Magnetic resonance imaging of hypoxic injury to the murine placenta

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Fetal growth restriction (FGR) complicates up to 8% of all pregnancies, adversely impacts perinatal outcome, and results in life-long complications for adult survivors (14). Placental insufficiency is the major cause of FGR in developed countries, where it remains prevalent despite improvements in obstetrical care. Diverse causes of placental injury, such as chronic hypertension, preeclampsia, connective tissue disease, diabetes, and abruption, alter placental perfusion and may cause hypoxic and reperfusion injury (42).

Doppler ultrasound is useful in assessing FGR-related blood flow changes in the preplacental (uterine) and postplacental (umbilical, fetal) vasculature (1, 21). While the detection of these changes has limited diagnostic value, changes in postplacental flow are presently used to assist clinicians in management and timing of delivery of pregnancies with an established diagnosis of FGR (1, 30). Notably, Doppler ultrasound detects umbilical vessel flow changes that result from parenchymal damage but cannot directly assess intraplacental perfusion abnormalities (1, 28, 30).

Magnetic resonance imaging (MRI) offers superb visualization of anatomy and allows quantitative analysis of spatial and temporal changes in tissue morphology. MRI studies of placental anatomy and perfusion have been conducted in different species (16, 29, 31, 35, 36, 44, 51). Contrast agents (typically gadolinium-based chelates) were noted to easily cross the placental barrier in both the mouse and human placenta, and appeared in the fetal bladder soon after maternal intravenous administration (8, 49, 52). Dynamic contrast-enhanced (DCE) MRI is a powerful imaging technique for characterizing tissue vasculature. In DCE-MRI experiments, time-resolved images are collected as contrast agent washes in and out of the tissue, providing insight into blood flow, fractional blood volume, and vascular permeability (18, 53).

Mice are particularly useful for studying placental biology. Akin to the human placenta, the mouse placenta is hemochorial, with maternal blood perfusing labyrinthine trophoblasts in a manner analogous to the branching villous tree that is bathed by the maternal human intervillous space (2, 10). In addition, the mouse is particularly amenable to targeted mutations, designed to analyze mechanisms underlying placental development and function (41). Not surprisingly, we previously found that several genes that are critical for murine placental development also regulate the differentiation of human trophoblasts (20, 39, 46, 47).

Hypoxia hinders the process of cytotrophoblast differentiation, induces apoptosis, and diminishes hormone production in the human placenta (9, 21, 25, 34). Exposure to hypoxia during murine pregnancy results in FGR (15, 27), although the precise mechanism underlying placental villous injury during hypoxia is unclear. Specifically, it is not known whether systemic hypoxia elicits preplacental hyperperfusion or causes placental parenchymal perfusion abnormalities. To elucidate mechanisms underlying placental hypoxic injury associated with FGR, we hypothesized that dynamic MR imaging could help to define hypoxia-induced placental dysfunction.

MATERIALS AND METHODS

Animals. Care for the mice and all procedures were conducted in the Association for Assessment and Accreditation of Laboratory Animal Care-approved facility of Washington University School of Medicine in accordance with the standards set forth in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All experimental procedures were approved by the Animal Studies Committee of Washington University.
Experiments were performed using pregnant C57BL/6 mice. Timed matings were carried out by pairing strain-matched males and nulligravida females for one night, with the morning after mating designated as embryonic day 0.5 (E0.5). Pregnancy was assumed based on a 10% weight gain on E10.5. Mice were kept under constant conditions until day E15.5. They were given a standard rodent chow and water ad libitum, and kept on a 12:12-h light-dark cycle in room air. Deliveries typically occur in these mice on E19.5. All procedures were initiated on E15.5, when the mice were allocated to one of three groups: 1) exposure to \( F_{O2} = 12\% \) between E15.5 and E18.5 (hypoxic or Hpx group), 2) exposure to normoxia with food restriction similar to the intake of hypoxic mice between E15.5 and E18.5 (N- FR group), and 3) exposure to normoxia with ad libitum feeding (N-AL group).

