis; Fig. 1D) released from explants cultured in normoglycemic conditions (5/5.5 mM) were as expected for healthy placental explant tissue [38], with peak average hCG levels of 44.13 ± 30.47 mIU/mL at 18 h and peak average LDH levels of 0.22 ± 0.017 U/mL at 24 h. This glucose profile (5/5.5 mM) represents *in vivo* levels in women with GDM that have appropriate control of glycaemia (normoglycaemia), that go on to deliver AGA infants [4].

We then assessed whether we could model *in vivo* profiles of pregnant women that experience periods of mild hyperglycaemia and mild hypoglycaemia. Culture of explants in 7 mM glucose resulted in fluctuations from 7 mM to 5.36 ± 0.36 mM over the culture period (Fig. 1A) which is comparable to temporal periods of mild hyperglycaemia, observed in women with GDM that go on to deliver an LGA infants [4]. In explants cultured in 5 mM glucose, levels fluctuated between 5 mM and 3.31 ± 0.07 mM (Fig. 1B), which is comparable to temporal periods of mild hypoglycaemia, seen in women with GDM at greater risk of delivering SGA infants [11,12]. Medium osmolality (Fig. 1B), hCG secretion (Fig. 1C) and LDH secretion (Fig. 1D) were not affected by these subtle changes in glucose. Taken together these data demonstrate that we can recapitulate normoglycaemic, mild hyperglycaemic and mild hypoglycaemic profiles observed *in vivo* in pregnant women with GDM in *ex vivo* human placental explants.

3.2. Mild hyperglycaemia alters placental transcriptomic pathways and functions associated with lipid metabolism and vascular development

Using this *ex vivo* human placental explant model, we assessed if the pathological fetal growth observed in pregnancies where only subtle, temporal changes in glucose occur, could likely be linked to changes in the placenta. RNA sequencing was conducted on placental tissue collected from pregnancies that delivered a male fetus, that had been cultured in normoglycaemic, mild hyperglycaemic and mild hypoglycaemic conditions (n = 5; Table 2). 61 transcripts, including 15 lncRNAs, were altered in mild hyperglycaemic (7 mM) conditions ($p < 0.01$ and a Log_2FC of $<$ -1 and $>$1, Fig. 2A; Supplementary Tables 1 and 2), compared to normoglycemic culture (5/5.5 mM).

FEA revealed that DEGs were associated with lipid metabolism and

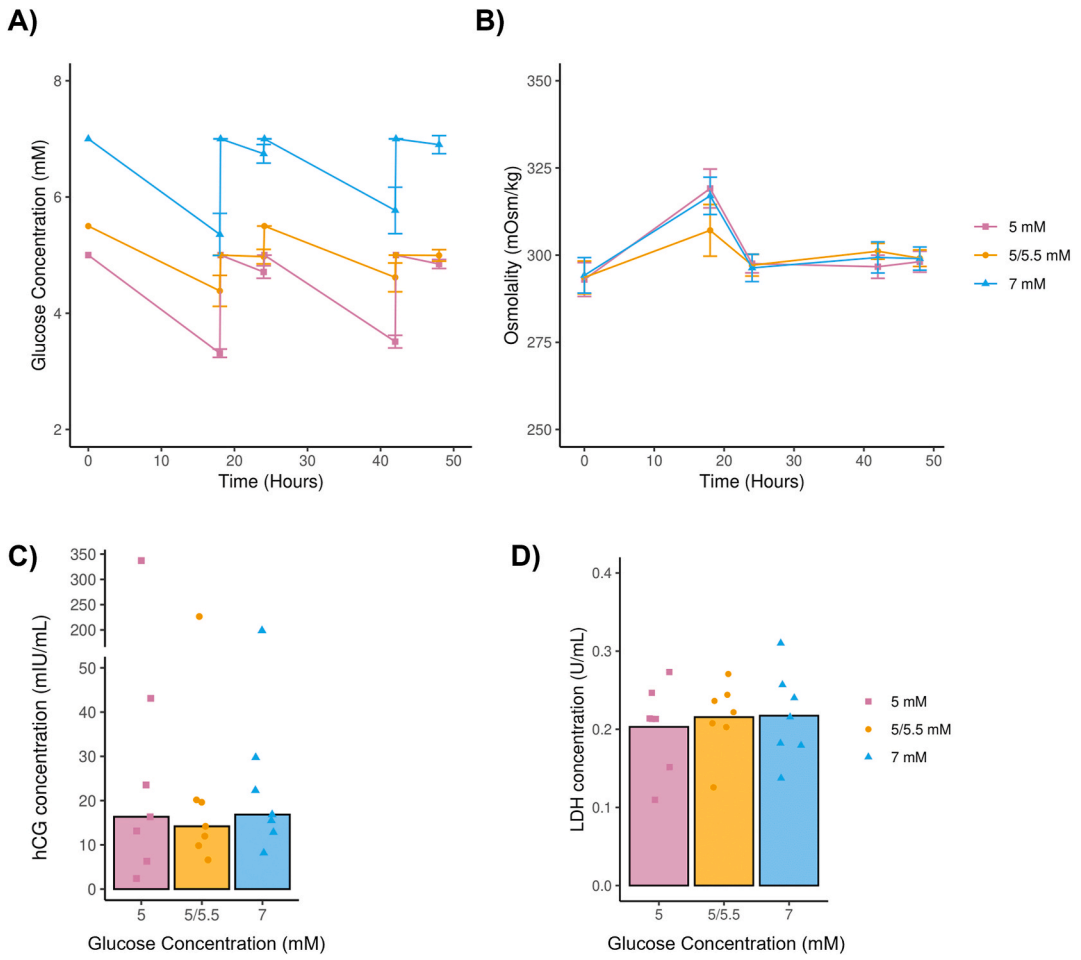

Fig. 1. Developing a model to mimic *in vivo* maternal glucose fluctuations in *ex vivo* human placental explants. Placental explants were exposed to either constant 5- or 7-mM glucose or fluctuating 5/5.5 mM glucose for 48 h. **A)** Glucose concentrations (mM) in culture medium, assessed using a GlucCell Monitoring System. Data is presented as the mean ± SEM (n = 9). **B)** Osmolality (mOsm/kg) of culture medium assessed using an osmometer. Data is presented as the mean ± SEM (n = 9) and statistical analysis was performed using a Two-Way ANOVA on log-transformed data with a Tukey's post-hoc test. **C)** Levels of human chorionic gonadotrophin (hCG), measured by ELISA, in the culture medium at 18 h (peak hCG release). Data was not normally distributed and is presented as the median (n = 7). Statistical analysis was performed using a Kruskall-Wallis with Dunn's post-hoc test. **D)** Levels of lactate dehydrogenase (LDH) measured by a colourimetric assay, in the culture medium at 24 h (peak LDH release). Data was normally distributed and is presented as the mean (n = 7). Statistical analysis was performed using a One-Way ANOVA, with Tukey's post-hoc test.

Table 2
Maternal and fetal demographic information for placental explant samples used for RNA sequencing (n = 5). Data is presented as the mean ± SEM. Abbreviations: BMI – body mass index. All explants were from elective caesarean section deliveries and appropriately grown for gestational age (AGA) pregnancies. [a]n = 4.

