Placental mitochondrial content and function in intrauterine growth restriction and preeclampsia

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Mandò C, De Palma C, Stampalija T, Anelli GM, Figus M, Novielli C, Parisi F, Clementi E, Ferrazzi E, Cetin I. Placental mitochondrial content and function in intrauterine growth restriction and preeclampsia. Am J Physiol Endocrinol Metab 306: E404–E413, 2014. First published December 17, 2013; doi:10.1152/ajpendo.00426.2013.—Intrauterine growth restriction (IUGR) and pregnancy hypertensive disorders such as preeclampsia (PE) associated with IUGR share a common placental phenotype called “placental insufficiency”, originating in early gestation when high availability of energy is required. Here, we assess mitochondrial content and the expression and activity of respiratory chain complexes (RCC) in placental cells of these pathologies. We measured mitochondrial (mtDNA) and nuclear respiratory factor 1 (NRF1) expression in placentas of IUGR and PE pregnancies complicated by IUGR and PE. To this purpose, we analyzed eight IUGR, six PE, and eight uncomplicated human pregnancies delivered by elective cesarean section. We found lower mRNA levels of complex II, III, and IV in IUGR cytotrophoblast cells but no differences at the protein level, suggesting a posttranscriptional compensatory regulation. mtDNA was increased in IUGR placentas. Both mtDNA and NRF1 expression were instead significantly lower in their isolated cytotrophoblast cells. Finally, cytotrophoblast RCC activity was significantly increased in placentas of IUGR fetuses. No significant differences were found in PE placentas. This study provides genuine new data into the complex physiology of placental oxygenation in IUGR fetuses. The higher mitochondrial content in IUGR placental tissue is reversed in cytotrophoblast cells, which instead present higher mitochondrial functionality. This suggests different mitochondrial content and activity depending on the placental cell lineage. Increased placental oxygen consumption might represent a limiting step in fetoplacental growth restriction, preventing adequate oxygen delivery to the fetus.

mitochondria; placenta; pregnancy; intrauterine growth restriction; preeclampsia

INTRAUTERINE GROWTH RESTRICTION (IUGR) is a multifactorial disease of pregnancy that markedly increases perinatal mortality and morbidity. In IUGR, the fetus fails to reach its growth potential (7). It may occur alone or associated with hypertensive disorders of pregnancy (HDP); preeclampsia (PE), gestational hypertension, and chronic hypertension. IUGR of placental origin without maternal clinical damage, and HDP associated with IUGR pregnancies share a common placental phenotype described as “placental insufficiency” that originates in early gestation, when the trophoblast invades the decidua. This process requires high availability of energy for cell growth, proliferation and metabolic activity (22).

Mitochondria are the main energy producers in the cell. They consume oxygen (O2) and produce ATP by electron transport and oxidative phosphorylation taking place across the respiratory chain complexes located in the mitochondrial inner membrane. Mitochondrial alterations occur in several disorders, including malignant and benign proliferations and inflammatory conditions (15, 59, 26). The metabolic activity of the placenta is sustained throughout gestation by increasing mitochondrial activity and biogenesis (29). Damage to this process could lead to excessive generation of reactive oxygen and nitrogen species (29), contributing to the placental damage that has been shown in pathologies such as IUGR, HDP (hypertensive disorders in pregnancy), gestational diabetes, and maternal obesity (35, 45, 20).

Mitochondrial dysfunctions also represent a critical factor for fetal programming in cases of placental insufficiency (29). In mouse embryo, altered mitochondrial function affects subsequent fetal and placental growth (57), and conversely, maternal undernutrition in rats induces impaired placental mitochondrial function in fetal and placental growth restriction (35).

We have previously reported higher mitochondrial DNA (mtDNA) levels in placenta and maternal blood of human IUGR (13, 28). mtDNA levels are largely recognized as a measure of the mitochondrial content (25, 36, 37, 58) and are regulated by different mitochondrial biogenesis activators in several tissues (41, 6). Nuclear Respiratory Factor 1 (NRF1) is one of the main players in the mitochondrial biogenesis pathway (51).

