Placental Insufficiency Leads to Development of Hypertension in Growth-Restricted Offspring

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Abstract—Low birth weight is a suggested risk factor for the development of hypertension. The purpose of the present study was to determine whether a model of intrauterine growth restriction produced in response to placental insufficiency in the pregnant rat was associated with marked elevations in blood pressure. Reduced uterine perfusion initiated in late gestation resulted in low-birth-weight offspring (5.8 ± 0.1 versus 6.6 ± 0.2 g, P < 0.05, growth-restricted versus control, respectively). Mean arterial pressure, as measured in conscious, chronically instrumented rats, was significantly elevated as early as 4 weeks of age (113 ± 3 versus 98 ± 2 mm Hg, P < 0.05) and was associated with significant decreases in body weight (66 ± 2 versus 81 ± 3 g, P < 0.05) in growth-restricted (n = 15) versus control (n = 15) rats. Marked elevations in arterial pressure at 8 weeks of age (male: 133 ± 3 versus 121 ± 6 mm Hg, P < 0.05; female: 137 ± 4 versus 112 ± 6 mm Hg, P < 0.01) were associated with sex-specific decreases in body weight (male: 251 ± 6 versus 275 ± 10 g, P < 0.05; female: 163 ± 6 versus 180 ± 6 g) in male growth-restricted (n = 12) versus male control (n = 9) rats and in female growth-restricted (n = 8) versus female control (n = 7) rats. At 12 weeks of age, hypertensive (144 ± 4 versus 131 ± 3 mm Hg, P < 0.05) male growth-restricted offspring (n = 10) had no alterations in glomerular filtration rate (2.3 ± 0.3 versus 2.2 ± 0.2 mL/min) compared with control (n = 10) offspring; even when adjusted for kidney weight (1.7 ± 0.3 versus 1.5 ± 0.3 mL/min · g⁻¹ kidney), despite marked decreases in body weight (305 ± 9 versus 343 ± 10 g, P < 0.05). These data suggest that placental insufficiency induced by reduced uterine perfusion in the pregnant rat results in low-birth-weight offspring predisposed to development of hypertension. (Hypertension. 2003;41:448-454.)

Key Words: hypertension, experimental ■ kidney ■ rat ■ arterial pressure ■ glomerular filtration rate

Hypertension is a multifactorial disorder that is thought to result from both genetic and environmental factor interactions. Numerous epidemiological studies have reported an association between low birth weight (LBW) and the risk of hypertension.¹² This association has been reported in a variety of populations from around the world and is further supported because elevations in blood pressure are also found in LBW children.³–⁵ Thus, the inverse relationship between LBW and hypertension suggests that factors present in the prenatal environment that affect fetal growth are responsible for the in utero programming of arterial blood pressure control.⁷–⁹

Nutrient and oxygen supply limitations are the components of the intrauterine environment that limit fetal growth and result in small-for-gestational-age newborns. Animal models of fetal malnutrition induced by maternal nutrition restriction support a role for programming of hypertension owing to an adverse fetal environment.¹⁰–¹² Woods et al¹³ report that protein restriction during the last third of gestation in the rat results in offspring with reduced renal function and hypertension. In the rat, nephrogenesis occurs during the last third of gestation and continues for several days after delivery.¹⁴,¹⁵ As the kidneys are known to play an important role in the long-term regulation of arterial pressure,¹⁶ this suggests that timing of the insult in utero is critical to the development of hypertension. Thus, fetal adaptations to oxygen and nutrient restriction that occur during a critical period of fetal renal development may result in long-term effects and increased risk for development of hypertension.