Body weight and food intake were recorded daily. Food matching between the N-FR and Hpx groups was based on daily assessment of food intake by mice in the Hpx group. For normobaric hypoxia exposure we used a Polymer Hypoxia Glove Box with a Purge Airlock system with \( CO_2 \) and \( O_2 \) control indicators (Coy Laboratory Products, Grass Lake, MI), which is specifically designed for experiments in live rodents and regulates ambient temperature, humidity, and gas composition. Oxygen concentration was maintained within a narrow preset range by introducing small amounts of nitrogen as required. The main chamber has a connected side chamber, equipped with a separate door to allow uninterrupted maintenance of a predefined \( F_{O2} \), when the mice and their food were weighed daily. Dams were euthanized by \( CO_2 \) asphyxiation following MR imaging on E18.5 (see below). Embryos and placentas were collected immediately after death, weighed, and processed for further analysis (see below).

**Imaging procedure.** All MR images were collected and analyzed in Washington University’s Biomedical MR Laboratory. Images were acquired with a small-animal MR scanner based on an Oxford Instruments (Oxford, UK) 4.7 tesla, 40-cm bore magnet. The magnet is equipped with Magnex Scientific (Oxford, UK) actively shielded, high-performance (10 cm ID, 60 gauss/cm, 100 \( \mu \)s rise time) gradient coils and Techron gradient power supplies and is interfaced with a Varian NMR Systems (Palo Alto, CA) INOVA console. Data were collected using a Stark Contrast (Erlangen, Germany) 3-cm-diameter birdcage radio frequency (RF) coil. Prior to the imaging experiments, mice were anesthetized with isoflurane and were maintained on an air/oxygen mixture (1:1–1.5% vol/vol) throughout data collection. Animal core body temperature was supported at 37 ± 1°C by circulation of warm air through the bore of the magnet. Dams in the Hpx group were removed from the hypoxia chamber 1 h before imaging on the morning of E18.5. Hence, none of the dams were hypoxic during scanning.

Sagittal, axial, and coronal scout images were collected to ensure correct positioning of the animal within the RF coil, allowing visualization of all fetoplacental units. Images were acquired with a field of view of 30 × 30 mm² (transaxial) or 40 × 40 mm² (sagittal, coronal) and a data matrix of 128 × 128, leading to an in-plane resolution of 234 \( \mu \)m (transaxial) or 313 \( \mu \)m (sagittal, coronal). Eighteen to twenty-two contiguous, 1-mm thick slices were acquired to cover all of the fetoplacental units.

DCE-MRI experiments were performed using a T₁-weighted two-dimensional multislice gradient echo sequence. Typical imaging parameters were: repetition time (TR) = 100 ms, echo time (TE) = 2 ms, flip angle (\( \alpha \)) = 30°, and eight signal averages; producing a temporal resolution of ~100 s. Two baseline images were acquired at the start of the time course and the contrast agent was administered prior to the collection of 28 additional images. Total imaging time for the DCE experiment was ~50 min.

**Contrast agents and contrast distribution controls.** A standard gadolinium chelate (gadoversetamide, OptiMark; Coviden, St. Louis, MO) injected on a weight-based protocol was used throughout. Injection was performed via a 28-gauge catheter that was placed into the internal jugular vein prior to the start of imaging. To characterize placentation transport to the fetus, a single bolus of 2.7 \( \mu \)g of undiluted contrast, corresponding to a gadolinium concentration of 2 mmol/kg, was administered over 10 s using a Harvard 2 dual syringe pump (Harvard Clinical Technology, South Natick, MA). To define the systemic distribution of contrast under different physiological conditions, DCE-MRI (1:10 dilution of contrast agent, 0.2 mmol/kg; 33 s temporal resolution) was performed on four groups of dams: normoxic pregnant, hypoxic pregnant, normoxic nonpregnant, and hypoxic nonpregnant. Gadolinium distribution was measured in the paraspinal muscles in these paradigms, and these data served as a reference for the placental flow results, as described below.

**DCE data analysis.** Regions of interest (ROIs) were drawn over the placentas, paraspinal muscle, and fetal kidneys by a single operator (T. M. Tomlinson) using ImageJ (http://rsbweb.nih.gov/ij). Although not blinded to the experimental group of the dam being analyzed, this operator followed standard procedures for unbiased ROI selection. These procedures were determined prior to data analysis. Placentas analyzed were those that could be easily visualized in sagittal images of the dam and around which ROIs could be clearly drawn. The coronal slice chosen for each placenta’s time course analysis was the one having the largest (midplacental) cross-section in the sagittal image. Similarly, for fetal kidney analysis, the kidney ROI was drawn on the coronal slice that provided the largest image of the kidney.