Whether an altered mitochondrial content leads to altered placental O2 consumption (V˙O2) still needs to be investigated. Regnault et al. (49) reported in an ovine model of IUGR that fetal O2 uptake is decreased while transplacental O2 partial pressure (PO2) gradient is increased, suggesting increased placental O2 extraction in IUGR.

Here, we investigated potential differences in mitochondrial content and function as well as in placental respiration in pregnancies complicated by IUGR and PE. To this purpose, we measured placental trophoblast cells mitochondrial content (mtDNA), NRF1 levels, and VO2, together with the respiratory...
chain complex gene and protein expression in IUGR, in maternal PE without IUGR, and in normal control pregnancies.

MATERIALS AND METHODS

Population. Pregnant patients were enrolled in the Units of Obstetrics and Gynecology at the L. Sacco Hospital and the V. Buzzi Children Hospital in Milan, Italy. The study was approved by the Institutional Ethics Committee, and all pregnant patients gave their informed consent.

Placental samples were collected at elective cesarean section from eight pregnancies with IUGR without HDP (IUGR group), six pregnancies with PE without IUGR (PE group), and eight normal pregnancies at term (control group). All samples were obtained from nonlaboring pregnant women.

Gestational hypertension was defined as blood pressure ≥140/90 mmHg in two measurements 4 h apart after the 20th week of pregnancy in a previously normotensive and nonproteinuric woman (5). PE was defined as gestational hypertension with proteinuria >300 mg/24 h.

IUGR fetuses were identified in utero through longitudinal sonographic measurements that demonstrated a reduction in fetal growth below the 5th percentile for local standards (54). Depending on gestational age, timing of delivery was based on abnormal ductus venosus pulsatility index (PI) (3), abnormal cardiotocography as previously described (40), or worsening maternal conditions in cases affected by HDP.

Controls had normal intrauterine growth as assessed by longitudinal ultrasound biometry at 20 wk, 30–32 wk, and term. The newborn weight at term was appropriate for gestational age (AGA) according to Italian standards for birth weight and gestational age (4). Cesarean sections before labor were performed for breech presentation, repeated cesarean section, or maternal request. Exclusion criteria were any maternal, placental, or fetal disease.

Placental samples collection and cytotrophoblast cells isolation. Placentas were collected immediately after cesarean section and sampled in different sites of the placental disc (central, median, and peripheral) by discarding the maternal decidua and collecting the underlying villi.

Samples were washed in PBS (Dulbecco’s phosphate-buffered solution; Euroclone, Milano, Italy) and processed for the isolation of cytotrophoblast cells or frozen in liquid nitrogen and then stored at −80°C until mtDNA and expression analysis.

Cytotrophoblast cells were isolated using the trypsin/deoxyribonuclease/dispase/Percoll method, as described (52). Reagents were supplied by Sigma-Aldrich (St. Louis, MO; Hank’s balanced salt solution, HEPES, trypsin, Percoll), BD-Becton-Dickinson (Bedford, MA; dispase), Roche, Mannheim, Germany (DNase), and Euroclone (gentamicin, fetal bovine serum, and Dulbecco’s modified Eagle’s medium-HG).

DNA, RNA, and protein extraction. Total RNA, DNA, and proteins were isolated from tissues and cytotrophoblast cells using TRizol reagent (Invitrogen, Life Technologies, Cergy, France) following the manufacturer’s instructions. RNA was treated with a DNA-free kit (Ambion, Austin, TX) to remove potentially contaminating DNA. RNA and DNA concentrations were measured by spectrophotometer (NanoDrop ND1000; NanoDrop Technologies, Wilmington, DE). Protein concentration was determined using the Micro BCA Protein Assay Kit (Thermo Scientific-Pierce Biotechnology, Rockford, IL), according to the manufacturer’s protocol.

mtDNA content in placental tissue and in isolated cytotrophoblast cells. The mtDNA content was measured by real-time PCR normalizing the quantity of a not-polyomorph mitochondrial gene (cytochrome B) with a single-copy nuclear gene (RNase P). It was analyzed in the placental tissue of 8 controls, 6 PE, 8 IUGR, and in the cytotrophoblast cells isolated from them. For each assay, 30 ng of total DNA were analyzed in triplicate by TaqMan Assay technology, 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The Sequence Detector software (Applied Biosystems) was used to analyze the data, and relative quantification values were calculated according to the 2−ΔΔCT method.