	Mean ± SEM
Maternal age (years)	35.80 ± 2.80
BMI at booking (kg/m^2)	26.82 ± 0.67[a]
Ethnicity	White British = 3
	Pakistani = 1
	Unknown = 1
Gestational age (days)	272.0 ± 2.0
Parity	P0 = 1
	P1 = 2
	P2 = 1
	P3 = 1
Birthweight (g)	3431.0 ± 105.17
Placental weight (g)	462.60 ± 27.19
Fetal Sex	Males = 5
Birthweight Centile	49.0 ± 11.54

transport pathways and functions, including lipid localisation (p = 0.0000952), lipid homeostasis (p = 0.00213) and lipid transporter activity (p = 0.00257), lipid catabolic process (p = 0.0166) GO terms (Fig. 2B; Supplementary Table 3) and IPA canonical pathways and functions, FXR/RXR Activation (p = 0.00234; Fig. 2C; Supplementary Table 4) and lipid metabolism (p = 0.00000758; Fig. 2D; Supplementary Table 5). Key DEGs within these pathways included FABP4, APOB, IL1B, NPC1L1, ESYT3. FEA also demonstrated that DEGs altered by mild hyperglycaemia were associated with vascular development pathways and functions, including cellular response to vascular endothelial growth factor (VEGF) stimulus (p = 0.000143), sprouting angiogenesis (p = 0.00125), regulation of vasculature development (p = 0.0114) GO terms (Fig. 2B; Supplementary Table 3) and IPA canonical pathways and functions, atherosclerosis signalling p = 0.0148; Fig. 2C; Supplementary Table 4), cardiovascular disease (p = 0.000000412; Fig. 2D; Supplementary Table 5). Key DEGs in these pathways and functions included IL1B, APOB, NR4A1, RAMP2 and JCAD.

3.3. Mild hypoglycaemia alters placental transcriptomic pathways and functions associated with ubiquitination and cell turnover

RNA sequencing revealed that mild hypoglycaemic (5 mM)

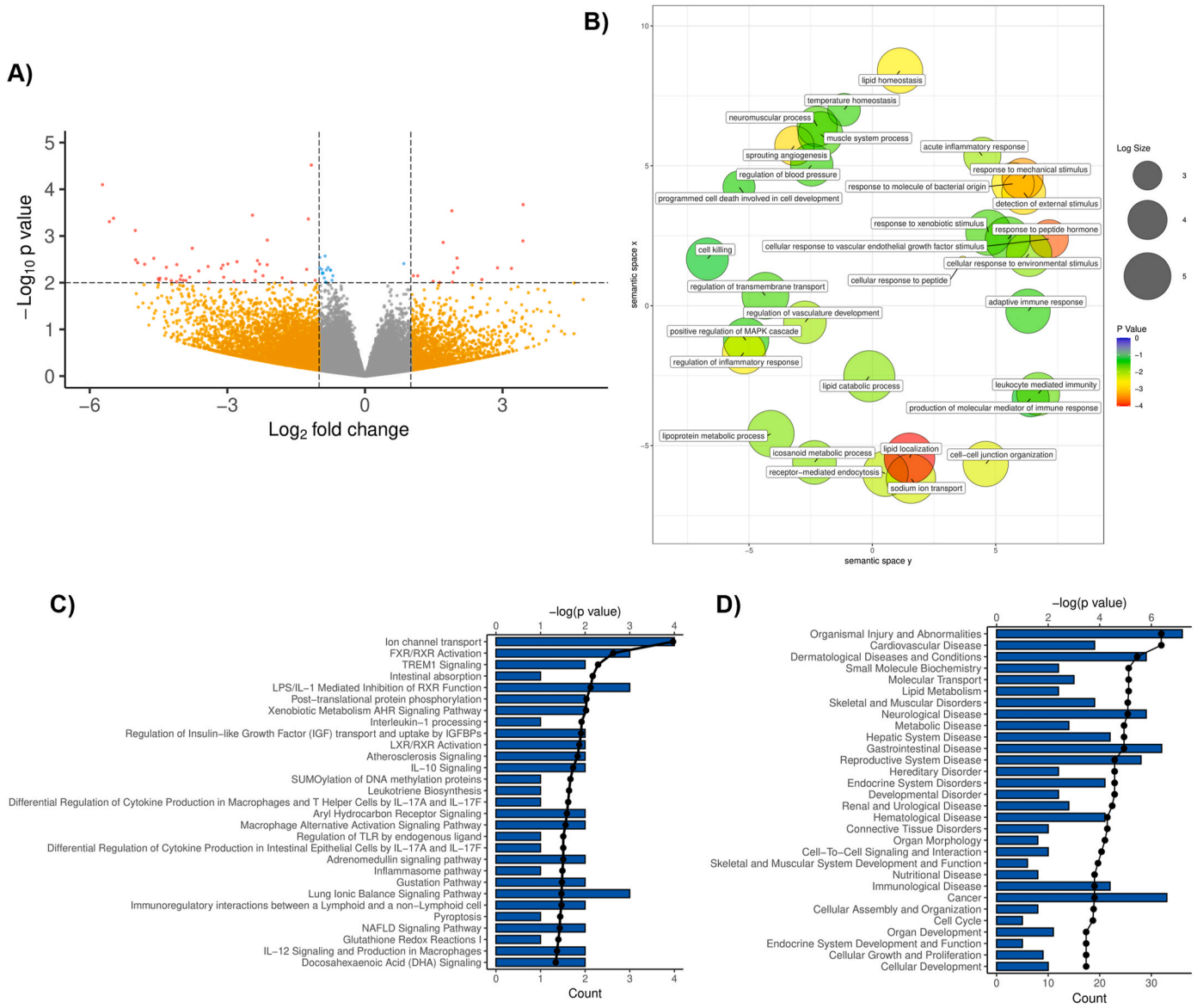

Fig. 2. Mild hyperglycaemia impacts the placental transcriptome. A) Volcano plot representing differentially expressed genes altered by 7 mM glucose (mild hyperglycaemia) compared to 5/5.5 mM glucose (normoglycaemia) in placental explants. Horizontal dashed line represents p = 0.01, the vertical dashed lines represent a Log_2FC of ±1. Colour key: non-significant = grey, significant p value = blue, significant Log_2FC = orange, significant p value and Log_2FC = red. Volcano plots were generated using EnhancedVolcano in R. **B)** Gene ontology (GO) biological process terms associated with 7 mM glucose in placental explants determined by over representation analysis (ORA). Bubble plot with colour based on the p value, where red is the most significant. Size of bubbles represent the generality of the GO terms, where smaller bubbles indicate more specific terms, and larger bubbles represent more general terms. Plot generated using REVIGO. **C)** Canonical pathways associated with 7 mM glucose in placental explants determined by ingenuity pathway analysis (IPA). Canonical pathways shown are those with a –log (p value) > 1.3; p < 0.05. The top y-axis represents the -log (p value), indicated by the black line plot, and the lower y-axis represents the number of genes counted for each of the represented canonical pathways, indicated by the bars. Pathways are ordered by most significant p-value. **D)** Diseases and functions associated with 7 mM glucose in placental explants determined by ingenuity pathway analysis (IPA). The top y-axis represents the -log (p value), indicated by the black line plot, and the lower y-axis represents the number of genes counted for each of the diseases and functions, indicated by the bars. Diseases and functions are ordered by most significant p-value. The top 30 diseases and functions are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

conditions altered 54 transcripts, including 10 lncRNAs (p < 0.01 and a Log_2FC of < -1 and >1, Fig. 3A & Supplementary Tables 6 and 7). FEA revealed that under mild hypoglycaemic conditions, DEGs were associated with the GO term, protein polyubiquitination (p = 0.00615; Fig. 3B; Supplementary Table 8), including several DEGs encoding ubiquitin ligase proteins (*SPSB4*, *TRIM34*, *TRIM71*), involved in protein degradation, via the ubiquitin proteasome pathway [39]. IPA predicted that DEGs altered by mild hypoglycaemia were also associated with canonical pathways and functions associated with cell turnover, including TRAIL signalling (p = 0.0115), necroptosis signalling pathway (p = 0.0182), caspase activation (p = 0.0204; Fig. 3C; Supplementary Table 9), cell death and survival (p = 0.00128) and cellular growth and proliferation (p = 0.00128) (Fig. 3D; Supplementary Table 10). Key DEGs in these pathways and functions included, *TMPO-AS1*, *TNFAIP6*, *TNFSF10* and *TRIM71*.

