Fetal Growth Restriction Induced by Transient Uterine Ischemia-Reperfusion: Differential Responses in Different Mouse Strains

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Abstract

We characterized fetal and placental growth and uterine and placental inflammation in pregnant C3H/HeOuj and C57BL/6J mice (strains with different sensitivities to metabolic and circulatory pathologies), using different uterine ischemia/reperfusion (I/R) protocols, to establish and refine a murine model of I/R-induced fetal growth restriction (FGR). Pregnant C3H/HeOuj mice on gestation day 15 were subjected to unilateral uterine I/R by (1) total blood flow restriction (TFR) by occlusion of the right ovarian and uterine arteries for 30 minutes, (2) partial flow restriction (PFR) by occlusion of only the right ovarian artery for 30 minutes, or (3) sham surgery. Pregnant C57BL/6J mice were treated the same, but on gestation day 14 and with TFR for only 5 minutes due to high sensitivity of C57BL/6J mice to I/R. Four days post-I/R, the animals were euthanized to determine fetal and placental weight and fetal loss and to assay placental myeloperoxidase (MPO) activity. In C3H/HeOuj mice, TFR/30 minutes induced significantly ($P < .05$) lower fetal and placental weights and higher placental MPO activity, compared to controls. The PFR/30 minutes produced the same effects except placental weights were not reduced. In contrast, in C57BL/6J mice, TFR for only 5 minutes was sufficient to induce FGR and increase fetal loss; while PFR/30 minutes lowered fetal but not placental weights and increased fetal loss but not placental MPO activity. In summary, we present the first published model of I/R-induced FGR in mice. We find that mice of different strains have differing sensitivities to uterine I/R, therefore differing I/R response mechanisms.

Keywords

fetal growth restriction, ischemia/reperfusion, mice, placenta, myeloperoxidase

Introduction

Fetal growth restriction (FGR) is an important cause of perinatal morbidity and mortality and of postnatal morbidity and life-long adverse outcomes.¹⁻³ Up to 40% of stillborn fetuses that were not malformed have been observed to be small for gestational age. Growth-restricted neonates exhibit increased risk of respiratory distress syndrome, intraventricular hemorrhage, and necrotizing enterocolitis.⁴⁻⁶ Growth-restricted newborns are nearly 3 times more likely to die in the neonatal period than normal weight newborns.⁷⁻⁹ Later in life, individuals who were growth restricted as fetuses have an increased propensity to develop metabolic syndrome and/or mental health abnormalities as well as diabetes, cardiovascular disease, infertility, and cancer.¹⁰⁻¹³ Despite intense investigation, the pathogenesis of FGR is not yet fully understood.

The importance of I/R phenomena in FGR is revealed by the study of uterine vascular remodeling during placentation. Ordinarily the cytotrophoblasts migrate into the maternal uterine

arterial vasculature, replacing smooth muscle, and allowing the vessels to become dilated and unresponsive to vasoactive hormones, to maintain optimum blood flow. Inadequate cytotrophoblast invasion and remodeling of the spiral arteries are believed to form the basis for abnormal placental development leading to preeclampsia, FGR, and early fetal loss.^{14,15} The

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musculature of the affected resistance arteries remains anatomically intact, with an intact nerve supply, retaining responsiveness to vasoconstrictors.^{16,17} The incomplete remodeling and resultant vasoconstriction produces decreased uteroplacental perfusion which contributes to an inability to meet the increasing placental and fetal demand for blood supply, leading to placental ischemia and areas of infarction. Additionally, while the normally developing placenta produces vascular endothelial growth factor and placental growth factor, which promote vascularization and vasorelaxation, the hypoxic/ischemic placenta produces antiangiogenic molecules such as the short form of fms-like tyrosine kinase one (sFlt-1) and the short form of endoglin, which further hinders vascular remodeling and blood supply to the placenta.¹⁸⁻²⁰ Thus, placental insufficiency and resultant ischemia play a central role in the development of FGR.