In the United States, a higher percentage of LBW babies are born relative to that of other Western countries, with the highest rates of LBW localized in the southern states and, in particular, within the black population.¹⁷ The highest rates of hypertension are also concentrated in the South, with a greater prevalence of hypertension within the black population.¹⁸ As intrauterine growth restriction (IUGR) within the Western world is more likely the result of impaired uteroplacental perfusion rather than of maternal malnutrition,¹⁹ we have used a model of in vivo placental insufficiency initiated in late gestation in the rat to examine the inverse relationship between LBW and hypertension. Specifically, a chronic reduction in uteroplacental perfusion in the pregnant rat,
induced by placement of a silver clip around the aorta below the renal arteries at the start of late gestation, resulted in reduced uterine perfusion by 35% to 45% and IUGR with birth at term. Thus, the purpose of the present study was to determine whether a chronic reduction in uterine perfusion initiated in the last third of gestation in the pregnant Sprague-Dawley rat was associated with growth-restricted offspring that are predisposed to hypertension.

Methods

Animals

All experimental procedures executed in this study were in accordance with National Institutes of Health guidelines for use and care of animals, with approval of all protocols by the Animal Care and Use Committee at the University of Mississippi Medical Center. Female timed-pregnant Sprague-Dawley rats were purchased from Harlan Sprague-Dawley Inc (Indianapolis, Ind) and housed 1 to a cage in a temperature-controlled room (23°C) with a 12-hour/12-hour light/dark cycle with food and water available ad libitum.

At day 14 of gestation, rats destined for reduced uterine perfusion were clipped as described below. All dams were allowed to deliver at term, with birth weight recorded within 12 hours. At this time, a control litter was size-matched per growth-restricted (IUGR) litter, with a minimum litter size of 8 pups, with all marked for identification by tattooing (Spaulding Special electronic Tattoo Marker, Spaulding and Rogers). Pups were weighed twice a week after birth and were weaned at 3 weeks of age. Mean arterial pressure (MAP) and renal hemodynamics were determined in conscious, chronically instrumented rats. Offspring from different litters were chosen at random, with simultaneous measure of control and IUGR offspring. In the initial study for measure of MAP, a total of 10 reduced-uterine-perfusion dams and 10 control dams were used with the following breakdown per experiment. At 4 weeks of age, a total of 7 male and 8 female controls and 8 male and 7 female IUGR offspring were used for measure of MAP and determination of organ weights. At 8 weeks of age, a total of 9 male and 7 female controls and 12 male and 8 female IUGR offspring were used for measure of MAP. For measure of MAP and determination of organ weights, rats were anesthetized with 2% isoflurane (WA Butler Co) and sacrificed by exsanguination, with all organs weighed. A second study for determination of renal hemodynamics in conjunction with measure of MAP, a total of 6 reduced-uterine-perfusion dams and 7 control dams were used with the following breakdown per experiment. At 8 weeks of age, 13 male control and 11 male IUGR offspring were used for determination of glomerular filtration rate (GFR), effective renal plasma flow (ERPF), renal vascular resistance (RVR), and MAP; 10 male control and 10 male IUGR were used at 12 weeks of age. For measure of fetal weights at day 19 of gestation, 8 litters per reduced uterine perfusion and 8 per control dam were used.

Reduced Uterine Perfusion in the Pregnant Rat

Eder and McDonald21 have previously reported that placement of a silver clip around the aorta below the renal arteries during mid-to-late gestation in the pregnant rat results in a 35% to 45% reduction in uterine perfusion. A modification of this model, as described previously, was used to induce IUGR.20 Briefly, at day 14 of gestation, rats were anesthetized with 2% isoflurane (WA Butler Co) delivered by anesthesia apparatus (Vaporizer for Forane Anesthetic, Ohio Medical Products). A silver clip (0.203-mm ID) was placed around the isolated abdominal aorta above the iliac bifurcation, with additional clips placed on both right and left branches of uterine arteries (0.100-mm ID). IUGR was not present in sham-operated control pregnant rats.

Results

Effect of IUGR on Birth Weight

Birth weight was significantly decreased in offspring from reduced uterine perfusion relative to offspring from control pregnant rats. Specifically, at birth IUGR were significantly smaller than their control offspring relative to offspring from control pregnant rats. Specifically, at birth IUGR were significantly smaller than their control offspring, combined for both male and female. Data shown is for IUGR versus control offspring, combined for both male and female. *P<0.01 vs control. All data are mean±SEM.