4. Discussion

In this study we have utilised human placental villous explants and fluctuating glucose concentrations to recapitulate *in vivo* CGM maternal

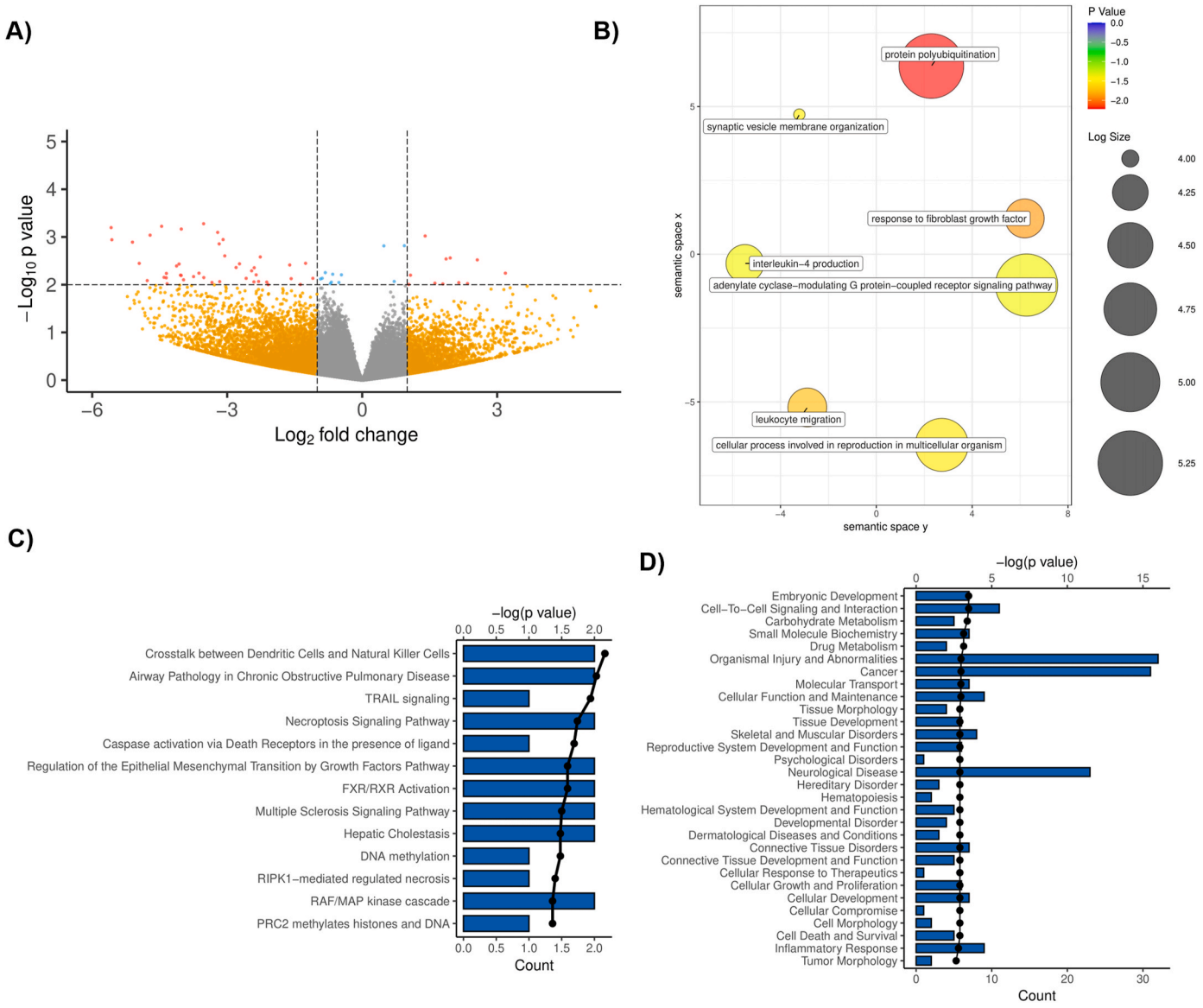

Fig. 3. Mild hypoglycaemia impacts the placental transcriptome. A) Volcano plot representing differentially expressed genes altered by 5 mM glucose (mild hypoglycaemia) compared to 5/5.5 mM glucose (normoglycaemia) in placental explants. Horizontal dashed line represents p = 0.01, the vertical dashed lines represent a Log_2FC of ±1. Colour key: non-significant = grey, significant p value = blue, significant Log_2FC = orange, significant p value and Log_2FC = red. Volcano plots were generated using EnhancedVolcano in R. **B)** Gene ontology (GO) biological process terms associated with 5 mM glucose in placental explants determined by over representation analysis (ORA). Bubble plot with colour based on the p value, where red is the most significant. Size of bubbles represent the generality of the GO terms, where smaller bubbles indicate more specific terms, and larger bubbles represent more general terms. Plot generated using REVIGO. **C)** Canonical pathways associated with 5 mM glucose in placental explants determined by ingenuity pathway analysis (IPA). Canonical pathways shown are those with a –log (p value) > 1.3; $p < 0.05$. The top y-axis represents the -log (p value), indicated by the black line plot, and the lower y-axis represents the number of genes counted for each of the represented canonical pathways, indicated by the bars. Pathways are ordered by most significant p-value. **D)** Diseases and functions associated with 5 mM glucose in placental explants determined by ingenuity pathway analysis (IPA). The top y-axis represents the -log (p value), indicated by the black line plot, and the lower y-axis represents the number of genes counted for each of the diseases and functions, indicated by the bars. Diseases and functions are ordered by most significant p-value. The top 30 diseases and functions are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

glucose profiles in pregnancies complicated by GDM. Here, we demonstrate that mild hyperglycaemia and mild hypoglycaemia associated with LGA and SGA outcomes, respectively, induce changes to the placental transcriptome with predicted functional consequences on lipid metabolism and transport, vascular development, and cell survival.

Whilst several *in vivo*, *in vitro* and *ex vivo* studies have shown that maternal hyperglycaemia alters placental development and function [14–16,18,20–24], many studies utilise supraphysiological concentrations of glucose (≥20 mM), which do not allow us to delineate the impact of subtle fluctuations in glucose on the placenta. Given that clinical research, using recent CGM technology, has demonstrated that maternal glucose fluctuates over the 24-h day, even in normoglycaemic pregnancies [8], and this been linked to adverse pregnancy outcomes in pregnancies complicated by diabetes [4,5], it's important to consider these findings when examining the impact of glucose on the placenta. This will aid in uncovering meaningful mechanistic insights into the potential mechanisms regulating placental development and function and fetal growth. Importantly, here we have also utilised human placental villous explants, which is advantageous over the use of single cells as they recapitulate the complex 3D environment in the placenta, as

well as cell-cell relationships, and including paracrine signalling between the trophoblast, mesenchymal stromal cells, Hofbauer cells and vasculature [40].