Inadequate uteroplacental perfusion is commonly observed in human FGR.²¹ One direct consequence of inadequate perfusion is ischemia/reperfusion (I/R) in the tissue involved. Histological examination frequently reveals ischemic lesions in the placenta from FGR-complicated pregnancies, and a correlation between the number and severity of ischemic lesions and the severity of FGR.²² The characteristics of these lesions are better represented by an I/R process than by hypoxia alone. The prevailing hypothesis is that the uterine vasculature that feeds the placenta retains sufficient responsiveness to vasoactive stimuli, in the setting of preeclampsia and/or FGR, and that the blood flow is highly variable and produces periods of hypoxia followed by reperfusion. Wide fluctuations in oxygen tension are capable of producing an I/R type of injury, including severe tissue damage and infarction.²³

To investigate underlying pathogenic mechanisms, animal models of I/R-induced FGR have been developed. Fetal growth restriction can be induced by uterine artery ligation in the rat.²⁴ More recently, it was found that transient, unilateral uterine I/R challenge results in FGR in both uterine horns of treated rats.^{25,26} In addition, prolonged restriction of blood flow to the uterus can cause preeclampsia-like symptoms including hypertension and nephropathy²⁷ as well as FGR. Further studies using the rat model have implicated inflammatory mediators—for example, leukocyte adhesion molecules,²⁸ reactive oxygen species (ROS) and thromboxane (TX),²⁹ platelet-activating factor (PAF),²⁶ and vasoactive agents (eg, increased endothelin and decreased nitric oxide)^{30,31}—in the pathogenesis of FGR.

The potential for genetic manipulation in the rat is limited compared to mice. Mouse models based on different strains of mice or based on genetic modification, on the other hand, lend themselves well to the study of genetic and molecular mechanisms in this disease and other diseases. Thus, a murine I/R model of FGR would be advantageous for in-depth mechanistic investigation.

To address this issue, we conducted a series of studies to examine the effect of uterine I/R on pregnant inbred mice of different strains, specifically C3H and C57BL/6, the 2 “general purpose” strains used most widely in biomedical research, to

develop a reliable model of FGR in these animals. C57BL/6 mice are susceptible to diet-induced obesity, type 2 diabetes, and atherosclerosis, whereas C3H mice are resistant to these metabolic and circulatory pathologies.³²⁻³⁴ A partial basis for these differences is a naturally occurring deletion in C57BL/6J mice, in the nicotinamide nucleotide transhydrogenase (*Nnt*) gene, resulting in an absence of NNT protein and leading to impaired glucose homeostasis and reduced insulin secretion.³⁵ Our hypothesis was that these strain differences would significantly affect the induction and outcomes of I/R-induced FGR in mice.

Materials and Methods

Animal Experiments

Virgin male and female mice of C3H/HeOuJ and C57BL/6J strains were purchased from Jackson Laboratories (Bar Harbor, Maine) and housed at the NorthShore University HealthSystem Research Institute Center for Comparative Medicine in 12-hour light/dark cycles with free access to a standard laboratory rodent diet and water. Animal care and the conduct of experiments were approved by the Animal Care and Use Committee of the Research Institute. Timed pregnancy was produced through overnight mating of 11- to 20-week-old females in estrus.

C3H/HeOuJ mice. On the 15th day of gestation, the pregnant females were randomly divided into 3 subgroups (6 to 8 in each subgroup). Under ketamine/xylazine anesthesia, the right uterine horn was exposed through a midline abdominal incision and was subjected to one of the following procedures:

- (1) Total blood flow restriction (TFR) by clamping both the uterine and ovarian arteries with 2 arterial clips. The uterine horn was then returned to the abdominal cavity, with the occlusion clips in place until their removal after 30 minutes for the initiation of reperfusion. After removal of the clips, the abdominal incision was closed with sutures followed by surgical staples.
- (2) Partial blood flow restriction (PFR) by clamping only the ovarian artery with a single arterial clip. The uterine horn was then returned to the abdominal cavity, with the occlusion clip in place until its removal after 30 minutes for the initiation of reperfusion. After removal of the clip, the abdominal incision was closed with sutures and staples.
- (3) Sham surgery performed in the same manner as for I/R but without artery clamping. Closure of the abdominal incision was performed 30 minutes after the exposure of the uterine horn.

Four days after the I/R surgery (gestation day 19), the animals were euthanized and dissected. The weight of each live fetus and attached placenta, and the number of resorptions and stillbirths in each horn of the uterus were documented. Tissue

samples of uterus and placenta were collected, snap frozen in liquid nitrogen, and stored at -80°C for further analysis.