NRF1 and respiratory chain complexes gene expression: real-time PCR. The mRNA levels of four subunits belonging to four respiratory chain complexes (RCC) were measured by real-time PCR (Applied Biosystems) in the cytotrophoblast cells isolated from 8 control, 6 PE, and 8 IUGR placentas. Analyzed genes were: NADH-dehydrogenase-1α subcomplex 9 [NDUFA9, complex I (CI); assay ID: Hs00245308_m1], succinate dehydrogenase complex subunit A [SDHA, complex II (CII); assay ID: Hs00188166_m1], ubiquinol-cytochrome c reductase core protein 1 [UQRC1, complex III (CIII); assay ID: Hs00163415_m1], cytochrome c oxidase subunit IV isoform 1 [COX4I1, complex IV (CIV); assay ID: Hs00971639_m1]. Moreover, we analyzed the gene expression of the nuclear respiratory factor 1 (NRF1; assay ID: Hs00192316_m1), a mitochondrial biogenesis activator, in both placental tissue and cytotrophoblast cells of 5 controls and 6 IUGR samples.

For each sample, 1 μg of total RNA was reverse-transcribed by the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) and obtained cDNA served as template for quantitative real-time PCR with TaqMan assays (Applied Biosystems). Gene expression levels were calculated using the geNorm method (55) relative to HPRT (hypoxanthine-guanine phosphoribosyltransferase; assay ID: Hs99999909_m1) and YWHAZ (tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein-ζ polypeptide; assay ID: Hs00237047_m1) genes, selected from a pool of tested housekeeping genes. All samples were reverse-transcribed in duplicate, and cDNA was run in triplicate to allow assessment of sample homogeneity and technical variability.

Respiratory chain complexes protein expression in cytotrophoblast cells: western blotting. Western blotting analyses were performed on proteins extracted from the same cytotrophoblast cells used for gene expression analysis. Proteins from each sample (50 μg) were precipitated overnight (ON) at −20°C in 2.5 μl of acetone and resolved under reducing conditions by SDS polyacrylamide gel electrophoresis. Separated proteins were transferred to a polycrylidyne difluoride (PVDF) membrane (iBlot Dry Blotting System, Invitrogen). All nonspecific bindings were blocked by membrane incubation in a blocking buffer [5% skimmed powdered milk in TBS-T (TBS, 0.3% Tween 20)] for 2.5 h at room temperature. Membranes were incubated ON in blocking buffer with mouse monoclonal antibodies against the four RCC subunits analyzed by real-time PCR (1:5,000 dilution; Molecular Probes, Invitrogen). After incubation with an anti-mouse HRP-conjugated antibody, protein bands were detected by the enhanced chemiluminescence method (ImmunStar Western C; Bio-Rad, Hercules, CA). All membranes were subsequently stripped and reprobed with anti-β-actin antibody (1:15,000 dilution; Sigma-Aldrich). Band intensity was measured by ImageJ software (freely available at http://rsbweb.nih.gov/ij/). For relative semiquantitative analysis, the protein expression values were normalized on β-actin. Data from each blot were referred to a sample chosen as reference to allow comparison among different experiments.

High-resolution respirometry. RCC efficiency was determined by high-resolution respirometry (HRR) on cytotrophoblast cells isolated from placentas of the IUGR group, three cases of the PE group, and seven controls. This technique allows to measure total mitochondrial V0 and to suggest the role of each mitochondrial complex in the process.