Culture of placental explants in 7 mM glucose, which fluctuated between normoglycaemic and mild hyperglycaemic levels, as observed *in vivo* in women with GDM that deliver LGA infants [4], impacted genes associated with vascular development. Predicted pathways and functions included regulation of vasculature development and sprouting angiogenesis. Interestingly, maternal diabetes in pregnancy has been associated with altered placental vascularisation [41,42], including increased capillary branching [43–45]. Indeed, altered vascularisation has previously been linked to glycaemic control, as Calderon et al. (2007) reported placental hypervascularisation in cases of mild hyperglycaemia, and hypovascularisation in GDM and overt diabetes [14]. These alterations in placental vasculature in pregnancies complicated by maternal diabetes are likely to impact on placental function, including its ability to transfer nutrients to the fetus and thus has the potential to result in complications of fetal growth. Indeed, this suggests that LGA outcomes associated with mild hyperglycaemia could potentially be attributed to vascular alterations in the placenta. Most studies investigating the placenta in pregnancies complicated by diabetes do not stratify based on birthweight. However, some studies have reported that FGR placentas have reduced vascularisation of the villi and intermediate and terminal villi in these areas had reduced vessel lumens [46] and recent studies have shown that the vasculo-syncytial membrane and syncytial basal membrane is increased in thickness in placentas from GDM pregnancies complicated by macrosomia (birthweight above 4000g) [47], further demonstrating that fetal growth complications may be linked to these alterations. Whilst it would be interesting to further investigate the impact of glucose on the vasculature in placental explants, studies suggest that vessel integrity is reduced during explant culture [48] and thus may not be a suitable model. Therefore, further study should employ other placental *in* vitro models, such as placental endothelial cells or placenta-on-a-chip [49].

Other predicted functional changes associated with mild hyperglycaemic (7 mM) conditions included metabolic pathways and functions, such as lipid homeostasis, lipid transporter activity and lipid metabolism. In the placenta, non-esterified fatty acids (NEFAs) are produced from maternal lipids by lipases located on the syncytiotrophoblast microvillous membrane and their transport across the placenta is mediated by fatty acid transporters (FATPs). Once in the cytoplasm, NEFAs bind to fatty acid binding proteins (FABPs) [50]. Alterations in placental FABPs and lipases have been reported to be altered in pregnancies complicated by maternal diabetes (reviewed by Castillo-Castrejon & Powell, 2017) [50], including *FABP4*, which was upregulated by mild hyperglycaemia in the present study, and has been found to be increased in placentas from obese women with GDM or Type 2 diabetes [51].

Additionally, glucose has been shown to promote placental triglyceride accumulation in placental explants, although, no differences in expression or localisation of lipases or FATPs were observed, however, in contrast to the present study, this study utilised supraphysiological levels of glucose [21]. Other predicted pathways associated with mild hyperglycaemia included retinoid X receptor (RXR) signalling; RXR is expressed in the human placenta, specifically within the trophoblast and is known to regulate the expression of fatty acid transport and metabolism proteins [52,53] as well as playing an important role in placental vascular development and trophoblast differentiation [54]. Altered RXR signalling has been linked to cardiovascular complications in diabetes [55] and genetic variants have been associated with GDM risk [56]. Our findings suggest that altered placental lipid metabolism in GDM, may be attributed to periods of mild hyperglycaemia. These alterations in lipid metabolism and homeostasis may lead to dysregulated levels of circulating lipids in pregnancy, which can lead to maternal dyslipidaemia, altered fetal growth, such as LGA, and increased risk of offspring metabolic disease later in life [57]. Collectively, these findings show that mild hyperglycaemia may be attributed to altered placental vascularisation and metabolic processes, which could indicate increased nutrient transport to the fetus, however, further studies are needed to explore this.

Whilst there are limited studies on the effect of hypoglycaemia on the placenta, there is some evidence that placental function is likely altered [58–63], including altered nutrient transporter expression [58,59,61] and endothelial dysfunction in HUVECs and endothelial cells from other systems [62,63]. In the present study, culture of placental explants in 5 mM glucose, resulted in tissue being exposed to fluctuations between normoglycemic and mild hypoglycaemic levels, as seen *in vivo* in women with GDM at greater risk of delivering SGA infants [11,12]. Transcriptomic changes in mild hypoglycaemia had predicted functional consequences associated with placental growth, including cell death and survival, cellular growth and proliferation and regulated cell death pathways, all of which are known to play important roles in placental development [64]. Altered placental growth has been widely reported in pregnancies complicated by GDM [65,66] and has been linked to changes in trophoblast turnover, including altered proliferation and apoptosis [67] and other studies have shown that alterations in placental weight and surface area are associated with birthweight [68] and increased placental apoptosis and reduced proliferation has been linked to intrauterine growth restriction (IUGR) [69,70]. This suggests that hypoglycaemia could potentially impact fetal growth through modulation of placental cell turnover. We also show that several genes encoding ubiquitin ligases and related proteins were altered by mild hypoglycaemia, including *SPSB4*, which has previously been shown to be altered in a streptozotocin-induced mouse model of diabetic pregnancy [71]. Altered expression of several ubiquitin-conjugating enzymes have been reported in trophoblast cells from GDM pregnancies and were associated with increased maternal fasting plasma glucose [72]. Whilst we did not explore the impact of changes in ubiquitin ligases in our study, other studies have shown that E3 ubiquitin ligases contribute to trophoblast function, including migration/invasion, turnover and differentiation [39]. Furthermore, altered ubiquitination in the placenta has also been linked to dysregulation of nutrient transporter expression in pregnancies complicated by growth restriction [73]. Hence, it is possible that the increased incidence of SGA in offspring exposed to periods of maternal hypoglycaemia may be due to perturbed placental growth, trophoblast turnover, regulated cell death, which may be a consequence of ubiquitination.

Additionally, it is also important to note from a tissue culture perspective that glucose is rapidly metabolised by placental explants and as glucose is not usually measured in *in vitro* and *ex vivo* studies there could be periods of hypoglycaemia in cultures that are not identified. Thus, assessing medium concentrations throughout culture to ensure they represent *in vivo* levels is an important consideration.

Whilst our study has established that acute exposure of term placental tissue to physiological levels of glucose associated with GDM impact the placental transcriptome, it would also be interesting to assess the impact of chronic exposure to physiological glucose in future studies, since we anticipate that chronic hyperglycaemia would likely have a greater impact on the placenta and fetal growth. This is consistent with poorer outcomes, including increased rates of LGA, in type 1 and type 2 diabetic pregnancies [1], where the placenta is exposed to maternal hyperglycaemia throughout gestation. Moreover, studies to assess the impact of physiologically relevant glucose levels in first trimester or early second trimester placenta are also warranted to enable full characterisation of the dynamic interplay between glucose levels and placental development across gestation.

An important consideration of our study is that whilst we show that glucose exerts changes to the placental transcriptome in pregnancies that delivered a male fetus, we have not assessed the impact on placentae from female fetuses. Adverse outcomes in pregnancies complicated by maternal diabetes, including increased rates of LGA, are reported to be more common when there is a male fetus [74–76] and it is

possible that this could be attributed to a differential response to glucose in male compared to female placentae. Studies reporting sex-specific differences in glucose regulation in other systems [77,78], and sex-specific differences in placental structure, function and gene/protein expression [79–82] further support this but this warrants further investigation.

Here we have considered the impact of glucose on the placenta, however, there are other components of diabetes that could also impact the placental response to glucose, including maternal weight, use of hypoglycaemic agents, such as metformin, ethnicity, the type of diabetes and underlying pathophysiology, birthweight and gestational age [42]. It would be interesting to assess whether these parameters impact the actions of glucose on the placenta as there is evidence that glucose metabolism is dynamic across gestation [83] and that the expression and location of glucose transporters are altered across gestation and in pregnancies complicated by pathological fetal growth [84–88]. Moreover, maternal BMI and birthweight have also been found to be correlated with placental hexokinase activity, a key enzyme involved in placental glucose metabolism [89].