All subsequent image analysis calculations were performed in Matlab (Mathworks, Natick, MA) by using programs developed in our laboratory. Assuming the fast-exchange limit, signal intensity data for placenta and muscle were converted to concentrations by standard methods (18) using Eq. 1 and Eq. 2.

\[
\frac{1}{T_1(t)} = -\frac{1}{TR} \ln \left[ \frac{S(t)}{S(0)} \left( e^{\frac{TR}{T_1}} - 1 \right) e^{-\frac{TR}{T_1}} + \cos(\alpha) \right] 
\]

\[
\frac{1}{T_1(t)} = \frac{1}{T_0(t)} + r_1[CA(t)] 
\]

Here, \( S(t) \) is the signal at time \( t \), \( S(0) \) is the signal in the absence of contrast agent (CA), \( T_1(t) \) is the longitudinal relaxation rate constant at time \( t \), \( T_0(0) \) is the longitudinal relaxation rate constant in the absence of CA, TR and \( \alpha \) are the repetition time and flip angle of the pulse sequence, respectively, \( [CA] \) is the concentration of the contrast agent, and \( r_1 \) is the relaxivity of the CA [4.4 mmol/s (40)]. Precontrast \( T_0(0) \) values for both murine paraspinal muscles and term placentas were determined from a series of \( T_1 \)-weighted spin-echo images.

In the fetal kidneys, in which \( T_1(0) \) could not be measured, relative signal increase, itself proportional to contrast agent concentration, was calculated (18).

\[
\frac{S(t) - S(0)}{S(t)} = [CA(t)] 
\]

Intradam variations in image intensity (placenta and fetal kidney) were partly attributed to variations in RF coil sensitivity and to fetal position within the coil. To account for these variations, a straightforward normalization procedure was adopted. This normalization assumed the same vascular input function for all placentas/fetuses within a given dam, a reasonable assumption given that each dam received a single bolus injection of contrast agent. For each placenta, an individual concentration vs. time curve was calculated, and an average concentration curve for all placentas within a specific dam was then computed. Next, each individual placenta’s concentration vs. time curve was compared analytically, on a point-by-point basis, with the placental average concentration curve for its corresponding dam. A single scale factor was derived for each placenta by performing a linear, least-squares minimization of the variation between these curves. Finally, for each placenta, all data values were multiplied by

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its computed scale factor. This normalization procedure has the effect of scaling the overall intensity without altering the shape of the individual placental concentration curves. An analogous procedure was followed for normalizing the relative signal intensity curves for each fetal kidney within a given dam.

Complete characterization of the complex vasculature of the placenta, which contains multiple permeable barriers and compartments, is beyond the scope of small-animal DCE-MRI. While a full kinetic analysis is not possible, the time course DCE data can still be quantified using a model-free parameter approach (18). In all cases, analysis of DCE data requires accurate knowledge of the concentration of the CA in the blood throughout the time course experiment, a factor known as the arterial input function (AIF). Traditionally, the AIF is measured from a large vessel within the image, taking care to avoid inflow and partial volume artifacts (18, 37, 38). However, determining the AIF directly is difficult in mice, due to small spatial dimensions and the effects of motion. Instead, in analogy to our model-free analysis of the DCE data, we can derive a model-free vascular input parameter from a reference tissue, such as muscle (23, 55). As outlined below, we then computed the ratio of CA concentration in the placenta to that in the reference tissue, as previously described (13).

Quantification of dynamic data was achieved using three model-free parameters: initial area under the curve (IAUC), final slope, and area under the curve (AUC) (18). The IAUC was evaluated for the first 1,200 s postinjection (IAUC1200) by calculating the areas under each placental concentration curve and the corresponding curve for paraspinal muscle from time 0 to 1,200 s postinjection. Placental values were then referenced to the muscle signal by forming the ratio IAUC1200, placenta/IAUC1200, muscle (13). The final slope was calculated by measuring the slope of each placental concentration curve from 1,300 s to 2,800 s postinjection. The complexity of transport contrast injection to the fetal kidneys, with numerous points of (bidirectional) transfer, prevents the calculation of CA concentration in these organs. Instead, AUC was calculated for each fetal kidney by calculating the area under its relative signal curve. As with IAUC in placenta, these values were normalized by forming the ratio AUCplacenta/AUCmuscle, where for each fetal kidney, AUCplacenta is the area under the relative signal vs. time curve for its corresponding placenta.