O2 suspension was measured by HRR with an Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria) in MitR06 medium (EGTA 0.5 mM, MgCl2·6H2O 3 mM, K-lactobionate 60 mM, taurine 20 mM, KH2PO4 10 mM, HEPES 20 mM, succrose 110 mM, BSA 1 g/l, catalase 280 μg/ml), according to HRR MitNNet protocols, Oroboros Instruments) at 37°C. MitR06 was equilibrated and then replaced by the cell suspension, which was continuously stirred at 460–600 rpm.
The experimental protocol started with routine respiration, defined as VO₂ of cell suspension without any substrate addition. Cells were then permeabilized with digitonin (40 µg/ml), allowing loss of plasma membrane barrier but maintaining intracellular membrane structure (mitochondria and ER) (1). CI was measured by the injection of glutamate (10 mM) and malate (2 mM) substrates in the presence of ADP 2.5 mM. A cytochrome c test was performed to test the intactness of mitochondrial outer membrane, since in case of damage it would be released with a consequent inhibition of respiration. Addition of succinate 10 mM in the presence of conventional substrates for CI supported the simultaneous, convergent electron flow. Addition of succinate 10 mM in the presence of conventional substrates for CI supported the simultaneous, convergent electron flow. CI was measured by the injection of TMPD (N,N,N',N'-tetramethyl-p-phenylenediamine 0.5 mM and ascorbate 2 mM).

Table 1. A: Maternal and fetal baseline demographic data of the 2 groups of cases and controls. Data are presented as average ± SE. *P < 0.017 vs. controls; **P < 0.001 vs. controls. B: prenatal and neonatal baseline data of cases and controls. Uterine and umbilical artery pulsatility index (PI) was assessed by Doppler velocimetry. PE, preeclampsia; IUGR, intrauterine growth restricted; A, abnormal; N, normal. C: blood pressure (BP) and urine protein values in PE women

### RESULTS

Maternal and fetal baseline demographic data of cases and controls are compared in Table 1A. Maternal age and BMI were not significantly different among the three groups. As expected, gestational age and fetal and placental weights were significantly lower in IUGR and PE groups than in controls.
Table 1B shows clinical and diagnostic characteristics of each single case. Table 1C presents maternal blood pressure and urine protein data of PE pregnancies.

**mtDNA in placental tissue and in isolated cytotrophoblast cells.** mtDNA levels were significantly higher in IUGR placental tissues ($P < 0.017$) than in controls, whereas no significant difference was observed between placentas of PE and controls (Fig. 1A).

mtDNA levels were instead significantly lower in IUGR cytotrophoblast cells isolated from the same placentas ($P < 0.017$) compared with controls. Again, no significant difference was found between PE and control cells (Fig. 1B).

**NRF1 gene expression in placental tissue and cytotrophoblast cells.** NRF1 gene expression was significantly lower in IUGR cytotrophoblast cells, with a 30% expression decrease compared with controls. NRF1 mRNA levels in placental tissue were on average 24% higher in IUGR compared with controls; however, the difference was not statistically significant (Fig. 2).

**Respiratory chain complexes gene and protein expression in cytotrophoblast cells.** mRNA expression of CII, CIII, and CIV in cytotrophoblast cells of IUGR was lower than in controls, though this difference did not reach statistical significance when corrected by the Bonferroni adjustment ($P = 0.04$). PE did not present any difference vs. controls in mRNA expression of the analyzed subunits of the respiratory chain complexes (Fig. 3A).

The protein expression of the same subunits was not significantly different between cytotrophoblast cells of IUGR and PE groups compared with controls (Fig. 3B).

**Cytotrophoblast cells V˙O2.** Figure 4 shows the V˙O2 levels per mitochondrion (Fig. 4A) and per number of cells (Fig. 4B) resulting from the activity of the respiratory chain complexes. Cytotrophoblast cells of the IUGR group showed significantly higher coupled V˙O2 compared with controls, both as total assessment (CI+CI) and at the level of the single respiratory complex CIV, normalized to mitochondrial content (Fig. 4A). CIV IUGR V˙O2 was also higher than that of PE. Interestingly, data not normalized to mtDNA showed higher CIV V˙O2 in cells from IUGR placentas, notwithstanding their lower content of mtDNA (Fig. 4B).

Again, opposite to the IUGR cases, cytotrophoblast cells of PE placentas did not show such changes, except for an increase...
in per-mitochondrion CII respiration that, however, did not reflect in enhanced overall VO₂.