In conclusion, our findings demonstrate that subtle changes in maternal glucose impact transcriptomic pathways and functions associated with lipid metabolism and transport, vascular development, and cell turnover, which are known to be altered in pregnancies complicated by GDM and/or fetal growth and therefore could potentially explain the adverse pregnancy outcomes associated with glucose fluctuations *in vivo*. This provides a foundation for further research into the functional consequences of these transcriptomic changes on placental and fetal development. These subtle fluctuations in maternal glucose, detected by CGM, would otherwise go undetected using standard methods of glucose monitoring and could have a significant impact on the placenta. Therefore, this also highlights the importance of employing CGM in GDM pregnancies. We also demonstrate that utilising physiological levels of glucose in *ex vivo* and *in vitro* studies and measuring glucose concentrations throughout culture is an important consideration.

CRediT authorship contribution statement

Abigail R. Byford: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Katy Walsh:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Dapeng Wang:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Chloe Baird-Rayner:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Virginia Pensabene:** Writing – review & editing, Supervision, Methodology. **Eleanor M. Scott:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization. **Karen Forbes:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization.

Data availability statement

The RNA sequencing data from this study are publicly available in the GEO database (GSE288239). Processed data (differentially expressed gene lists) are available in the supplementary files.

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Declaration of competing interest

None to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2025.06.006.

References

[1] N.S. Clement, A. Abul, R. Farrelly, H.R. Murphy, K. Forbes, N.A.B. Simpson, E. M. Scott, Pregnancy outcomes in type 2 diabetes: a systematic review and meta-analysis, Am. J. Obstet. Gynecol. (2024), https://doi.org/10.1016/j.ajog.2024.11.026.

[2] W. Ye, C. Luo, J. Huang, C. Li, Z. Liu, F. Liu, Gestational diabetes mellitus and adverse pregnancy outcomes: systematic review and meta-analysis, The BMJ (2022), https://doi.org/10.1136/bmj-2021-067946.

[3] C.A. Crowther, J.E. Hiller, J.R. Moss, A.J. McPhee, W.S. Jeffries, J.S. Robinson, Effect of treatment of gestational diabetes mellitus on pregnancy outcomes, N. Engl. J. Med. 352 (2005) 2477–2486, https://doi.org/10.1056/NEJMoa042973.

[4] G.R. Law, A. Alnaji, L. Alrefaii, D. Endersby, S.J. Cartland, S.G. Gilbey, P. E. Jennings, H.R. Murphy, E.M. Scott, Suboptimal nocturnal glucose control is associated with large for gestational age in treated gestational diabetes mellitus, Diabetes Care 42 (2019) 810–815, https://doi.org/10.2337/dc18-2212.

[5] G.R. Law, G.T.H. Ellison, A.L. Secher, P. Damm, E.R. Mathiesen, R. Temple, H. R. Murphy, E.M. Scott, Analysis of continuous glucose monitoring in pregnant women with diabetes: distinct temporal patterns of glucose associated with large-for-gestational-age infants, Diabetes Care 38 (2015) 1319–1325, https://doi.org/10.2337/dc15-0070.

[6] D.S. Feig, L.E. Donovan, R. Corcoy, K.E. Murphy, S.A. Amiel, K.F. Hunt, E. Asztalos, J.F.R. Barrett, J.J. Sanchez, A. de Leiva, M. Hod, L. Jovanovic, E. Keely, R. McManus, E.K. Hutton, C.L. Meek, Z.A. Stewart, T. Wysocki, R. O'Brien, K. Ruedy, C. Kollman, G. Tomlinson, H.R. Murphy, J. Grisoni, C. Byrne, K. Davenport, S. Neoh, C. Gougeon, C. Oldford, C. Young, L. Green, B. Rossi, H. Rogers, B. Cleave, M. Strom, J.M. Adelantado, A. Isabel Chico, D. Tundidor, J. Malcolm, K. Henry, D. Morris, G. Rayman, D. Fowler, S. Mitchell, J. Rosier, R. Temple, J. Turner, G. Canciani, N. Hewapathirana, L. Piper, A. Kudirka, M. Watson, M. Bonomo, B. Pintaudi, F. Bertuzzi, G. Daniela, E. Mion, J. Lowe, I. Halperin, A. Rogowsky, S. Adib, R. Lindsay, D. Carty, I. Crawford, F. Mackenzie, T. McSorley, J. Booth, N. McInnes, A. Smith, I. Stanton, T. Tazzeo, J. Weisnagel, P. Mansell, N. Jones, G. Babington, D. Spick, M. MacDougall, S. Chilton, T. Cutts, M. Perkins, E. Scott, D. Endersby, A. Dover, F. Dougherty, S. Johnston, S. Heller, P. Novodorsky, S. Hudson, C. Nisbet, T. Ransom, J. Coolen, D. Baxendale, R. Holt, J. Forbes, N. Martin, F. Walbridge, F. Dunne, S. Conway, A. Egan, C. Kirwin, M. Maresh, G. Kearney, J. Morris, S. Quinn, R. Bilous, R. Mukhtar, A. Godbout, S. Daigle, A. Lubina, M. Jackson, E. Paul, J. Taylor, R. Houlden, A. Breen, A. Banerjee, A. Brackenridge, A. Briley, A. Reid, C. Singh, J. Newstead-Angel, J. Baxter, S. Philip, M. Chlost, L. Murray, K. Castorino, D. Frase, O. Lou, M. Pragnell, Continuous glucose monitoring in pregnant women with type 1 diabetes (CONCEPTT): a multicentre international randomised controlled trial, Lancet 390 (2017) 2347–2359, https://doi.org/10.1016/S0140-6736(17)32400-5.

[7] K. Kristensen, L.E. Ögge, V. Sengpiel, K. Kjölhede, A. Dotevall, A. Elfvin, F.K. Knop, N. Wiberg, A. Katsarou, N. Shaat, L. Kristensen, K. Berntorp, Continuous glucose monitoring in pregnant women with type 1 diabetes: an observational cohort study of 186 pregnancies, Diabetologia (Berl.) 62 (2019) 1143–1153, https://doi.org/10.1007/s00125-019-4850-0.

[8] C. Durnwald, R.W. Beck, Z. Li, E. Norton, R.M. Bergenstal, M. Johnson, S. Dunnigan, M. Banfield, K. Krumwiede, J. Sibayan, P. Calhoun, A.L. Carlson, Continuous glucose monitoring profiles in pregnancies with and without gestational diabetes mellitus, Diabetes Care 47 (2024) 1333–1341, https://doi.org/10.2337/dc23-2149.

[9] E.M. Scott, D.S. Feig, H.R. Murphy, G.R. Law, Continuous glucose monitoring in pregnancy: importance of analyzing temporal profiles to understand clinical outcomes, Diabetes Care 43 (2020) 1178–1184, https://doi.org/10.2337/dc19-2527.

[10] A.R. Byford, K. Forbes, E.M. Scott, Glucose treatment targets in pregnancy - a review of evidence and guidelines, Curr. Diabetes Rev. 18 (2022), https://doi.org/10.2174/1573399818666220422083935.

[11] O. Langer, J. Levy, L. Brustman, A. Anyaegbunam, R. Merkatz, M. Divon, Glycemic control in gestational diabetes mellitus-How tight is tight enough: small for gestational age versus large for gestational age? Am. J. Obstet. Gynecol. 161 (1989) 646–653, https://doi.org/10.1016/0002-9378(89)90371-2.

[12] E.S. Vadakekut, S.J.B. McCoy, M.E. Payton, Association of maternal hypoglycemia with low birth weight and low placental weight: a retrospective investigation, J. Am. Osteopath. Assoc. 111 (2011) 148–152, https://doi.org/10.7556/jaoa.2011.111.3.148.