Figure 1. Measure of birth weight in a rat model of IUGR induced by reduced uterine perfusion. Data shown is for IUGR versus control offspring, combined for both male and female. *P<0.01 vs control. All data are mean±SEM.

Measurement of Renal Hemodynamics and Arterial Pressure in Conscious Rats

In brief, all rats undergoing surgical procedures were anesthetized with 2% isoflurane, as described above, and were surgically instrumented with catheters in the femoral vein, carotid artery, and bladder, as previously described.22 Renal function and MAP measurements were performed in conscious, chronically instrumented rats, as described previously.22 Briefly, GFR and ERPF were calculated from radioactivity of Glofil ([125I] sodium iothalamate, Questcor Pharmaceuticals) and concentration of para-aminomophurate, respectively, in plasma and urine. On completion of MAP determination, total body and organ (kidney, brain, liver, and heart) weights were recorded.

Statistical Analyses

GB-STAT, version 6.5, was used for all statistical analysis. All data are expressed as mean±SEM. Comparisons of control offspring with IUGR were analyzed by using factorial ANOVA followed by the Scheffé test. A value of P<0.05 was considered statistically significant.

Effect of IUGR on MAP

As early as 4 weeks of age, a significant increase in arterial pressure was evident in growth-restricted offspring compared with controls (113±3 versus 98±2 mm Hg, P<0.05, IUGR...
versus control, respectively) (Figure 3). However, marked elevations in MAP were not sex specific, as significant decreases in arterial pressure were present at 4 weeks of age in both male (114±4 versus 97±4 mm Hg, P<0.05) and female (113±4 versus 99±2 mm Hg, P<0.05) growth-restricted offspring compared with controls, respectively (Figure 3). At 8 weeks of age, marked elevations in MAP were still apparent in the growth-restricted offspring (134±2 versus 117±4 mm Hg, P<0.01, IUGR versus control, respectively), an observation that remained non–sex specific (133±3 versus 121±6 mm Hg, P<0.05, male IUGR versus male control, respectively; 137±4 versus 112±6, P<0.01, female IUGR versus female control, respectively) (Figure 3). However, at 12 weeks of age, sex-specific differences in MAP were observed, as male growth-restricted offspring exhibited a significant increase in arterial pressure (158±1 versus 135±2 mm Hg, P<0.01), whereas female IUGR did not (139±4 versus 126±3 mm Hg; IUGR versus control, respectively) (Figure 3).

**Effect of IUGR on Body Weight**

At 4 weeks of age, marked increases in arterial pressure were associated with significant decreases in body weight in both male (65±2 versus 87±7 g, P<0.05) and female (68±3 versus 76±1 g, P<0.05) growth-restricted offspring relative to control, respectively. At 8 weeks of age, hypertensive male growth-restricted offspring retained a marked decrease in body weight relative to control (251±6 versus 275±10 g, P<0.05, IUGR versus control, respectively). However, hypertensive female growth-restricted offspring at 8 weeks of age exhibited a nonsignificant decrease in body weight (163±6 versus 180±6 g, IUGR versus control, respectively). A marked reduction in body weight was still evident at 12 weeks of age in the hypertensive male growth-restricted offspring (307±9 versus 348±11 g, P<0.05, IUGR versus control, respectively). Despite the significant difference in body weight, 24-hour food and water intake did not differ at either 8 or 12 weeks of age, even with adjustment for body weight (data not shown).