In the IUGR cases with abnormal umbilical PI (PIumb), VO₂ per mitochondrion was higher than in controls for all reported complexes (Fig. 4C). This resulted in higher CIV and total VO₂ also overall in the cell (Fig. 4D). IUGR cases with normal PIumb were instead similar to controls except for an increased per-mitochondrion respiration on complex IV that, however, did not reflect on the cell overall total respiration.

Finally, although not significantly different from controls, VO₂ levels were higher in more severe cases of PE (data not shown).

We did not find any sex difference for VO₂ levels (data not shown).

**Data correlations.** In our population, mtDNA content of placental tissue inversely correlated both with fetal (R² = 0.2, P = 0.03) and placental (R² = 0.3, P = 0.008) weight (Fig. 5, A and B). The mtDNA content of both placental tissue and isolated cytotrophoblast cells did not correlate with gestational age (data not shown). The trophoblast cells’ mtDNA inversely correlated with normalized total (CI+CII) VO₂ (R² = 0.3, P = 0.02), and this was sustained by the single respiratory complexes (CI: R² = 0.3, P = 0.02; CIV: R² = 0.6, P < 0.001; Fig. 6, A–C).

Both fetal and placental weights inversely correlated with cytotrophoblast mitochondrial VO₂ (data not shown).

Finally, total VO₂ values, regardless of their normalization on mtDNA or cell number, inversely correlated with gestational age (data not shown).

**DISCUSSION**

In this study, we investigated the content and function of placental mitochondria by measuring 1) mtDNA and NRF1 gene expression levels in placental tissue and cytotrophoblast cells, and 2) gene and protein expression of respiratory chain complexes and their VO₂ in cytotrophoblast cells. These analyses were performed in placentas of IUGR fetuses, of pregnant women affected by PE without IUGR, and in uncomplicated pregnancies. All pregnancies were terminated by cesarean section before labor.

**Mitochondrial content in placental tissue and cytotrophoblast cells.** We found that mtDNA content was higher in placentas of the IUGR group than in control placentas. This is in agreement with our previously reported results (28) and complies with NRF1 gene expression levels, which were increased, though not significantly, in IUGR placentas. On the other hand, mtDNA placental content of the PE group was not significantly different from control placentas. Opposite to what we observed in whole placental tissue samples, both mtDNA content and NRF1 gene expression levels were significantly lower in isolated cytotrophoblast cells of the IUGR group compared with controls, accounting for a decreased mitochondrial content in these cells.

The mitochondrial content increase in IUGR placental tissue, opposite to its reduction in cytotrophoblast cells isolated from the same placentas, is a genuine new, remarkable result. Since in this study we investigated mtDNA levels only in placental cytotrophoblast cell lineage, further cell types (namely syncytiotrophoblast, fetal vascular, and placental stroma cells such as mesenchymal cells, mesenchymal derived macrophages, and fibroblasts) may be responsible for the mitochondrial content increase in the whole placental tissue.

Changes in mitochondrial content in different lineages of placental cells may possibly be related to the increasing transplacental PO₂ gradient between uterine and umbilical venous blood, which we previously described in IUGR pregnancies (39) and which was also reported in IUGR animal models (49).

The regulation of mitochondrial biogenesis and function by O₂ concentration has been previously observed in other animal tissues (19, 60). Different O₂ concentrations may thus lead to different mitochondrial content in placental cells.

Endothelial cells are most likely to be the cells responsible for the overall higher placental tissue mtDNA content found in IUGR. Indeed, fetal vascular mitochondrial variations might occur in utero in response to intrauterine O₂ content (29).

We could speculate that the higher mitochondrial activity we observed in trophoblast cells of IUGR fetuses might cause additional fetal vascular damage via an excess of reactive oxygen species (ROS). Such damage could eventually trigger additional mitochondrial biogenesis in fetal and placental endothelial cells.