[13] R.K. Harrison, V. Saravanan, C. Davitt, M. Cruz, A. Palatnik, Antenatal maternal hypoglycemia in women with gestational diabetes mellitus and neonatal outcomes, J. Perinatol. 42 (2022) 1091–1096, https://doi.org/10.1038/s41372-022-01350-4.

[14] I.M.P. Calderon, D.C. Damasceno, R.L. Amorin, R.A.A. Costa, M.A.M. Brasil, M.V. C. Rudge, Morphometric study of placental villi and vessels in women with mild hyperglycemia or gestational or overt diabetes, Diabetes Res. Clin. Pract. 78 (2007) 65–71, https://doi.org/10.1016/j.diabres.2007.01.023.
[15] M. Higgins, P. Felle, E.E. Mooney, J. Bannigan, F.M. McAuliffe, Stereology of the placenta in type 1 and type 2 diabetes, Placenta (Eastbourne) 32 (2011) 564–569, https://doi.org/10.1016/j.placenta.2011.04.015.
[16] U. Weiss, M. Cervar, P. Puerstner, O. Schmut, J. Haas, R. Mauschitz, G. Arikan, G. Desoye, Hyperglycaemia in vitro alters the proliferation and mitochondrial activity of the choriocarcinoma cell lines BeWo, JAR and JEG-3 as models for human first-trimester trophoblast, Diabetologia (Berl.) 44 (2001) 209–219, https://doi.org/10.1007/s001250051601.
[17] H. Li, Y. Gu, Y. Zhang, M.J. Lucas, Y. Wang, High glucose levels down-regulate glucose transporter expression that correlates with increased oxidative stress in placental trophoblast cells in vitro, J. Soc. Gynecol. Invest. 11 (2004) 75–81, https://doi.org/10.1016/j.jsgi.2003.08.002.
[18] L. Ji, Z. Chen, Y. Xu, G. Xiong, R. Liu, C. Wu, H. Hu, L. Wang, Systematic characterization of autophagy in gestational diabetes mellitus, Endocrinology 158 (2017) 2522–2532, https://doi.org/10.1210/en.2016-1922.
[19] L. Zhang, X. Yu, Y. Wu, H. Fu, P. Xu, Y. Zheng, L. Wen, X. Yang, F. Zhang, M. Hu, H. Wang, X. Liu, J. Qiao, C. Peng, R. Gao, R. Saffery, Y. Fu, H. Qi, C. Tong, M. D. Kilby, P.N. Baker, Gestational diabetes mellitus-associated hyperglycemia impairs glucose transporter 3 trafficking in trophoblasts through the downregulation of AMP-activated protein kinase, Front. Cell Dev. Biol. 9 (2021). https://www.frontiersin.org/articles/10.3389/fcell.2021.722024.
[20] Z.J.W. Easton, X. Luo, L. Li, T.R.H. Regnault, The impact of hyperglycemia upon BeWo trophoblast cell metabolic function: a multi-OMICS and functional metabolic analysis, PLoS One 18 (2023), https://doi.org/10.1371/journal.pone.0283118.
[21] C.H. Hulme, A. Nicolaou, S.A. Murphy, A.E.P. Heazell, J.E. Myers, M. Westwood, The effect of high glucose on lipid metabolism in the human placenta, Sci. Rep. 9 (2019) 14114, https://doi.org/10.1038/s41598-019-50626-x.
[22] C.H. Hulme, A. Stevens, W. Dunn, A.E.P. Heazell, K. Hollywood, P. Begley, M. Westwood, J.E. Myers, Identification of the functional pathways altered by placental cell exposure to high glucose: lessons from the transcript and metabolite interactome, Sci. Rep. 8 (2018) 1–11, https://doi.org/10.1038/s41598-018-22535-y.
[23] T. Sáez, P. de Vos, J. Kuipers, L. Sobrevia, M.M. Faas, Fetoplacental endothelial exosomes modulate high D-glucose-induced endothelial dysfunction, Placenta (Eastbourne) 66 (2018) 26–35, https://doi.org/10.1016/j.placenta.2018.04.010.
[24] L. Zheng, M. Li, H. Li, High glucose promotes and aggravates the senescence and dysfunction of vascular endothelial cells in women with hyperglycemia in pregnancy, Biomolecules 14 (2024) 329, https://doi.org/10.3390/biom14030329.
[25] Perinatal Institute, Gestation related optimal weight (GROW) calculator. https://www.gestation.net/cc/about.htm, 2022.
[26] S. Chen, Y. Zhou, Y. Chen, J. Gu, Fastp: an ultra-fast all-in-one FASTQ preprocessor, in: Bioinformatics, Oxford University Press, 2018, pp. i884–i890, https://doi.org/10.1093/bioinformatics/bty560.
[27] NCBI, GRCh38 - hg38 - genome - assembly - NCBI. https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.26/, 2013.
[28] A. Dobin, C.A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut, M. Chaisson, T.R. Gingeras, STAR: ultrafast universal RNA-seq aligner, Bioinformatics 29 (2013) 15–21, https://doi.org/10.1093/bioinformatics/bts635.
[29] Y. Liao, G.K. Smyth, W. Shi, featureCounts: an efficient general purpose program for assigning sequence reads to genomic features, Bioinformatics 30 (2014) 923–930, https://doi.org/10.1093/bioinformatics/btt656.
[30] M.I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, Genome Biol. 15 (2014) 550, https://doi.org/10.1186/s13059-014-0550-8.
[31] J.R. Stevens, J.S. Herrick, R.K. Wolff, M.L. Slattery, Power in pairs: assessing the statistical value of paired samples in tests for differential expression, BMC Genom. 19 (2018) 953, https://doi.org/10.1186/s12864-018-5236-2.
[32] D. Smedley, S. Haider, B. Ballester, R. Holland, D. London, G. Thorisson, A. Kasprzyk, BioMart - biological queries made easy, BMC Genom. 10 (2009), https://doi.org/10.1186/1471-2164-10-22.
[33] Y. Liao, J. Wang, E.J. Jaehnig, Z. Shi, B. Zhang, WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs, Nucleic Acids Res. 47 (2019) W199–W205, https://doi.org/10.1093/nar/gkz401.
[34] A. Krämer, J. Green, J.J. Pollard, S. Tugendreich, Causal analysis approaches in ingenuity pathway analysis, Bioinformatics 30 (2014) 523–530, https://doi.org/10.1093/bioinformatics/btt703.
[35] H. Wickham, ggplot2: Elegant Graphics for Data Analysis, Springer International Publishing, Cham, 2016, https://doi.org/10.1007/978-3-319-24277-4.
[36] S. Xu, M. Chen, T. Feng, L. Zhan, L. Zhou, G. Yu, Use ggbreak to effectively utilize plotting space to deal with large datasets and outliers, Front. Genet. 12 (2021), https://doi.org/10.3389/fgene.2021.774846.
[37] K. Blighe, S. Rana, E. Turkes, B. Ostendorf, M. Lewis, EnhancedVolcano: publication-ready volcano plots with enhanced colouring and labeling. https://github.com/kevinblighe/EnhancedVolcano, 2020.
[38] C.M. Simán, C.P. Sibley, C.J.P. Jones, M.A. Turner, S.L. Greenwood, The functional regeneration of syncytiotrophoblast in cultured explants of term placenta, Am. J. Physiol. Regul. Integr. Comp. Physiol. 280 (2001) 1116–1122, https://doi.org/10.1152/ajpregu.2001.280.4.r1116.
