Long-Term Circulatory Consequences of Perinatal Iron Deficiency in Male Wistar Rats

Stephane L. Bourque, Marina Komolova, Kanji Nakatsu, Michael A. Adams

Abstract—Perinatal iron deficiency (PID) has been reported to induce developmental abnormalities, including cardiovascular complications in rats. These complications are believed to be “programmed” by an aberrant perinatal environment because the changes persist long after the insult is corrected (ie, despite subsequent iron replenishment). Little is known about the mechanisms by which PID affects blood pressure in the offspring, although the kidney is likely to play a central role. The objective of this study was to investigate the circulatory complications of PID and the putative role of the kidney involved therein. Before and throughout gestation, female Wistar rats were fed either a low-iron diet (3 ppm/10 ppm Fe) or an iron-enriched diet (225 ppm Fe). After giving birth, all of the dams were placed on a standard grain-based diet. At 24 hours postpartum, hematocrits and hemoglobin levels from offspring of iron-deficient mothers were 60% and 59% of control values, respectively. Adult PID animals had greater mean arterial pressures (110 versus 106 mm Hg) and systolic blood pressures (129 versus 124 mm Hg) than controls, as assessed by radiotelemetry. The relationship between renal arterial pressure and renal interstitial hydrostatic pressure, assessed in anesthetized rats, was blunted by 41% in the PID group compared with controls. In addition, arterial pressure changes were significantly greater in response to altered sodium in the PID animals compared with controls. These data confirm that PID adversely affects blood pressure control, which seems to be mediated, at least in part, by altered intrarenal hemodynamic properties. (Hypertension. 2008;51:1-2.)

Key Words: anemia • iron deficiency • fetal development • blood pressure • telemetry • kidney

Iron deficiency ranks among the World Health Organization’s top 10 global health risks and is considered a significant health risk in both developing and industrialized countries. This is not surprising given that the worldwide incidence of iron deficiency is estimated to be 66% to 80%. Although iron deficiency significantly affects all populations, the group most at risk is pregnant women. The enhanced risk profile in pregnancy is a consequence of increased erythropoiesis because of blood volume expansion in the mother and increased iron use from the growing fetal-placental unit. Overt iron deficiency manifests as anemia in more than half of the pregnant women in developing countries and 20% to 40% of women in Western countries.

Perinatal iron deficiency (PID) can adversely impact the growth and development of the offspring, resulting in cardiovascular complications in later life. Specifically, studies in rats have shown that inadequate iron supply during early development can produce hypertension, even when iron levels are subsequently normalized. In fact, Lisle et al demonstrated that PID produced both a nephron deficit and elevated blood pressure in adult offspring. Although the effects of PID on renal function were not investigated, the study by Lisle et al implicates the developing kidney as a potential target for perinatal insult.

There is a large body of evidence that suggests that the kidney plays a critical role in establishing the long-term set point of arterial pressure by modulating sodium and water excretion (and, hence, blood volume). The most compelling evidence for this hypothesis involves the transplantation of kidneys from hypertensive animals into normotensive animals, which confers on the recipient the hypertensive phenotype. Furthermore, we have shown, using similar kidney cross-transplant experiments, that pharmacological manipulations that persistently alter the renal vascular structure and function are sufficient to confer long-term changes in blood pressure, independent of changes in systemic vascular tone. Thus, changes in renal vascular resistance properties, at least in part, are likely to play a crucial role in determining the set point of blood pressure control.

Together, these studies provide a clear rationale for investigating the role of the kidney in the development of PID-induced hypertension. The objective of this study was 3-fold: (1) to determine the long-term effects of PID on the circulatory phenotype using direct measurements of blood pressure by radiotelemetry; (2) to determine the impact of PID on the intrinsic hemodynamic properties of the kidney; and (3) to assess renal function by characterizing changes in arterial pressure during low-, normal-, and high-sodium intake.
Methods

Animals and Treatments

The experimental protocols described herein were approved by the Queen’s University Animal Care Committee. Eighteen 6-week-old female Wistar rats were purchased from Charles River (Saint-Constant, Quebec, Canada) and housed in the Queen’s University Animal Care Facility. Dams were housed in individual plastic cages with a stainless-steel mesh covering, which held their food and water bottle. Rats had ad libitum access to food and water. The animal care facility maintained a 12 hour/12 hour light/dark cycle and an ambient temperature of 23°C. Animals were allowed to acclimate for 1 week before experimentation.