Moreover, the increased maternal-fetal O₂ gradient (39, 40) may expose endothelial and trophoblast cells to two extremes of O₂ environment with opposite changes in mitochondrial biogenesis.
The difference between mtDNA content of cytotrophoblast cells and total placental tissue in IUGR fetuses might also be due to differential exposure to progesterone. This and other steroids regulate mitochondrial biogenesis and might play a role in the pathogenesis of IUGR in humans. Partial progesterone withdrawal in rats induces placental and fetal growth restriction (34). Receptor regulation of miometrial progesterone was also proved in IUGR guinea pigs (38). Thus, progesterone production, which occurs in the syncytiotrophoblast but not in the cytotrophoblast cell, may lead to different mitochondrial levels in these different cell lineages of the IUGR placenta.

Gene and protein expression of respiratory chain complexes and their $V_{O2}$ in cytotrophoblast cells. When we moved from mtDNA content to mitochondrial function, we observed lower,
although not significantly, mRNA levels of the subunits of CII, CIII, and CIV in cytotrophoblast cells of the IUGR group compared with controls. Their expression was also not significantly different at the protein level.

In contrast, mitochondrial bioenergetics efficiency represented by \( \dot{V}O_2 \) was higher in cytotrophoblast cells of the IUGR group vs. controls. Again, PE cells presented only minor alterations in CII activity, which did not impact on overall mitochondrial activity.

Thus, despite the protein content of RCC subunits not being altered, their activity was significantly increased in IUGR cytotrophoblast cells. This may have been due to a different RCC assembly in the so-called supercomplexes. Supercomplex assembling is required for RCC stability and functionality by organizing electron flux and ameliorating the use of available substrates (23, 27, 56). A more efficient supercomplex assembly might explain the increase in respiratory capacity of IUGR cells. This hypothesis needs further investigation.

Moreover, although the mitochondrial content in cytotrophoblast cells of the IUGR group was significantly lower than in controls, these same mitochondria were able to sustain higher total cellular respiration rate.

In addition, when all samples were considered together, \( \dot{V}O_2 \) was inversely correlated both with mtDNA content of cytotrophoblast cells and with fetal and placental weights.

These findings all together might suggest a functional compensatory effect to the decreased mitochondrial content: the more the growth restriction the higher the mitochondrial \( \dot{V}O_2 \).

Another remarkable new observation derives from the analysis of data according to umbilical artery Doppler velocimetry (PI\(_{\text{umb}}\)). We have previously reported that these IUGR cases are associated with cerebral metabolic and maturation changes driven by hypoxia, as shown by in utero MR spectroscopy (8) and postnatal MR imaging (47). \( \dot{V}O_2 \) presented a significantly higher increase compared with controls in IUGR with abnormal PI\(_{\text{umb}}\) than in IUGR fetuses with normal PI\(_{\text{umb}}\). These
findings underline the role of umbilical artery Doppler velocimetry as a marker of severity in IUGR.

These data suggest that possible compensatory mitochondrial mechanisms occur in IUGR to sustain fetal growth under conditions of severe placental vascular insufficiency. Further studies are needed to elucidate the consequences of these findings, especially as regards possible enhanced ROS generation, and in different phases of placental development.

Likewise, it would be important to investigate the mitochondrial dynamic processes of fusion and fission and their relationship with placental development. In other cell types, mitochondrial elongation has been shown to positively correlate with enhanced mitochondrial VO₂ and provide energy useful for tissues development (11).

Regnault et al. (49) suggested that in IUGR the higher transplacental P₀₂ gradient and the lower umbilical vein P₀₂ are the result of a lower ratio between low and high hindrance sites in the placental epithelium. The higher rate of VO₂ that we found in cytotrophoblast cells of IUGR human fetuses can contribute to generate high hindrance sites in the placenta, together with possible local decreases in the IUGR transplacental diffusion distance. This possibility has been demonstrated to date in a mouse model of IUGR (14).

Thus, our data suggest that altered O₂ delivery to IUGR fetuses might also be due to increased VO₂ within trophoblast cells, possibly representing one of the key factors leading to growth restriction. However, we cannot exclude that placental changes may be the result of reduced fetal VO₂ due to the slower rate of growth and thus decreased need for oxygen. We indeed have previously reported a significant reduction of fetal VO₂ even on a per kilogram basis in IUGR (46).