[39] J. Feng, H. Yin, Baturuhu, Y. Dai, F. Dai, J. Xu, Z. Chen, Y. Liu, Research progress of E3 ubiquitin ligase regulating biological behavior of human placental trophoblast cells, Front. Endocrinol. 14 (2023), https://doi.org/10.3389/fendo.2023.1124041.
[40] J.L. James, A. Lissaman, Y.N.S. Nursalim, L.W. Chamley, Modelling human placental villous development: designing cultures that reflect anatomy, Cell. Mol. Life Sci. 79 (2022), https://doi.org/10.1007/s00018-022-04407-x.
[41] A. Byford, C. Baird-Rayner, K. Forbes, Don't sugar coat it: the effects of gestational diabetes on the placental vasculature, Biochem (Lond) 43 (2021) 34–39, https://doi.org/10.1042/bio_2021_117.
[42] M. Owen, M.G. Kennedy, R. Quilang, E. Scott, K. Forbes, The role for miRNAs in Pregnancies complicated by Maternal Diabetes, Clin. Sci. (2024), https://doi.org/10.1042/CS20230681.
[43] M. Jirkovská, T. Kučera, J. Kaláb, M. Jadrníček, V. Niedobová, J. Janáček, L. Kubínová, M. Moravcová, Z. Žižka, V. Krejčí, The branching pattern of villous capillaries and structural changes of placental terminal villi in type 1 diabetes mellitus, Placenta (Eastbourne) 33 (2012) 343–351, https://doi.org/10.1016/j.placenta.2012.01.014.
[44] M. Jirkovská, L. Kubínová, J. Janáček, M. Moravcová, V. Krejčí, P. Karen, Topological properties and spatial organization of villous capillaries in normal and diabetic placentas, J. Vasc. Res. 39 (2002) 268–278, https://doi.org/10.1159/000063692.
[45] O. Björk, B. Persson, Villous structure in different parts of the cotyledon in placentas of insulin-dependent diabetic women: a morphometric study, Acta Obstet. Gynecol. Scand. 63 (1984) 37–43, https://doi.org/10.3109/00016348409156271.
[46] T.O. Junaid, P. Brownbill, N. Chalmers, E.D. Johnstone, J.D. Aplin, Fetoplacental vascular alterations associated with fetal growth restriction, Placenta (Eastbourne) 35 (2014) 808–815, https://doi.org/10.1016/j.placenta.2014.07.013.
[47] J. Wei, T. Dong, M. Chen, X. Luo, Y. Mi, Unique ultrastructural alterations in the placenta associated with macrosomia induced by gestational diabetes mellitus, Mater. Fetal Med. 6 (2024) 164–172, https://doi.org/10.1097/FM9.0000000000000240.
[48] J. Aplin, S. Swietlik, J. Charnock, M. Khalid, M. Westwood, E. Johnstone, Vascular regression in the fetoplacental vascular bed, and its possible implications for fetal growth restriction, Placenta (Eastbourne) 36 (2015) 472, https://doi.org/10.1016/j.placenta.2015.01.389.
[49] M. Cherubini, S. Erickson, K. Haase, Modelling the human placental interface in vitro—a review, Micromachines (Basel) 12 (2021), https://doi.org/10.3390/mi12080884.
[50] M. Castillo-Castrejon, T.L. Powell, Placental nutrient transport in gestational diabetic pregnancies, Front. Endocrinol. 8 (2017) 1–9, https://doi.org/10.3389/fendo.2017.00306.
[51] C.M. Scifres, B. Chen, D.M. Nelson, Y. Sadovsky, Fatty acid binding protein 4 regulates intracellular lipid accumulation in human trophoblasts, J. Clin. Endocrinol. Metab. 96 (2011), https://doi.org/10.1210/jc.2010-2084.
[52] Q. Wang, H. Fujii, G.T. Knipp, Expression of PPAR and RXR isoforms in the developing rat and human term placentas, Placenta (Eastbourne) 23 (2002) 661–671, https://doi.org/10.1053/plac.2002.0855.
[53] Y. Barak, Y. Sadovsky, T. Shalom-Barak, PPAR signaling in placental development and function, PPAR Res. (2008), https://doi.org/10.1155/2008/142082.
[54] O. Wendling, P. Chambon, M. Mark, Retinoid X receptors are essential for early mouse development and placentogenesis, Dev. Biol. 96 (1999) 547–551, https://doi.org/10.1073/pnas.96.2.547.
[55] J. Pan, R. Guleria, S. Zhu, K. Baker, Molecular mechanisms of retinoid receptors in diabetes-induced cardiac remodeling, J. Clin. Med. 3 (2014) 566–594, https://doi.org/10.3390/jcm3020566.
[56] X.Y. Yu, L.P. Song, H.T. Zheng, S.D. Wei, X.L. Wen, B. Huang, D. Bin Liu, Association between functional genetic variants in retinoid X receptor-α/γ and the risk of gestational diabetes mellitus in a southern Chinese population, Biosci. Rep. 41 (2021), https://doi.org/10.1042/BSR20211338.
[57] V. Nikolova, G. Papacleovoulou, E. Bellafante, L. Borges Manna, E. Jansen, S. Baron, S. Abu-Hayyeh, M. Parker, C. Williamson, Changes in LXR signaling influence early-pregnancy lipogenesis and protect against dysregulated fetoplacental lipid homeostasis, Am. J. Physiol. Endocrinol. Metabol. 313 (2017) E463–E472, https://doi.org/10.1152/ajpendo.00449.2016.
[58] N.P. Illsley, M.C. Sellersb, R.L. Wrighta, Glycaemic regulation of glucose transporter expression and activity in the human placenta, Placenta (Eastbourne) 19 (1998) 517–524, https://doi.org/10.1016/S0143-4004(98)91045-1.
[59] M.C. Gordon, P.D. Zimmerman, M.B. Landon, S.G. Gabbe, D.A. Kniss, Insulin and glucose modulate glucose transporter messenger ribonucleic acid expression and glucose uptake in trophoblasts isolated from first-trimester chorionic villi. https://doi.org/10.1016/0002-9378(95)91332-7, 1995.
[60] V.F.H. Jensen, A.M. Mølck, J. Nowak, M. Wohlfarth, E. Nüsken, D. Demozay, K. D. Nüsken, I.B. Bøgh, Placental nutrient transporters adapt during persistent maternal hypoglycaemia in rats, PLoS One 17 (2022), https://doi.org/10.1371/journal.pone.0265988.
[61] V.F.H. Jensen, A.M. Mølck, J. Lykkesfeldt, I.B. Bøgh, Effect of maternal hypoglycaemia during gestation on materno-foetal nutrient transfer and embryo-foetal development: evidence from experimental studies focused primarily on the rat, Reprod. Toxicol. 77 (2018) 1–24, https://doi.org/10.1016/j.reprotox.2018.01.007.
[62] J. Wang, A. Alexanian, R. Ying, T.J. Kizhakekuttu, K. Dharmashankar, J. Vasquez-Vivar, D.D. Gutterman, M.E. Widlansky, Acute exposure to low glucose rapidly induces endothelial dysfunction and mitochondrial oxidative stress role for AMP kinase, Arterioscler. Thromb. Vasc. Biol. 32 (2012) 712–720, https://doi.org/10.1161/ATVBAHA.111.227389/-/DC1.
[63] A. He, D. Zuo, X. Liang, Y. Guo, L. Suxin, Y. Xia, Hypoglycemia increases endothelial-dependent vasodilation through suppressing phosphorylation at