C57BL/6J mice. The procedure for anesthesia and surgery was the same as used for the C3H/HeOuJ animals but with the following changes, due to increased sensitivity of this strain to uterine I/R:

- (a) The surgery was performed on the 14th day of gestation instead of the 15th day.
- (b) The animals were divided into 3 subgroups (8 to 10 in each subgroup) to receive one of the following procedures:
 - (1) TFR as described for C3H animals, but the duration was reduced to 5 minutes, followed by closure of the abdominal incision 30 minutes later;
 - (2) PFR as described for C3H animals, that is, 30-minute occlusion of the ovarian artery.
 - (3) Sham surgery as described for the C3H animals.
- (c) The fetal growth data and tissue sample collection were performed 4 days post-I/R, that is, on the 18th day of gestation instead of the 19th day.

Myeloperoxidase Activity

Myeloperoxidase (MPO, a marker enzyme for activated neutrophils, shown to be linearly related to neutrophil number in the intestine³⁶) was assayed according to a method published previously.³⁷ In brief, tissues were homogenized in 0.05 M potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide (Sigma, St. Louis, MO) and EDTA (5 mM), and sonicated. An aliquot was mixed with substrate (*o*-dianisidine HCl [Sigma] + H_2O_2 in potassium phosphate buffer) and its optical density was read at 460 nm. A standard curve was constructed with serial dilutions of human MPO (Sigma).

Statistical Analyses

Results are presented as mean $\pm$ standard error of the mean (SEM). Analysis of variance (ANOVA), with Newman-Keuls posttest, was employed for the comparison of multiple groups. Linear regression was used to test correlation between variables. All statistical analyses were 2-tailed and results were considered statistically significant at $P < .05$.

Results

Effect of TFR on C3H/HeOuJ and C57BL/6J Mice

TFR produced FGR in both C3H/HeOuJ and C57BL/6J mice but with differing tolerance. In this study, we examined the effect of TFR in mice. To make the ischemic challenge comparable to the rat model, in C3H/HeOuJ mice the I/R procedure was applied on the 15th day of pregnancy, which corresponds to gestation day 17 in the rat (term = 22 days, compared with 19 to 21 days

for mice). The results in Figure 1A show that this procedure induced FGR in C3H/HeOuJ mice. The treatment was well tolerated by the animals. No significant difference was found in the rate of fetal loss (resorption/stillbirth) between the I/R-treated group and the sham-operated group (Figure 1C).

C57BL/6J mice, however, responded quite differently to uterine I/R by TFR. It was observed in preliminary experiments that mice of this strain are very sensitive to surgical intervention on gestation day 15. Assuming the impact of the flow-restricting procedure might be less hazardous at an earlier gestation day when smaller fetuses demand less blood supply, we shifted the I/R challenge to the 14th day of gestation and achieved a stable sham control. However, in preliminary experiments, the prolonged TFR (well tolerated by the C3H/HeOuJ animals) was too severe for the C57BL/6J animals. The 30-min flow deprivation caused total fetal loss in most treated mice, and in some cases, the death of the dams as well. We could achieve survival of the fetuses only for a much shorter duration of the arterial clamping. We adopted 5 minutes for our experimental protocol, and the effect is illustrated in Figure 1B. The mean weight of the surviving fetuses was significantly lower in comparison with the mean fetal weight in the sham-operated group. However, even at 5 minutes, TFR still caused high fetal loss (Figure 1D). Over 50% of the fetuses were lost as either resorptions or stillbirths—mean (SEM) = 66% (13.7%) in the I/R-challenged (ipsilateral) horn and 57% (12.8%) in the unchallenged (contralateral) horn, as compared to 18.8% (9.8%) and 15.8% (9.4%) in the respective ipsilateral and contralateral horns of sham-operated mice.

TFR caused reduction in placental weight in C3H/HeOuJ mice but not in C57BL/6J mice. The placental weights of C3H/HeOuJ mice exposed to TFR (30 minutes) were lower than those of sham-operated controls, although only I/R(R) and sham(R) reached statistical significance (Figure 1E). Both I/R(R) and I/R(L) did not differ significantly from each other. In contrast, no significant difference was found in placental weights between the C57BL/6J mice subjected to TFR (5 minutes) and their sham-operated controls (Figure 1F).