The changes we observed in IUGR might be influenced by an array of causes, such as caloric restriction. Indeed, strong links have been previously described between caloric restriction and changes in the mitochondrial machinery of different tissues (12, 30). Although maternal BMI was not different among groups in our study population, IUGR placentas are known to present in fact many characteristics leading to caloric restriction and mitochondrial defects, such as poor expression and activity of nutrient transporters (7, 10, 18, 33, 53), low activity of the glutamine-glutamate metabolism, and poor control of mitochondrial lipid peroxidation (33, 50), with increased sensitivity to mitochondrial oxidative stress and ROS production. Moreover, micronutrient imbalance might also be involved in IUGR mitochondrial alterations (9, 32).

Several mechanisms may be implicated in altered mitochondrial function. Estrogen-related receptors (ERRs), involved in mitochondrial biogenesis and functions (31, 48), also regulate estrogen production (21). Their possible increase, leading to mitochondrial biogenesis alterations in IUGR, may thus also lead to higher estradiol levels in IUGR placentas, contributing to abnormal placental vascularization and fetal maturation in IUGR pregnancies (42, 44).

Finally, we should stress the remarkable finding that, in our limited number of cases of PE, which were not associated with IUGR, most of the changes at stake in mitochondrial content and function were not different from controls. This might suggest that mitochondrial dysfunctions may be involved in IUGR pathophysiology but not in PE.

**Limitations**

Placental VO₂ is a complex feature resulting from the activity of different cell lineages. However, besides descriptive histological data (24), we are not aware of studies that have quantified the proportion of different cell types within the placenta and their relative contribution to placental VO₂. In this study, VO₂ data were obtained only in cytotrophoblast cells due to the protocol needed to isolate a proper amount of single cells from the entire placental tissue. Thus, we can only speculate about the possible contribution of cytotrophoblast cells to the total placental VO₂, and we cannot exclude that different cell types may give different results.

Another possible limit in this study is the different gestational age between cases and controls. This is a limit of all studies investigating human IUGR and PE compared with control-term placentas. However, to the best of our possible experimental design, we did not observe any significant negative correlation between gestational age and the VO₂ of CIV, which presents the highest significant difference between IUGR and controls among respiratory chain complexes.

Gestational age correlates to fetal weights but may independently alter mitochondrial number and/or function because of differences in hormone secretion and potential changes in placental VO₂ as gestational age advances. No studies have addressed the influence of gestational age on placental or trophoblast cells VO₂ in humans. Mayeur et al. (35) reported different placental VO₂ between normal and growth-restricted rats at the same gestational age, suggesting a role of mitochondrial dysfunction in the placental etiopathogenesis of IUGR pregnancies independent of gestational age. Moreover, few animal studies have been published about the influence of gestational age on the VO₂ of uteroplacental and fetal tissues in vivo. These studies report different conclusions: in pregnant ponies, both uteroplacental and per-kilogram fetus uptakes of oxygen did not seem to change from mid- to late gestation (17). In sheep the fetal oxygen uptake per kilogram was instead increased at the end of gestation (2). However, since significantly lower values of umbilical vein flow were found in human severely growth-restricted fetuses compared with normally growing fetuses of comparable gestational age (16), we may hypothesize lower fetal oxygen uptake in IUGR due to higher placental VO₂ rates, accounting for hypoxic fetuses in human IUGR.

Different results observed in PE are intriguing, although the limited number of cases studied for the VO₂ values invokes caution in the interpretation of these results.

**Conclusions**

The results we report in this study shed genuine new data into the complex physiology of placental oxygenation in IUGR fetuses. Mitochondrial content is higher in IUGR total placental tissue compared with normal pregnancies at term. This difference is reversed in cytotrophoblast cells of IUGR fetuses, which instead present higher mitochondrial functionality. These findings suggest different mitochondrial features depending on the placental cell lineage. We suggest that increased placental O₂ consumption by placental tissue may represent a limiting step in fetal growth restriction, preventing adequate O₂ delivery to the fetus. This limitation has potential
consequences on fetal O$_2$ consumption both in animal models and in human IUGR.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