All of the purified diets were obtained from Research Diets Inc. The diets used before and throughout gestation were based on the AIN-93G rodent diet, which has been described elsewhere.13 The control and iron-deficient diets were identical in composition, with the exception of the amount of added ferric citrate, which was adjusted to obtain the following iron concentrations: control diet, 225 ppm; low-iron diet, 3 ppm (no added ferric citrate); and moderately low-iron diet, 10 ppm. The standard grain-based rodent chow (Laboratory Diet) had an iron concentration of 270 ppm.

During the acclimatization period, all of the dams were placed on the purified control diet. Ten females were then randomly selected and placed on the low-iron diet, whereas the remaining 8 females were maintained on the control diet. After 2 weeks on their respective diets, dams were bred naturally (ie, without synchronization) with each dam for 4 consecutive days; those that did not mate within this period were excluded from the study. Beginning at the time of mating, all of the dams in the low-iron group were then changed to the moderately low-iron diet. This was accomplished by housing 1 male with each dam for 4 consecutive days; those that did not mate within this period were excluded from the study. At 24 hours postpartum, all of the litters were reduced to 10 males to standardize postnatal conditions; in litters that contained <10 males, the difference was made up with females. One control litter only was culled pups. For details on tissue collection methods, please see the data supplement.

Data Analyses

Neonatal offspring Hcts, Hb levels, and organ weights from each litter were pooled, and the means were calculated and presented as a 5-day average. High- and low-sodium MAP values were obtained by radiotelemetry. Body weights and organ weights were monitored twice weekly.

Table 1. Summary of Weights, Hematological Indices of Iron Status, and Pregnancy Outcomes in Control and Iron-Deficient Dams

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Iron-Deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BW (7 wk), g</td>
<td>195.6±3.9</td>
<td>188.0±3.1</td>
</tr>
<tr>
<td>BW gain (pregnancy), g</td>
<td>60.7±4.3</td>
<td>58.3±2.8</td>
</tr>
<tr>
<td>BW gain (pregnancy), g per pup</td>
<td>6.7±0.4</td>
<td>6.0±0.2</td>
</tr>
<tr>
<td>Pups per litter</td>
<td>15.1±1.9</td>
<td>16.1±0.9</td>
</tr>
<tr>
<td>Percentage of male pups</td>
<td>46.8±3.0</td>
<td>46.4±3.0</td>
</tr>
<tr>
<td>Hct (pregnancy)</td>
<td>0.47±0.01</td>
<td>0.44±0.01*</td>
</tr>
<tr>
<td>Hb (pregnancy), g/dL</td>
<td>134±5</td>
<td>121±3*</td>
</tr>
<tr>
<td>Hct (1 d after parturition)</td>
<td>0.41±0.01</td>
<td>0.30±0.01†</td>
</tr>
<tr>
<td>Hct (1 d after parturition), g/dL</td>
<td>114±5</td>
<td>82±5†</td>
</tr>
<tr>
<td>Hct (7 d after parturition)</td>
<td>0.47±0.01</td>
<td>0.47±0.01</td>
</tr>
<tr>
<td>Hb (7 d after parturition), g/dL</td>
<td>123±7</td>
<td>128±3</td>
</tr>
</tbody>
</table>

Control: n=7; PID: n=9. BW indicates body weight.
*P<0.05; †P<0.001 vs control values.

Conscious Hemodynamic Assessments

Starting at 10 weeks of age, mean arterial pressure (MAP) and systolic blood pressure (SBP) were continuously monitored in the offspring using radiotelemetry data acquisition (Data Sciences International), as described previously.17 For details, please see the data supplement.

For the sodium challenge experiments, blood pressure from rats (13 weeks of age) implanted with radioembolometric transducers was recorded at baseline levels (normal sodium intake) for 5 days, and the rats were placed on a low-sodium treatment regimen for 5 days and subsequently placed on a high-sodium treatment regimen for 5 days. The low-sodium regimen consisted of ad libitum access to a low-sodium (0.04% Na+) purified diet (Research Diets Inc.), based on the AIN-76A rodent diet, as well as tap water. The high-salt treatment regimen consisted of ad libitum access to the standard grain-based rodent chow described above (0.4% Na+), as well as drinking water supplemented with 1% NaCl (wt/vol). The normal-salt treatment consisted of the grain-based rodent chow and tap water. Body weights, as well as food and water intake, were monitored daily during these treatments.

In Vivo Assessments of Renal Vascular Properties

Intrarenal hemodynamic assessments were performed in anesthetized 10-week–old male PID and control offspring, based on a method described previously.17 For details, please see the data supplement.