Threonine 495/497 site of endothelial nitric oxide synthase, Microvasc. Res. 133 (2021), https://doi.org/10.1016/j.mvr.2020.104075.
[64] H. Chen, Y. Chen, Q. Zheng, The regulated cell death at the maternal-fetal interface: beneficial or detrimental? Cell Death Discov. 10 (2024) https://doi.org/10.1038/s41420-024-01867-x.
[65] E.M. Strøm-Roum, A.M. Jukic, A. Eskild, Offspring birthweight and placental weight—does the type of maternal diabetes matter? A population-based study of 319 076 pregnancies, Acta Obstet. Gynecol. Scand. 100 (2021) 1885–1892, https://doi.org/10.1111/aogs.14217.
[66] E. Taricco, T. Radaelli, M.S. Nobile de Santis, I. Cetin, Foetal and placental weights in relation to maternal characteristics in gestational diabetes, Placenta (Eastbourne) 24 (2003) 343–347, https://doi.org/10.1053/plac.2002.0913.
[67] M.B. Aires, A.C.V. Dos Santos, Effects of maternal diabetes on trophoblast cells, World J. Diabetes 6 (2015) 338–344, https://doi.org/10.4239/wjd.v6.i2.338.
[68] R.L. Balihallimath, V.S. Shirol, A.M. Gan, N.K. Tyagi, M.R. Bandankar, Placental morphometry determines the birth weight, J. Clin. Diagn. Res. 7 (2013) 2428–2431, https://doi.org/10.7860/JCDR/2013/7478.3564.
[69] S.C. Smith, P.N. Baker, E.M. Symonds, Increased placental apoptosis in intrauterine growth restriction, Am. J. Obstet. Gynecol. 177 (1997) 1395–1401, https://doi.org/10.1016/s0002-9378(97)70081-4.
[70] C.-P. Chen, R. Bajoria, J.D. Aplin, Decreased vascularization and cell proliferation in placentas of intrauterine growth-restricted fetuses with abnormal umbilical artery flow velocity waveforms, Am. J. Obstet. Gynecol. 187 (2002) 764–769, https://doi.org/10.1067/mob.2002.125243.
[71] J.M. Salbaum, C. Kruger, X. Zhang, N.A. Delahaye, G. Pavlinkova, D.H. Burk, C. Kappen, Altered gene expression and spongiotrophoblast differentiation in placenta from a mouse model of diabetes in pregnancy, Diabetologia (Berl.) 54 (2011) 1909–1920, https://doi.org/10.1007/s00125-011-2132-6.
[72] M.F. Bari, S. Ngo, C.C. Bastie, A.M. Sheppard, M. Vatish, Gestational diabetic transcriptomic profiling of microdissected human trophoblast, J. Endocrinol. 229 (2016) 47–59, https://doi.org/10.1530/JOE-15-0424.
[73] Y.Y. Chen, F.J. Rosario, M.A. Shehab, T.L. Powell, M.B. Gupta, T. Jansson, Increased ubiquitination and reduced plasma membrane trafficking of placental amino acid transporter SNAT-2 in human IUGR, Clin. Sci. 129 (2015) 1131–1141, https://doi.org/10.1042/CS20150511.
[74] M. Persson, H. Fadl, Perinatal outcome in relation to fetal sex in offspring to mothers with pre-gestational and gestational diabetes-a population-based study, Diabet. Med. 31 (2014) 1047–1054, https://doi.org/10.1111/dme.12479.
[75] A. García-Patterson, A. Aulinas, L. Sojo, G. Ginovart, J.M. Adelantado, A. de Leiva, R. Corcoy, Poorer perinatal outcome in male newborns of women with pregestational diabetes mellitus, Diabet. Med. 28 (2011) 436–439, https://doi.org/10.1111/j.1464-5491.2011.03227.x.
[76] M. Maghalian, Z. Alizadeh-Dibazari, M. Mirghafourvand, Impact of fetal sex on neonatal outcomes in women with gestational diabetes mellitus: a systematic review and meta-analysis, BMC Pregnancy Childbirth 25 (2025) 110, https://doi.org/10.1186/s12884-025-07250-7.
[77] F. Mauvais-Jarvis, Gender differences in glucose homeostasis and diabetes, Physiol. Behav. 187 (2018) 20–23, https://doi.org/10.1016/j.physbeh.2017.08.016.
[78] B. Tramunt, S. Smati, N. Grandgeorge, F. Lenfant, J.F. Arnal, A. Montagner, P. Gourdy, Sex differences in metabolic regulation and diabetes susceptibility, Diabetologia (Berl.) 63 (2020) 453–461, https://doi.org/10.1007/s00125-019-05040-3.
[79] R. Sood, J.L. Zehnder, M.L. Druzin, P.O. Brown, Gene expression patterns in human placenta, Proc. Natl. Acad. Sci. 103 (2006) 5478–5483. http://smd.stanford.edu.
[80] S. Jiang, A.M. Teague, J.B. Tryggestad, C.E. Aston, T. Lyons, S.D. Chernausek, Effects of maternal diabetes and fetal sex on human placenta mitochondrial biogenesis, Placenta (Eastbourne) 57 (2017) 26–32, https://doi.org/10.1016/j.placenta.2017.06.001.
[81] C.S. Rosenfeld, Sex-specific placental responses in fetal development, Endocrinology (United States) 156 (2015) 3422–3434, https://doi.org/10.1210/en.2015-1227.
[82] Z.R. Ren, S.S. Luo, X.Y. Qin, H.F. Huang, G.L. Ding, Sex-specific alterations in placental proteomics induced by intrauterine hyperglycemia, J. Proteome Res. 23 (2024) 1272–1284, https://doi.org/10.1021/acs.jproteome.3c00735.
[83] W.W. Hay, Placental-fetal glucose exchange and fetal glucose metabolism, Trans. Am. Clin. Climatol. Assoc. 117 (2006).
[84] M.U.B. Nicholas P. Illsley, Human placental glucose transport in fetoplacental growth and metabolism, Biochim. Biophys. Acta Mol. Basis Dis. 1866 (2018) 139–148, https://doi.org/10.1016/j.bbadis.2018.12.010.
[85] T. Jansson, M. Wennergren, N.P. Illsley, Glucose transporter protein expression in human placenta throughout gestation and in intrauterine growth retardation, J. Clin. Endocrinol. Metab. 77 (1993) 1554–1562, https://doi.org/10.1210/jcem.77.6.8263141.
[86] L.B. James-Allan, J. Arbet, S.B. Teal, T.L. Powell, T. Jansson, Insulin stimulates GLUT4 trafficking to the syncytiotrophoblast basal plasma membrane in the human placenta, J. Clin. Endocrinol. Metab. 104 (2019) 4225–4238, https://doi.org/10.1210/jc.2018-02778.
[87] C. Janzen, M.Y.Y. Lei, J. Cho, P. Sullivan, B.-C. Shin, S.U. Devaskar, Placental glucose transporter 3 (GLUT3) is up-regulated in human pregnancies complicated by late-onset intrauterine growth restriction, Placenta (Eastbourne) 34 (2013) 1072–1078, https://doi.org/10.1016/j.placenta.2013.08.010.
[88] P.J. Stanirowski, D. Szukiewicz, A. Majewska, M. Wątroba, M. Pyzlak, D. Bomba-Opoń, M. Wielgoś, Differential expression of glucose transporter proteins GLUT-1, GLUT-3, GLUT-8 and GLUT-12 in the placenta of macrosomic, small-for-gestational-age and growth-restricted foetuses, J. Clin. Med. 10 (2021), https://doi.org/10.3390/jcm10245833.
[89] M.U. Sajjad, T. Henriksen, M.C.P. Roland, G. Haugen, T.M. Michelsen, Maternal body mass index, birthweight, and placental glucose metabolism: evidence for a role of placental hexokinase, Am. J. Obstet. Gynecol. 230 (2024) 677.e1–677.e10, https://doi.org/10.1016/j.ajog.2023.10.043.
[90] F Supek, M. Bošnjak, N. Škunca, T. Šmuc, REVIGO summarizes and vsualizes Long lists of gene ontology terms, PLoS One 6 (7) (2011) e21800.