Placental histology. Each placenta was divided in half by making a midline incision immediately adjacent to the cord insertion site, from the chorionic to the basal plate. One half was randomly selected for routine histology processing, following fixation overnight in fresh 4% paraformaldehyde. Tissues were dehydrated with alcohol, embedded in paraffin blocks, and cut into 5-μm sections. The sections were stained using standard hematoxylin and eosin staining and qualitatively evaluated for changes in the labyrinthine, spongiotrophoblast, and giant cell layers as routinely performed in our lab (47). To enhance assessment of the spongiotrophoblast layer, parallel slides from a portion of the placentas were stained with periodic acid Schiff staining (4, 47).

Assessment of gene expression using quantitative RT-PCR. The second half of each harvested placenta was snap frozen in liquid nitrogen and stored at −80°C for RNA isolation. RNA was extracted as previously described (47). Complementary DNA was prepared from 1 μg RNA in 50 μl reaction volume using the TaqMan Gold RT-PCR kit with the supplied random hexamer primers (Applied Biosystems, Foster City, CA). Quantitative PCR was performed in duplicate using 3 μl samples of cDNA and 12.5 μl SYBR Green PCR Master Mix (Applied Biosystems) in a total reaction volume of 25 μl that contained 300 nM of forward and reverse primers. Reactions were run and analyzed using Applied Biosystems Geneamp 7300 Sequence Detection Systems. Dissociation curves were performed on all reactions to ensure amplification of a single product with the appropriate melting temperature. The transcript levels were normalized to the housekeeping gene L32 RNA levels, and the data expressed as fold change relative to a single N-AL control placenta. The fold increase relative to control cultures was determined by the ΔΔCt method (26). All PCR primer pairs were checked for specificity using BLAST analysis. The list of primers is provided in Table 1.

Statistics. We calculated our sample size based on attainment of 20% reduction in embryo weight in the Hpx group compared with the N-AL group. To achieve this difference at 80% power and α = 0.05 required at least 12 dams in each paradigm. Data are expressed as means ± SD, and statistical significance was determined using the one-way ANOVA and Bonferroni post hoc test. Significance was determined at P < 0.05. For the data in Fig. 4, which are further analyzed in Table 2, we plotted binned data and confirmed symmetrical distributions about the means.

RESULTS

Maternal food consumption and weight. We first sought to examine the effect of normobaric hypoxic environment on maternal food intake and weight gain. We exposed pregnant mice to FIO2 = 12% or to standard atmosphere over a period of 3 days at the end of pregnancy (E15.5–E18.5) with ad libitum supply of chow. We found that dams in the Hpx group (n = 22) exhibited lower food intake on the first day in hypoxia (Fig. 1A). Although food intake by hypoxic dams was increased in the subsequent 2 days in hypoxia, it remained lower than that of the N-AL dams (n = 25). Therefore, to control for food intake, we generated a third group, N-FR dams (n = 15), in which food was restricted to match exactly that of hypoxic dams (Fig. 1A). As expected, maternal weight gain in the N-AL group over the 3 days of the experiment was markedly higher than that of N-FR or Hpx groups (Fig. 1B).

Fetal and placental weights. As shown in Fig. 2A, murine pups (n = 126) harvested on E18.5 from Hpx dams were significantly smaller than pups from the N-FR dams (n = 99), which, in turn, were significantly smaller than those from N-AL dams (n = 113). We also observed a small, but statistically significant, difference in mean placenta weights from each of the three cohorts, with the largest placentas originating from the N-AL dams and the smallest from the N-FR dams (Fig. 2B). Not surprisingly, the placental-to-fetal ratio was greatest in the Hpx group (Fig. 2C). We found no significant differences in mean litter size (N-AL = 7.5, N-FR = 8.3, Hpx = 8.4). Fetal survival was > 95% in all three groups.