TFR increased MPO activity in both strains. Ischemia/reperfusion is known to cause an inflammatory response that may lead to pathological effects in the associated tissue.³⁸ In this study, we examined the activity of MPO, a marker enzyme of activated neutrophils, in placental tissue as an index of the intensity of the I/R-induced inflammatory response.

Total blood flow restriction induced placental MPO activity (suggesting neutrophil sequestration) differentially in the 2 strains, as demonstrated in Figure 2A and B. In the C3H/HeOuJ strain, the placental MPO activity was significantly higher in I/R-challenged mice compared to sham-operated mice (Figure 2A). But in the C57BL/6J animals, no difference was observed in placental MPO activity (Figure 2B).

Figure 1. Fetal and placental weights and fetal loss after total blood flow restriction (TFR). Comparison of fetal weights (A, B), fetal loss (C, D), and placental weights (E, F) in C3H/HeOuJ mice at gestation day 19 (A, C, E) and C57BL/6J mice at gestation day 18 (B, D, F) with (ischemia/reperfusion, I/R) and without (sham) unilateral uterine I/R by TFR. Responses of the right (R, ischemic) and left (L, nonischemic) uterine horns are displayed separately. Results are displayed as mean $\pm$ standard error of the mean (SEM). * $P < .05$ compared with respective sham-operated horn, by analysis of variance (ANOVA).

Effect of PFR on C3H/HeOuJ and C57BL/6J Mice

PFR also produces FGR in both C3H/HeOuJ and C57BL/6J mice. Since TFR produces high fetal loss in C57BL/6J animals, it seemed possible that this effect might be alleviated by shortening the duration of the blood flow deprivation. Shortened exposure to the ischemic challenge did in fact improve fetal survival, and it also proved adequate to induce FGR. This model was even further stabilized by employing PFR, in which only 1 of the 2 major arteries supplying the uterine tissue, the ovarian artery, is occluded. Thus, consistent FGR with lower fetal loss was achieved.

The result shows that PFR was sufficient to induce FGR in both C3H/HeOuJ (Figure 3A) and C57BL/6J mice (Figure 3B). Partial blood flow restriction still caused significantly increased fetal loss in C57BL/6J animals (Figure 3D), but the effect was significantly milder (reduced by 23% in the ipsilateral horn and by 42% in the contralateral horn) compared to the effect of TFR. In C3H/HeOuJ mice, however, similar to the results following TFR, the incidence of fetal loss following PFR was not significantly different between sham-operated and I/R-treated animals (Figure 3C).

Figure 2. Myeloperoxidase (MPO) activity after total blood flow restriction (TFR). Comparison of placental MPO activity in C3H/HeOuJ (A) and C57BL/6J (B) mice with (ischemia/reperfusion, I/R) and without (sham) unilateral uterine I/R by TFR. Responses of the right (R, ischemic) and left (L, nonischemic) uterine horns are displayed separately. Results are displayed as mean $\pm$ standard error of the mean (SEM). * $P < .05$ compared with respective sham-operated horn, by analysis of variance (ANOVA).

PFR caused no significant reduction in placental weight in either C3H/HeOuJ mice or C57BL/6J mice. In contrast to the results with TFR, PFR caused no significant reduction in placental weights in C3H/HeOuJ mice (Figure 3E). There was also no significant difference in placental weights in C57BL/6J mice following PFR compared to sham treatment (Figure 3F), as was observed with TFR.

PFR increased MPO activity in both C3H/HeOuJ and C57BL/6J mice. Partial flow restriction, as with TFR, also caused increased placental MPO activity in C3H/HeOuJ mice (Figure 4A), but not in C57BL/6 mice (Figure 4B).

Correlation of MPO Activity and Fetal Loss

Comparisons of the placental MPO activity with fetal loss were achieved by linear regression analysis (Figure 5). Placental MPO activity and fetal loss were significantly correlated only in C57BL/6J mice and only for the TFR group ($P < .01$). There was no statistically significant correlation between MPO activity and fetal weight in either of the 2 mouse strains.