**Effect of IUGR on Renal Function**

As arterial pressure was significantly elevated in growth-restricted offspring compared with control offspring, additional animals were examined for measure of renal hemodynamic parameters. Marked elevations in arterial pressure were again consistently observed in male IUGR offspring at both 8 (142±4 versus 127±4 mm Hg, P<0.05) and 12 (144±4 versus 131±3 mm Hg, P<0.05) weeks of age (IUGR versus control, respectively). However, the significant increase in MAP in male growth-restricted offspring was associated with nonsignificant differences in both GFR and ERPF. Specifically, neither GFR (2.1±0.3 versus 2.7±0.5 mL/min) nor GFR adjusted for kidney weight (1.7±0.3 versus 2.4±0.6 mL·min⁻¹·g⁻¹ kidney) differed significantly at 8 weeks of age in growth-restricted offspring compared with control, respectively. ERPF (12.1±1.8 versus 10.3±1.5 mL/min) and ERPF adjusted for kidney weight (8.6±1.4 versus 8.1±1.8 mL·min⁻¹·g⁻¹ kidney) were also not significantly altered (IUGR versus control, respectively). At 12 weeks of age neither GFR (2.3±0.3 versus 2.2±0.2 mL/min) nor ERPF (7.3±1.8 versus 8.8±1.9 mL/min) differed, even when adjusted for kidney weight (GFR: 1.7±0.3 versus 1.5±0.3 mL·min⁻¹·g⁻¹ kidney [Figure 4]; ERPF: 5.3±0.7 versus 6.3±1.0 mL·min⁻¹·g⁻¹ kidney) for IUGR offspring.

**Figure 2.** Growth progression in a rat model of IUGR induced by reduced uterine perfusion. Data shown is for IUGR versus control offspring, combined for both male and female, at 12 weeks of age. All data are mean±SEM.

**Figure 3.** Measure of MAP in a rat model of IUGR induced by reduced uterine perfusion. Data shown is for both male and female IUGR versus control offspring, at 4, 8, and 12 weeks of age. *P<0.05 vs male control; †P<0.05 vs female control; ‡P<0.01 vs control; and §P<0.01 vs control. All data are mean±SEM.

**Figure 4.** Measure of RVR and GFR adjusted to kidney weight in a rat model of IUGR induced by reduced uterine perfusion. Data shown is for male IUGR versus male control at 12 weeks of age. All data are mean±SEM.
versus control, respectively. In addition, RVR was not significantly altered at either 8 weeks of age (19.8 ± 6.4 versus 14.9 ± 3.1 mm Hg) or 12 weeks of age (14.3 ± 2.4 versus 10.4 ± 1.6 mm Hg; IUGR versus control, respectively) (Figure 4). Twenty-four–hour urinary sodium excretion in hypertensive IUGR offspring was not significantly altered compared with control offspring at either 8 (1.3 ± 0.2 versus 1.2 ± 0.2 mEq/d) or 12 (3.8 ± 0.5 versus 2.4 ± 0.6 mEq/d) weeks of age (IUGR versus control, respectively).

### Effect of IUGR on Organ Weights

Growth restriction induced by placental insufficiency was associated with alterations in organ weights (Table). At 4 weeks of age, brains were significantly smaller in male IUGR offspring (1.39 ± 0.02 versus 1.45 ± 0.01 g, \( P < 0.05 \), IUGR versus control, respectively). However, the brain-to–body weight ratio was significantly increased (21.8 ± 0.88 versus 17.1 ± 0.80 g/kg, \( P < 0.01 \); male IUGR versus male control, respectively). A similar observation was noted with both kidney weight and kidney-to–body weight ratio in the male growth-restricted offspring (kidney: 0.42 ± 0.02 versus 0.44 ± 0.03 g, \( P > 0.05 \); kidney/body weight: 6.49 ± 0.25 versus 5.17 ± 0.25 g/kg, \( P < 0.05 \); male IUGR versus male control, respectively). In addition, a significant reduction in liver size was observed in male IUGR rats at 4 weeks of age (3.22 ± 0.09 versus 4.13 ± 0.26 grams, \( P < 0.01 \); male IUGR versus male control, respectively). At 12 weeks of age, only a significant decrease in brain weight was noted in male growth-restricted offspring (1.74 ± 0.02 versus 1.95 ± 0.02 grams, \( P < 0.01 \); male IUGR versus male control, respectively).