Fetoplacental MR and DCE imaging. Representative contrast-enhanced MR images of mouse fetuses and placentas on E18.5 are shown in Fig. 3. Maximal placental enhancement by the contrast agent was observed at 7–10 min following contrast injection, with fetal enhancement lagging behind that shown in the placenta.

### Table 1. Primer sequences used for standard PCR expression analysis

<table>
<thead>
<tr>
<th>Name</th>
<th>Accession No.</th>
<th>Direction (Site)</th>
<th>Sequence, 5′-3′</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gcm1</td>
<td>NM_008103</td>
<td>F(35-54)</td>
<td>AGAGATATCTGAGGTCGCGAGATT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R(140-129)</td>
<td>CTGTCTCGGAGTTGAGATG</td>
</tr>
<tr>
<td>Phlda2</td>
<td>NM_009434</td>
<td>F(209-231)</td>
<td>GAGGCTTGTGGTTGCGACTCTGGCTTT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R(267-289)</td>
<td>GTTGGCTGAGGATGCAGTGACCA</td>
</tr>
<tr>
<td>Tpbpa</td>
<td>NM_009411</td>
<td>F(194-169)</td>
<td>AACCTCTTTTATCCTTCGCTCTGCA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R(394-415)</td>
<td>AGACTGATGCTTCGGTATAT</td>
</tr>
<tr>
<td>Mash2</td>
<td>U77628</td>
<td>F(484-460)</td>
<td>GCTGATGCGAGGCTGGAGGTAAATC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R(427-267)</td>
<td>AGCCGCGATGAAGATGATAGATC</td>
</tr>
<tr>
<td>Plf1</td>
<td>NM_031191</td>
<td>F(388-376)</td>
<td>CACTCTACATGAGTCGCAGAGG</td>
</tr>
</tbody>
</table>

F: forward; R: reverse.
of the placenta. Control experiments revealed that pregnancy did not noticeably impact the systemic distribution of contrast agent. Muscle-derived measures of vascular gadolinium concentration were used for referencing all of the placental data (see MATERIALS AND METHODS).

DCE analysis of transplacental gadolinium distribution in each of the three experimental groups was limited to placentas that were reliably identified in coronal sections. T1(0) values for placenta (1.8 s) and paraspinal muscle (1.4 s) were computed from representative T1 maps of the placenta and paraspinal muscle (reference tissue). CA concentrations, as a function of time, were calculated as described in MATERIALS AND METHODS. Fig. 4 depicts representative concentration curves for one dam from each of the three experimental groups. IAUC was calculated for all placentas on a per dam basis. This calculation revealed a statistically significant difference in IAUC for the N-AL and N-FR placentas compared with the Hpx placentas.

We anticipated that both maternal and fetal blood flow might contribute to contrast clearance from the placenta. Noting enhancement of the fetal kidneys early in the experimental time course, we sought to assess contrast accumulation in the fetal kidneys as a surrogate for fetal clearance of placental contrast. The adjusted fetal kidney AUC was lower in the Hpx group than in the N-AL group (Fig. 6), suggesting diminished transfer of contrast agent to the Hpx fetuses.

Placental histology and gene expression. To determine whether the perfusion changes assessed using DCE imaging were associated with discrete placental morphological changes, we examined the placentas from the three experimental groups using hematoxylin and eosin and periodic acid Schiff staining. While the placentas from the Hpx and N-FR groups were smaller than those from N-AL, we could not detect any differences in thicknesses of the labyrinth, spongiotrophoblast, or giant cell layers. Similarly, we did not

Table 2. Quantitative analysis of signal intensity in dynamic contrast-enhanced placentas

<table>
<thead>
<tr>
<th></th>
<th>N-AL</th>
<th>N-FR</th>
<th>Hpx</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAUC</td>
<td>1.87±0.35</td>
<td>2.28±0.40</td>
<td>1.93±0.59</td>
</tr>
<tr>
<td>Final slope</td>
<td>-2.59×10^-3±0.86×10^-5</td>
<td>-2.93×10^-3±1.12×10^-5</td>
<td>-1.34×10^-3±1.67×10^-5</td>
</tr>
</tbody>
</table>

Values are ± SD; n = 56/group. N-AL, normoxic ad libitum; N-FR, normoxic food restricted; Hpx, hypoxic; IAUC, initial area under the curve. *P < 0.05 vs. the other 2 experimental conditions.