Discussion

Uterine I/R induces FGR and fetal loss in several species of animals.^{25,26,39,40} This study refines the mouse as a model of I/R-induced FGR. This development is significant because it further opens the door to FGR studies in a species that is readily amenable to genetic manipulation for the study of molecular pathways. The following factors are of particular importance to the successful study of I/R-induced FGR in the mouse.

Parturition Date of the Animals and the Window of Challenge

Fetal growth restriction models utilizing transient uterine I/R are produced by imposing the ischemic challenge in the early

portion of logarithmic growth, a period when fetal development is especially sensitive to blood flow restriction, due to high demand to sustain accelerated growth. The developers of the rat models put the window of challenge at approximately three-quarters of the gestation (the 17th day of a 22-day gestation).²⁵ We applied this principle to mice and made the following adjustments.

- Mice are more sensitive than rats to I/R challenge late in the third trimester. We have found that at least some strains of mice (such as C57BL/6J in this study) have low tolerance for uterine I/R if it occurs later than the 14th day of gestation. Later administration of uterine I/R can cause total loss of the fetuses or even death of the mother. This is not because the intensity of the I/R challenge is too high; even sham surgery can lead to fetal loss or FGR in these animals if administrated on the 15th day of gestation.
- Mice are less homogeneous in their date of parturition across strains. Unlike rats, which have parturition on the 22nd day of gestation, the parturition date of mice varies from 19 to 21 days of gestation from strain to strain. Taking this fact into consideration, it is not practical to restrict the challenge to a fixed gestation date in all mice when inducing FGR with uterine I/R. It is more appropriate to fit it into a "gestation stage," for example, 68% to 72% of the full term, to get comparable results in different strains of mice. For the strains that tolerate uterine I/R poorly (in this study, C57BL/6J), shifting the window of challenge earlier in gestation leads to less pregnancy loss. This shift may be especially important in strains that demonstrate earlier parturition compared to other strains. For the strains used in our experiments, the difference in gestation length is minimal (half a day), with C57BL/6 mice averaging 19.3 days and C3H mice averaging 19.8 days.⁴¹

Figure 3. Fetal and placental weights and fetal loss after partial blood flow restriction (PFR). Comparison of fetal weights (A, B), fetal loss (C, D), and placental weights (E, F) in C3H/HeOuj mice at gestation day 19 (A, C, E) and C57BL/6J mice at gestation day 18 (B, D, F) with (ischemia/reperfusion, I/R) and without (sham) unilateral uterine I/R by PFR. Responses of the right (R, ischemic) and left (L, nonischemic) uterine horns are displayed separately. Results are displayed as mean $\pm$ standard error of the mean (SEM). * $P < .05$ compared with respective sham-operated horn, by analysis of variance (ANOVA).

Sensitivity of the Animals to I/R and the Intensity of Challenge

We have demonstrated that mice of different strains have different sensitivity to uterine I/R. The C57BL/6J mouse has been shown to develop a more intense inflammatory response when exposed to proinflammatory stimuli,⁴² in accord with our current observation that the I/R-induced inflammatory reaction is more severe in C57BL/6J (as measured by placental MPO activity) in comparison with their C3H/HeOuj counterparts. Our study has demonstrated that when I/R is used to induce

FGR, its “dose” should also be tailored to get the intended response. The adjustment of the “dose” for I/R challenge can be realized by altering either the duration of ischemia or the degree of blood flow restriction.

In addition to the above guidelines for development of FGR modalities by transient uterine I/R, our study has demonstrated the importance of particular I/R-related parameters in the pathogenesis of this disease. We have shown that I/R, even at a relatively mild level (as in the case of PFR), or for a short time (5 minutes), and with just part of the uterine tissue subjected to ischemia (only 1 of the 2 uterine horns), is adequate to generate

Figure 4. Myeloperoxidase (MPO) activity after partial blood flow restriction (PFR). Comparison of placental MPO activity in C3H/HeOuJ (A) and C57BL/6J (B) mice with (ischemia/reperfusion [I/R]) and without (sham) unilateral uterine I/R by PFR. Responses of the right (R, ischemic) and left (L, nonischemic) uterine horns are displayed separately. Results are displayed as mean $\pm$ standard error of the mean (SEM). ** $P < .01$, *** $P < .001$, compared with respective sham-operated horn, by analysis of variance (ANOVA).