Data Analyses

For intrarenal hemodynamic assessments, linear regression analysis was performed by the ordi-
there were no differences between groups in the number of pups born per litter or the proportion of males and females. There were, however, clear signs of negative impact in the PID group. For example, 1 litter in the PID group did not survive 24 hours. In addition, there were 2 deaths in 2 separate PID litters within the first 14 days. Tissues from these animals were excluded from subsequent analysis. In contrast, there were no perinatal deaths in the control group.

During the 2-week treatment period before conception, dams fed the low-iron diet had a modest decrease in Hcts (93% of control; \( P < 0.05 \)) and Hb levels (91% of control; \( P < 0.05 \); Table 1). Hcts and Hb levels fell to <75% \( P < 0.001 \) of controls 24 hours after parturition but had returned to control levels within 7 days (Table 1). Conversely, Hcts and Hb levels in pups of iron-deficient dams were 60% \( P < 0.001 \) and 59% \( P < 0.001 \) of control values at birth, respectively, and remained significantly decreased until after PD14 (Table 2). At PD21, Hcts in the PID offspring remained >10% below controls \( P < 0.05 \), but Hb levels were no longer significantly depressed. Control pups, but not PID pups, had significant decreases in Hcts and Hb levels after birth \( P < 0.01 \) at all times compared with PD1.

Body weights of offspring in the PID group were >10% lower than those of the control group throughout the study period \( P < 0.05 \); Figure 1). After a marked decrease in relative body weight during the first postnatal week (Figure 1, inset), PID pups underwent 2 periods of “catch-up” growth (when absolute weight gain was greater in the PID group), 1 preweaning (PD10 to PD21) and 1 postweaning (beyond PD24). Heart weights (normalized to body weight) were 29% higher in the PID offspring at PD1 compared with control offspring \( P < 0.01 \); Table 2). These differences persisted until PD21. There were no observed differences in kidney weights (normalized to body weight) between the control and PID offspring between PD1 and PD21 (Table 2).

Blood pressure data, expressed as MAP and SBP, starting at ~11 weeks of age (after the 10-day recovery period after surgery), were moderately but significantly elevated in the PID group compared with control group over a 10-day period (Figure 2). The mean 6-hour MAP values for the control and PID animals over the 10-day period were 105.8 \( \pm 0.8 \) mm Hg versus 110.7 \( \pm 1.5 \) mm Hg, respectively \( P < 0.05 \); the average SBP values were 124.0 \( \pm 0.7 \) mm Hg versus 129.3 \( \pm 2.0 \) mm Hg, respectively \( P < 0.05 \).

A summary of intrarenal hemodynamic parameters assessed in control and PID animals at ~10 weeks of age is presented in Table 3. The mean RAP in the PID group under anesthesia was found to be ~12 mm Hg higher than controls \( P < 0.05 \). Consistent with the elevated pressure, left ventricular weights (normalized to body weight) were 10.4% larger in the PID group compared with controls \( P < 0.05 \); right ventricular weights (normalized to body weight) were not statistically different between groups. Despite the increased RAP, the resting mean RIHP was not different. In contrast, the slope of the overall \( \Delta RAP-\Delta RIHP \) relationship (Figure 3) was blunted by 41% in the PID group \( 0.062 \pm 0.005; \ r^2 = 0.87 \) compared with controls \( 0.10 \pm 0.02; \ r^2 = 0.95; P < 0.01 \). Likewise, assessment of the slope of the \( \Delta RAP-\Delta RIHP \) relationship between the more physiologically relevant RAP interval of 25 to 25 mm Hg revealed a similar blunting of 45.5% in the PID animals \( 0.054 \pm 0.006; \ r^2 = 0.76 \) compared with controls \( 0.099 \pm 0.010; \ r^2 = 0.80; P < 0.01; \) data not shown). The slope of the RAP-RIHP relationship, when not normalized to baseline pressures, was 24% blunted in the PID offspring \( 0.098 \pm 0.004; \ r^2 = 0.85 \) compared with controls \( 0.075 \pm 0.005; \ r^2 = 0.73; P < 0.01 \); Figure 3, inset).