Fig. 1. Influence of hypoxia on dams’ food intake and weight change. A: daily food intake in grams for each experimental group during the 3 experimental days (E15.5–E18.5, n = 113 for N-AL, n = 99 for N-FR, and n = 126 for Hpx). B: dams’ weight change during the 3 experimental days (E15.5–E18.5, n = 8 for N-AL, n = 11 for N-FR, and n = 8 for Hpx). N-AL, normoxic ad libitum group; N-FR, normoxic food-restricted group; Hpx, hypoxic group. Values are means ± SD; *P < 0.05 compared with the other paradigms (ANOVA with Bonferroni correction for multiple comparisons).

Fig. 2. Influence of hypoxia on fetal and placental weight. Measurements were performed during E15.5–E18.5 (n = 113 for N-AL, n = 99 for N-FR, and n = 126 for Hpx). Values are means ± SD; *P < 0.05 compared with the other paradigms (ANOVA with Bonferroni correction for multiple comparisons).
identify any differences in labyrinth density, dilatation of the labyrinthine blood spaces, or any other discrete structural alterations (data not shown). To expand our assessment of region-specific changes, we measured the expression of several placental genes that are expressed in specific layers of the murine placenta. As shown in Fig. 7, the expression of labyrinthine Gcm1 and Phlda2 was increased in placentas from Hpx dams relative to those derived from the N-FR controls. No significant differences were found between these two groups in the expression level of spongiotrophoblastic Tpbpa and Mash2, or in the level of Plf (primarily giant cells), although some of the genes exhibited decreased expression in one or both injured groups relative to the N-AL controls.

**DISCUSSION**

We used DCE-MRI with gadolinium contrast to assess placental perfusion in a mouse model of hypoxia at the end of pregnancy. We found that the initial uptake of contrast was similar for the Hpx and N-AL mice. The uptake was delayed in the Hpx mice compared with the N-FR mice, possibly reflecting reduced perfusion in hypoxia-exposed mice. Importantly, the clearance of contrast was reduced in the Hpx placentas, which manifested as a lower final slope of the gadolinium concentration vs. time curve in the Hpx group relative to the two normoxic controls. We emphasize that these results were normalized to the arterial input function, derived by assessing

![Image of MRI images](image_url)

**Fig. 3.** Representative 4.7 tesla in vivo MRI images of control (N-AL) mice on E18.5. Respiratory-gated spin-echo images were collected following intraperitoneal administration of the contrast agent. Transaxial image of murine placentas (A; arrows) and coronal image of murine fetuses (B; arrows) are shown. Enhancement of the chorionic plate preceded enhancement of the remainder of the placental parenchyma and occurred within 30 s of contrast agent injection in all 3 experimental groups.

![Graph of contrast kinetic curves](graph_url)

**Fig. 4.** Effect of hypoxia on placental and paraspino muscle contrast kinetic curves. Each dynamic contrast-enhanced (DCE) time course curve represents 1 dam and a set of placentas from that dam for 1 of the 3 experimental groups, showing the concentration of gadolinium contrast in these tissues during the course of imaging. Solid color lines represent different placentas within each dam. For each dam, an average concentration vs. time curve was computed for all placentas in that dam. Individual placental concentration vs. time curves were normalized based upon this average concentration curve, as detailed in the text. Note that negative concentration values immediately after contrast administration stem from a T2* effect (signal loss due to magnetic susceptibility), associated with the bolus of gadolinium.
perfusion of a reference tissue, thereby correcting to general alterations in perfusion.

The initial appearance of contrast in the chorionic plate is consistent with rapid labyrinth perfusion by maternal blood, delivered directly from the uterine spiral arteries via central arterial canals into labyrinthine sinusoids (2). Our data suggest that the clearance of contrast from the adjacent labyrinth was slowed in Hpx placentas. Whereas our findings are consistent with the notion that hypoxia influences preplacental vasoconstriction (17), our data illuminate hypoxia-induced changes within the labyrinth, the site of maternal-fetal exchange in the murine placenta (7, 32). Finally, we found that the accumulation of contrast in the kidneys of fetuses of Hpx dams was reduced. While we cannot rule out a direct influence of hypoxia on fetal renal function, our findings are consistent with diminished fetal perfusion.