### Discussion

Associations between LBW and later disease such as hypertension have been found in a variety of populations from around the world, and are independent of influences such as obesity and smoking, suggesting that increases in blood pressure may be linked to impaired fetal growth. Fetal growth, regardless of its genetic component, can be constrained by its environment or fetal undernutrition. Fetal undernutrition or availability of nutrients and oxygen is a major contributor to perinatal programming of hypertension and may result from either maternal undernutrition or inadequate delivery of nutrients to the fetus. Numerous investigators have shown that maternal undernutrition induced by protein restriction during gestation in the rat results in offspring that are hypertensive. However, as a strong correlation between hypertension and LBW is found in well-nourished populations, fetal undernutrition as a result of placental insufficiency, rather than maternal undernutrition, may be the major cause of IUGR in the Western world.

A major goal of the present study was to determine whether a model of placental insufficiency induced in late gestation by a reduction in uterine perfusion in the pregnant rat resulted in IUGR offspring born at term that were predisposed to development of hypertension. In the present study, placental insufficiency was induced by a chronic reduction in uterine perfusion initiated at day 14 of gestation in the pregnant rat. Offspring from the reduced uterine perfusion pregnant rats had significantly lower birth weights compared with those of offspring from control pregnant rats. Specifically, birth weight was reduced by 12% in IUGR offspring compared with control. In addition, MAP, as measured by carotid arterial catheter in conscious animals, was significantly increased as early as 4 weeks of age in both male and female IUGR offspring (17 and 15 mm Hg increase, \( P < 0.05 \), respectively). A significant increase in MAP was evident at 12 weeks of age in male IUGR offspring (23 mm Hg increase, \( P < 0.05 \)) and was associated with a marked reduction in body weight, present since birth. In female IUGR offspring, MAP was significantly elevated at 8 weeks of age (25 mm Hg increase, \( P < 0.05 \)), yet marked differences in body weight were no longer apparent. Thus, in
the present study, significant elevations in MAP were associated with equivalent or significant decreases in body weight. Furthermore, IUGR induced in this model of placental insufficiency was associated with sex-specific differences, as at 12 weeks of age, male IUGR offspring maintained a significant elevation in MAP, whereas female IUGR offspring had a nonsignificant elevation. Sexual dimorphism is also apparent in the protein restriction model of fetal undernutrition as male offspring of modest protein-restricted dams are hypertensive, whereas both male and female offspring are hypertensive under conditions of more severe protein restriction during gestation.

The kidneys are known to play a major role in the long-term regulation of arterial pressure through pressure natriuresis, whereby changes in renal perfusion pressure lead to alterations in sodium and water balance. Support for a key role of the kidneys in the association of fetal undernutrition and the intrauterine programming of hypertension is suggested from studies of fetal undernutrition, induced by maternal protein restriction during pregnancy in the rat, in which importance of the timing of the insult is noted. Specifically, investigators found that protein restriction administered during the first third of gestation only resulted in offspring with normal renal morphology. However, protein restriction encompassing late gestation, during the period of nephrogenesis, resulted in hypertensive offspring that exhibited a significant decrease in glomerular or nephron number and kidney-to-body weight ratio. Alterations in renal structure in the rat, as induced by placental insufficiency, have been noted, as partial ureteric artery ligation initiated at day 17 resulted in a 30% reduction in nephron number together with a significant reduction in kidney weight in 2-week-old growth-restricted offspring. Thus, models of fetal undernutrition in the rat, whether initiated in late gestation by either maternal undernutrition or placental insufficiency, are associated with a deficit in nephron number and reduced kidney weight and, therefore, evidence for impaired fetal renal development.