Figure 5. Myeloperoxidase (MPO) activity/fetal loss correlation. (A) Regression analysis for C3H/HeOuJ placental MPO activity/fetal loss showed no significant correlation for any of the groups. (B) Regression analysis for C57BL/6J placental MPO activity/fetal loss shows a significant correlation only for the total blood flow restriction (TFR) group ($P < .01$). Regression lines in B: sham (---), partial blood flow restriction (PFR) (—), TFR (—).

FGR (and fetal loss) in the animals tested. The following comparisons highlight the importance of these parameters.

Ipsilateral (Blood Flow Restricted) Versus Contralateral (No Blood Flow Restriction)

As can be seen in all the figures, we found no significant difference between the results from the ipsilateral (challenged) horns and the contralateral (unchallenged) horns in all the indices examined. This result, which is consistent with rat models, carries the same implication as suggested by the rat studies, that is, unilateral uterine I/R-induced FGR is more due to systemic pathogenic mechanism(s) than due to local events. Evidence of a systemic effect is also shown by the fact that I/R-exposed C57BL/6 mice in a 30-minute TFR protocol exhibited bilateral FGR. This bilateral FGR finding is not surprising because the 2 horns are both exposed to the same circulating

mediators following the initiating, transient, pathogenic challenge. It should be noted that unilateral challenge has the benefit of comparing the uterine horns to determine whether there are differences in some parameters being measured.

TFR versus PFR

This study has demonstrated that FGR can be induced in mice through both I/R protocols tested, TFR and PFR. Total blood flow restriction is by nature more severe and, as anticipated, causes a more profound outcome, especially in a strain with higher sensitivity to inflammatory stimuli. This might be advantageous when a more intense effect is preferred for some research purposes (eg, the study of placental injury in C3H/HeOuJ mice, which is observed with statistical significance only in the TFR group). However, if survival of the fetuses is a major concern, such as in the study of fetal and placental

changes near term, or if early molecular events are being investigated, PFR might be preferred. Questions might be raised about the homogeneity of the pathogenic mechanism(s) of the 2 modalities, and this question needs to be explored carefully in subsequent studies. Most of the effects observed in the I/R-challenged groups appeared in both TFR and PFR groups with differences only in the intensity of their manifestation. The only exception is the reduction in placental weight observed in the TFR-treated mice but not in the PFR-treated C3H/HeOuJ mice. This may reflect the limited ability of the placenta to accommodate the higher level of insult caused by TFR compared with the relatively lower challenge caused by PFR.

C3H/HeOuJ Versus C57BL/6J Mice

This study has revealed profound differences in the response of mice with different genetic background to I/R. CH3/HeOuJ and C57BL/6J mice differ in at least 3 specific ways in their response to transient, unilateral uterine I/R:

- (a) Tolerance to TFR: C3H/HeOuJ mice can tolerate TFR for 30 minutes without severe consequence other than FGR. However, in C57BL/6J mice, even 5 minutes of TFR results in over 60% fetal loss.
- (b) Fetal loss as a major outcome: In C3H/HeOuJ animals, no obvious increase in fetal loss is observed in I/R-challenged groups, by either TFR or PFR. But in C57BL/6J mice, fetal loss is significantly higher in all I/R-challenged groups, by both TFR and PFR. Linear regression analysis shows that the level of fetal loss is significantly correlated with the intensity of the inflammatory response (MPO activity) in placental tissue in C57BL/6J mice (the more sensitive strain) following 5-minute TFR (the most intense ischemic challenge in this strain).
- (c) Involvement of the placenta: Placental weight alterations can indicate pathophysiological changes during development. Decreased tissue weight could be a result of injury, and increased weight a reflection of tissue edema and/or hyperplasia if it appears together with inflammation. In C3H/HeOuJ animals, I/R challenge induces both placental weight reduction and pronounced inflammation in placental tissue (reflected by tissue MPO activity), although the former is significant only in the TFR group. In contrast, neither of these 2 phenomena is observed in C57BL/6J animals, either TFR or PFR treated. These results suggest possible difference(s) in the pathological mechanism(s) involved in the development of growth restriction in these 2 strains of mice after I/R challenge. For example, they may imply that the placenta in 1 strain compared to the other is more resistant to ischemic challenge (both duration and intensity). C57BL/6J mice have a naturally occurring deletion in the *Nnt* gene, resulting in an absence of NNT protein and leading to impaired glucose homeostasis and reduced