Table 2. Neonatal Offspring Information at PD1, 7, 14, and 21

<table>
<thead>
<tr>
<th>Variable</th>
<th>PD1</th>
<th>PD7</th>
<th>PD14</th>
<th>PD21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Hct</td>
<td>0.39±0.07</td>
<td>0.33±0.07</td>
<td>0.29±0.07</td>
<td>0.32±0.09</td>
</tr>
<tr>
<td>PID Hct</td>
<td>0.23±0.07†</td>
<td>0.21±0.01†</td>
<td>0.25±0.06†</td>
<td>0.27±0.01*</td>
</tr>
<tr>
<td>Control Hb, g/dL</td>
<td>106±3</td>
<td>80±3</td>
<td>71±2</td>
<td>66±3</td>
</tr>
<tr>
<td>PID Hb, g/dL</td>
<td>62±3‡</td>
<td>54±2‡</td>
<td>56±2‡</td>
<td>59±3</td>
</tr>
<tr>
<td>Control HW/BW, g/kg</td>
<td>5.7±0.2</td>
<td>6.5±0.3</td>
<td>4.9±1.0</td>
<td>4.7±0.2</td>
</tr>
<tr>
<td>PID HW/BW, g/kg</td>
<td>7.4±0.3†</td>
<td>8.7±0.4‡</td>
<td>6.4±0.2‡</td>
<td>5.6±0.2‡</td>
</tr>
<tr>
<td>Control KW/BW, g/kg</td>
<td>5.1±0.2</td>
<td>6.0±0.2</td>
<td>5.1±0.1</td>
<td>4.8±0.2</td>
</tr>
<tr>
<td>PID KW/BW, g/kg</td>
<td>5.1±0.2</td>
<td>6.2±0.2</td>
<td>5.4±0.1</td>
<td>5.1±0.1</td>
</tr>
</tbody>
</table>

Control: n=4 litters; PID: n=6 litters. PD indicates postnatal day; PID, perinatal iron-deficient; HW, heart weight; KW, kidney weight.

* \( P < 0.05 \); † \( P < 0.01 \); ‡ \( P < 0.001 \) vs control values at same postnatal day.
Table 3. Physical and Renal Properties of Offspring at 10 Weeks of Age

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>PID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>486.0±18.0</td>
<td>396.4±16.5†</td>
</tr>
<tr>
<td>Hct</td>
<td>0.54±0.02</td>
<td>0.50±0.01</td>
</tr>
<tr>
<td>LV/BW, g/kg</td>
<td>1.70±0.052</td>
<td>1.95±0.10*</td>
</tr>
<tr>
<td>RV/BW, g/kg</td>
<td>0.55±0.05</td>
<td>0.65±0.04</td>
</tr>
<tr>
<td>KW/BW, g/kg</td>
<td>3.88±0.13</td>
<td>3.70±0.30</td>
</tr>
<tr>
<td>Baseline RAP, mm Hg</td>
<td>102.1±3.5</td>
<td>113.9±3.4*</td>
</tr>
<tr>
<td>Baseline RIHP, mm Hg</td>
<td>6.96±0.30</td>
<td>5.90±0.55</td>
</tr>
</tbody>
</table>

Control: n=5; PID: n=5. LV indicates left ventricle; RV, right ventricle; KW, kidney weight; BW, body weight.

*P<0.05; †P<0.01 vs control values.

Discussion

The major findings in the offspring after the maternal iron restriction intervention during pregnancy include the following: (1) severe decreases in hematoologic indices; (2) marked cardiac hypertrophy; (3) a moderate but persistent elevation in arterial pressure; (4) alterations in the hemodynamic properties of the kidney; and (5) an increased sensitivity of arterial pressure to changes in dietary sodium intake. These findings suggest that iron deficiency during periods of growth and development has a detrimental impact on circulatory function that persists in adulthood and that may, at least in part, be mediated by changes in renal function.

The treatment paradigm adopted in this study was one in which iron deficiency was induced primarily during the gestational period, because the dams were placed on an iron-replete diet immediately after giving birth, allowing them to recover Hb levels and Hcts within 7 days (Figure 1). With this approach, we avoided confounding factors associated with continued anemia in the mothers during the nursing phase. Specifically, milk production in the iron-deficient mother seems to be adversely affected with respect to iron, energy, and fat content. Despite returning the iron-deficient dams to an iron-replete diet after birth, their pups remained anemic for the entire fostering phase. This is consistent with previous reports that rat milk is low in iron content, even in mothers with normal iron status. Indeed, Hcts and Hb levels in control animals steadily decreased throughout lactation, suggesting diminished iron supply during the fostering phase in these animals as well. It may be that progressive iron deficiency in the control offspring in the immediate postnatal period is part of the natural pattern of development, although the mechanisms have not been investigated. In the present studies, although the magnitude of the iron deficiency was greater in the PID neonates, the specific impact of this period of development remains to be elucidated.