We induced FGR using global maternal hypoxia for 3 days, as described previously (11, 48, 54). Importantly, the level of FGR, placental weight, and mean placenta-to-fetus ratio achieved in our studies was similar to that achieved by others (11, 22, 24). Furthermore, we did not observe an increase in fetal mortality, which might have introduced an additional variable. Because there is a plateau in murine placental growth after E14 (50), it is not surprising that we observed a minimal effect of E15.5 injury on placental size. We note that DCE-MRI measurements were performed in standard atmospheric oxygen. Rather than hemodynamic changes that might be found during hypoxia, our results reflect tissue changes that were caused by hypoxia, but detected after the insult. We also did not rule out the possibility that some of the observed changes could have reflected reoxygenation injury, which might have taken place immediately prior to imaging. Nevertheless, we find it less likely that the FGR, CA dynamics changes, and altered gene expression could have been explained by changes that occurred over a brief reoxygenation period (2 h or less at the end of the experiment).
In vivo dynamic imaging of fetoplacental perfusion in mice poses a significant technological challenge (44, 51). First, high-quality DCE-MRI data must be acquired for several fetoplacental units within each pregnant dam. Second, these data must be referenced to an appropriate measure of vascular input, derived in our case from paraspinal muscle reference tissue. Third, a detailed evaluation of the fetus via imaging of the fetal kidney requires a higher dose of contrast agent than is commonly administered when imaging the dam. Despite these challenges, we were able to perform a quantitative analysis of temporal changes in contrast-medium concentration in both the placenta and fetal kidney. The accuracy of our measurements was limited by the subjective choice of ROIs, defined for analysis of the DCE images. In addition, partial volume effects could have contributed to errors in these measurements. Because of their small size and fetal movements, measurements of fetal kidney were particularly prone to this limitation. Finally, heterogeneity among mice within a given experimental group and among the fetoplacental units within each dam accounts, in part, for the variability in measurements that was independent of the experimental paradigm.

Our analysis of placental histology was restricted to morphological changes that might be identified using standard staining techniques. The lack of clear evidence for tissue damage likely reflects the short duration of injury that was inflicted at a relatively late point in gestation, after the period of rapid placental growth. Using quantitative PCR to probe for tissue response to hypoxia, we found that the expression of Phlda2, a maternally imprinted gene that is expressed mainly in the labyrinth and negatively regulates placental growth (5, 43), was increased in placentas from the Hpx group compared with N-FR controls. In Hpx placentas we also detected a small yet statistically significant increase in the expression of Gcm1, a gene associated with labyrinthine layer branching and differentiation (3, 6, 33). In contrast, we did not detect any significant change in the expression of Tppha, Mash2, or Pif1 between the Hpx and N-FR groups (12, 19). Although the role of these gene products in placental response is presently uncertain, our results point to concomitant changes in expression of labyrinthine genes that are expressed in regions of placental exchange function. These changes might be a part of a placental adaptive response to hypoxic injury. Notably, our analysis was restricted to histological changes, and therefore, we cannot rule out mild changes in labyrinthine fetal or maternal blood spaces that might have been uncovered using more refined stereology or electron microscopy. Interestingly, using pregnant mice prior to full placental development (E7.5–E10.5), Schaffer et al. (45) showed that short-term normoboric hypoxia (6–24 h) influenced primarily the giant cells and spongiotrophoblasts, with no evidence for hypoxic changes in the labyrinth. These differences may reflect the fact that our mice were exposed to hypoxia for a longer interval during the embryonic maturation phase, not the developmental period.

Perspectives and Significance

Although placental insufficiency is one of the leading causes of perinatal morbidity and mortality worldwide, our limited understanding of its pathophysiology has hindered the development of new diagnostic tools for this condition or successful prophylactic or therapeutic interventions designed to mitigate fetoplacental injury. DCE-MRI is a novel imaging tool for in vivo assessment of placental function. The combination of DCE-MRI and gene-expression analysis in placentas exposed to hypoxic injury may illuminate unrecognized pathways in placental adaptation to hypoxic injury. In addition to its research use to interrogate patterns of blood flow in the injured placenta, DCE-MRI may have clinical utility for the detection of placental injury in women at risk for FGR.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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