insulin secretion.³⁵ This metabolic difference may have a profound effect on placental capability to maintain normal function under ischemic stress. C57BL/6J mice are also deficient in type 2 secretory phospholipase A2 (PLA2G2A), due to the insertion of an extra thymidine residue in exon 3, resulting in a frame shift mutation.⁴³ PLA2G2A is an antibacterial peptide of the Paneth cells in the small intestine and is also a rate-limiting step in the extracellular production of lysophospholipid, an intermediate in the proinflammatory PAF synthetic pathway. PAF is a neutrophil chemoattractant. Deficiency of PLA2G2A could, then, be partially responsible for the reduced MPO activity in C57BL/6J placentas. Our results have shown that FGR may develop with and without severe placental injury, although they do not allow us to determine whether FGR in this model is dependent on placental function, and, indeed, whether reduced placental weight or increased inflammation is a cause, an effect, or an epiphenomenon related to development of FGR.

These findings have important implications for I/R-induced pathology and the consequent inflammatory response in the placenta. In the early development of I/R-induced FGR animal models, complete flow deprivation for longer periods up to an hour was often adopted.^{24,25} Our findings may provide new insight for the evaluation of the role of brief or incomplete I/R-related events in FGR pathogenesis in humans. Transient, partial restriction of perfusion to a limited portion of the uterine and placental tissue is not rare in human pregnancy.²² It may be the result of many mechanisms, from inadequate trophoblast invasion leading to insufficient spiral artery remodeling, to disturbance in the regulation of tissue perfusion by vasoactive mediators produced by inflammatory events, all of which may serve as potential pathogenic stimuli. The study reported herein is the first to demonstrate that total blood flow deprivation is not required for FGR to develop in animals. This model then provides an avenue for refining the study of various pathogenic pathways in FGR.

In addition to the role of inadequate uterine and placental perfusion in FGR, our study also provides supporting evidence that the pathogenesis of FGR and fetal loss may be linked to each other by a common failure of local perfusion, possibly due to a circulating factor, as we have shown in a rat model of I/R-induced FGR,²⁶ given that both ipsilateral and contralateral sides are affected. Fetal growth restriction and fetal loss can be induced simultaneously by uterine I/R,²⁴ and this study further demonstrates that the severity of the outcome is dependent on the extent of perfusion reduction and the duration of ischemic insult. An implication of this observation is that these 2 outcomes might be manifestations of differing severity in the same disease continuum. This hypothesis could be approached not just using I/R-related models, but also several complementary models in developmental pathology, for example, administration of lipopolysaccharide⁴⁴ or antiphospholipid

antibodies.⁴⁵ In these models, both FGR and fetal loss result simultaneously.

The point of confluence for these ostensibly discrete pathogenic mechanisms is inflammation-driven vascular events. The I/R-induced damage is by nature an outcome of an inflammatory response⁴⁰ that shares many factors in common with immune response-caused pathology, and this is particularly true when the challenge is transient. Our study shows that I/R challenge causes a significant inflammatory response in the placenta, even in the PFR scenario. Other studies, using the rat model of I/R-induced FGR, have implicated inflammation in the pathogenesis of FGR by demonstrating that inflammatory mediators—for example, adhesion molecules,²⁸ ROS and TX,²⁹ and PAF²⁶—as well as vasoactive agents (endothelin,²⁶ TX,²⁹ and PAF²⁶) give rise to the development of FGR and fetal loss. Of note, Girardi et al⁴⁶ showed that neutrophils and complement component C5a each mediate fetal loss in an anti-phospholipid antibody model of FGR. We have previously shown C5 mediation of uterine I/R-induced fetal loss and FGR.⁴⁰ In the study reported herein, we show neutrophil involvement in this same model of I/R-induced fetal loss.

The studies reported herein are the first to demonstrate that FGR and fetal loss can be differentially induced by different modalities of I/R in mice, mice of different strains have different tolerances to uterine I/R, and the pathogenic mechanism for FGR may be different between C3H and C57BL/6 mice.

Declaration of Conflicting Interests

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