The enlarged hearts in the PID animals during the neonatal period are consistent with reports by others and may be an adaptive response to anemia during gestation and the neonatal periods. Indeed, fetal anemia has been shown to increase heart weight and cardiac output in sheep. In the present study, the increased cardiac weight (which may result from hyperplastic and/or hypertrophic cardiomyocyte growth) may be linked to increases in cardiac output, a circulatory change that would facilitate perfusion of fetal tissues during development. As suggested by Lewis et al, this adaptation would be expected to limit the generation of hypoxia.

Consistent with most models of fetal programming, the PID offspring had lower birth weights than controls. Interestingly, the PID pups underwent 2 periods of catch-up growth, one during the preweaning phase and one in the postweaning phase [Figure 1, inset]. These periods of catch-up growth in the PID offspring have been proposed to...
be an important predisposing factor for long-term cardiovascular disease associated with fetal programming. However, similar iron deficiency-induced fetal programming effects have been reported by others in the absence of this catch-up growth phase. Regardless, it is clear that there is decreased growth in the PID animals during the first 2 weeks (when renal maturation is completed), and this may have further adversely affected the circulation. As indicated above, the precise role of these postnatal changes is presently unresolved.

The finding, using radiotelemetry, that arterial pressure was significantly elevated in the adult offspring after PID confirms previous results in which SBP was assessed using the indirect tail-cuff method. These discrepancies may be because of a number of factors, including the following: (1) differences in the timing and degree of iron deficiency in the mothers and offspring; (2) strain-specific differences (eg, Rowett Hooded-Lister, Sprague Dawley, and Wistar [present study]); and, most importantly, (3) SBP, measured via the tail-cuff methodology, affected by restraint and thermal stress. Indeed, it may be that programmed animals are more responsive to such stressors compared with nonprogrammed animals. In light of this evidence, the current validation of this cardiovascular phenotype in this model of programming using direct conscious, chronic determinations of arterial pressure is an important foundation for future studies.

The key finding that PID altered the intrarenal hemodynamic properties, namely, the RAP-RIHP relationship, may explain, in part, the long-term elevations in arterial pressure observed in these animals. The kidney is fundamental in establishing the set point of long-term arterial pressure by regulating sodium and fluid balance. Fluctuations in arterial pressure around the long-term level induce changes in perfusion of the poorly autoregulated medullary vessels and, consequently, cause changes in RIHP, which ultimately influence the set point of arterial pressure at which sodium and water balance occur. That is, a decrease in the responsiveness and set point of the RAP-RIHP relationship can impact the pressure-natriuresis mechanism such that greater changes in arterial pressure are required to generate corresponding changes in RIHP to regain the sodium and water balance.

The blunting of the RAP-RIHP relationship may also explain, in part, the altered responsiveness in handling low- and high-sodium intake in the PID animals. As depicted in the dietary sodium-MAP relationship (Figure 4, bottom), control offspring will increase sodium and fluid excretion in response to minor changes in arterial pressure. However, adult PID offspring would require greater changes in MAP to regain sodium balance. Indeed, in other rodent models in which blood pressure is salt-sensitive (eg, neonatal RAS-inhibited rat, spontaneously hypertensive rat, Dahl salt-sensitive rat, and atrial natriuretic peptide [mouse]), a similar blunting of the RAP-RIHP relationship is observed (Reference 30–33).

Although the specific mechanisms by which PID adversely impacts intrarenal hemodynamics and kidney function are beyond the scope of this study, decreased responsiveness of RHP to changes in RAP has previously been linked to alterations in renal interstitial compliance, as well as changes in the medullary circulation. PID could potentially affect the development of the renal interstitium and medullary vessels during development via changes in overall growth, nephron endowment, development of the renal tubules and associated vasculature, expression of tubular transporters (eg, Na\(^+\)/K\(^+\)-ATPase and Na\(^+\)-H\(^+\) co-transporter), modified renin-angiotensin system activity, and changes in the expression and activities of vasoactive species (eg, NO synthase, sGC, 20-HETE, etc). Indeed, because iron is an integral component of numerous signaling and effector molecules, it is likely that the etiology of the adverse programming effects observed in the present study is multifaceted and complex.
Perspectives
The adverse programming effects of iron deficiency, solely during the perinatal period, on the long-term circulatory phenotype further demonstrate the importance of the developmental origins of health and disease. The concept emphasized by the present study is that subtle changes in the status of maternal nutrition during pregnancy can influence the long-term health of the fetus. Although programming effects have been associated with a number of macronutrient and micronutrient deficiencies, given the worldwide prevalence of iron deficiency, as well as its propensity to afflict pregnant women, it may represent an especially important risk factor for long-term cardiovascular disease.

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Disclosures
None.

References
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