Fetal undernutrition is identified with 2 patterns of growth restriction. Proportionate or symmetric growth restriction, characteristic of developing countries, is generally indicative of fetal undernutrition present throughout gestation. Disproportionate or asymmetric growth restriction, a pattern more common to the Western world, is suggestive of fetal undernutrition restricted to the last third of gestation. In our model of placental insufficiency, a pattern of asymmetric growth was present in male, but not female, IUGR offspring. Specifically, the brain-to-body weight ratio was significantly increased at 4 weeks in male IUGR offspring, supporting the brain-sparing feature of fetal programming. However, reduced uterine perfusion initiated in late gestation resulted in reduced kidney weight at 4 weeks of age in male IUGR offspring (P<0.05). In addition, at 4 weeks of age, liver and brain weights were also significantly reduced in male IUGR offspring; at 12 weeks of age, brain weight only was markedly reduced. Maternal protein restriction has been shown to result in offspring with impaired gluconeogenesis and glucose handling. As LBW is related to the occurrence of syndrome X, reduced liver size at 4 weeks of age in the male IUGR offspring may also contribute to the development of hypertension in these animals. In addition, as brain weights were reduced at both 4 and 12 weeks of age in the male IUGR offspring, limitations in the intrauterine environment in this model of placental insufficiency may also result in neural fetal programming, leading to the development of hypertension. Specifically, alterations in peripheral sympathetic nervous control could result in impaired renal development, as evidence suggests that the renal nerves play an important role in renal development through stimulation of the renin-angiotensin system. Thus, asymmetric growth restriction resulting from limited growth of selective organs in late gestation, owing to alterations in nutrient supply to rapidly developing organs, may result in improper development and subsequent hypertension.

As the kidneys play an important role in the long-term control of arterial pressure, underlying renal mechanisms may be responsible for mediating the hypertension in IUGR offspring produced in response to reduced uterine perfusion. Mechanisms mediating hypertension in fetal programming may involve a decrease in GFR as a result of decreased nephron number, an increase in tubular reabsorption, or degrees of both. A significant reduction in glomerular number, as induced by gestational maternal protein restriction, is associated with a significant reduction in GFR normalized to kidney weight. However, although GFR and GFR normalized to kidney weight tended to be reduced at 8 weeks of age in IUGR offspring relative to control, placental insufficiency induced by reduced uterine perfusion at day 14 of gestation was not associated with significant reductions in either GFR or GFR normalized to kidney weight. In addition, 24-hour determination of sodium excretion did not differ significantly, despite a marked increase in arterial pressure in IUGR offspring. Thus, the normal regulatory systems that control pressure natriuresis may be altered in this model of LBW induced by placental insufficiency. Indeed, preliminary studies in our laboratory indicate that the pressure natriuresis relationship is altered in the hypertensive IUGR offspring. Many known regulatory mechanisms control sodium balance, and any alterations in natriuretic factors, such as NO, or antinatriuretic factors, such as the renal sympathetic nervous system and angiotensin II, in addition to alterations in physical factors, can result in abnormal pressure natriuresis, thus leading to hypertension. Further studies will be necessary to delineate the role of these regulatory systems in mediating the altered renal handling of sodium in the hypertensive IUGR offspring produced in response to placental insufficiency in the pregnant rat.

Perspectives
Fetal malnutrition, owing to an insufficient nutrient delivery via reduced uteroplacental perfusion or maternal undernutrition, limits fetal growth and results in an increased risk for development of hypertension and cardiovascular disease. The present study provides further support for an inverse relationship between birth weight and hypertension, as placental insufficiency initiated at mid-to-late gestation in the pregnant rat resulted in growth-restricted offspring predisposed to the development of hypertension. However, the marked increases...
in arterial pressure observed in growth-restricted offspring were not associated with significant decreases in GFR or GFR normalized to kidney weight. Thus, in this model of IUGR and hypertension, although marked elevations in arterial pressure may be mediated in part by a reduction in GFR, inappropriate tubular sodium reabsorption may also play an important role. The control of renal sodium excretion is multifactorial and involves humoral, neural, and physical mechanisms. Thus, regulatory systems such as the renin-angiotensin system, the renal sympathetic nervous system, or alterations in expression of renal sodium transporters may contribute to reduced pressure natriuresis and subsequent hypertension. Therefore, this model of IUGR will allow for examination of the underlying mechanisms that may be responsible for mediating the hypertension induced by in utero programming owing to placental insufficiency and resulting in fetal undernutrition constrained to late gestation.

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