Reduced Uteroplacental Blood Flow Alters Renal Arterial Reactivity and Glomerular Properties in the Rat Offspring

Marijke W. Sanders, Gregorio E. Fazzi, Ger M.J. Janssen, Peter W. de Leeuw, Carlos E. Blanco, Jo G.R. De Mey

Abstract—Fetal malnutrition and hypoxia may modify organ system maturation and result in cardiovascular diseases in the adult. We tested whether intrauterine stress (IUS) leads to persistent alterations of renal biology. In rats, intrauterine stress was induced by ligation of the uterine arteries at day 17 of pregnancy. Renal arteries of the 21-day-old male offspring were isolated to study pharmacological reactivity. Kidneys were dissected to analyze renal structure and \( \beta \)-adrenoceptor expression. At 21 days of age, half of the animals underwent unilateral left nephrectomy. At the age of 12 weeks, rats were instrumented for blood pressure monitoring, blood sampling, and renal function measurements. After IUS, litter size and birth weight were reduced, whereas the hematocrit was increased. Renal arterial responses to \( \beta \)-adrenoceptor stimulation and sensitivity to adenylyl cyclase activation were increased, along with the renal expression of \( \beta_2 \)-adrenoceptors. At 21 days and at 6 months of age, the number and density of the glomeruli were reduced, whereas their size was increased. The filtration fraction and urinary albumin concentration were increased 12 weeks after intrauterine stress. In control rats, removal of the left kidney at 21 days of age did not affect kidney function and blood pressure. However, after IUS, the remaining right kidney failed to compensate for the loss of the left kidney, and blood pressure was increased. In conclusion, prenatal stress transiently modifies renal arterial reactivity and results in long-lasting adverse effects on renal structure and function and on renal compensatory mechanisms. (Hypertension. 2004;43:1283-1289.)

Key Words: hypertension ■ kidney ■ glomerular filtration rate ■ nephrectomy ■ pregnancy ■ rats ■ renal artery receptors, adrenergic beta

Cardiovascular, endocrine and metabolic diseases can emerge as a consequence of intrauterine stress (IUS). A suboptimal fetal environment is often the result of placental insufficiency, which leads to an inadequate delivery of nutrients and oxygen to the fetus. Many animal models have been established that support a link between prenatal conditions and the development of disease in the adult. These include maternal caloric and protein restriction, exposure to hypoxia, and ligation of the uterine arteries to mimic placental insufficiency.

Hypoxia and malnutrition during fetal life were reported to affect the renin-angiotensin system, the hypothalamic-pituitary-adrenal axis, vascular endothelial function and sympathetic innervation, and the development of organs like the kidney. The number of glomeruli can be permanently reduced by influences during fetal life. These structural changes may contribute to renal dysfunction and hypertension later in life. Other aspects of renal organogenesis, such as renal vascular development and particularly adrenoceptor-mediated renovascular responses, might also be influenced by disturbed fetal growth. In the rat, an unfavorable intrauterine environment, induced by uterine artery ligation, modified postnatal renal arterial adrenergic reactivity in a regionally selective manner. This led us to the hypothesis that IUS results in long-lasting alterations of renovascular function and glomerular structure, which may blunt structural and functional compensatory mechanisms in the kidneys and lead to the development of hypertension later in life.

To test this hypothesis, IUS was induced by bilateral ligation of the uterine arteries at day 17 of pregnancy in rats. At 21 days of age, when nephrogenesis is completed and the vasculature displays most of its pharmacological properties, renal structure and arterial reactivity were evaluated. To investigate the long-term consequences of IUS for the adult offspring, the animals were instrumented for measuring glomerular filtration rate, renal blood flow, and blood pressure. Some of the experiments in 12-week-old animals were performed after unilateral nephrectomy at 21 days of age.

Methods

Experiments were approved by the local ethical committee for animal research of the University of Maastricht. Wistar rats had free...
access to pelleted food and tap water and were maintained on a 12-hour light/dark cycle at 21°C.

**Induction IUS**

The induction of IUS was performed as described. Additionally, for identification purposes, a toe was amputated from the offspring at birth and blood was collected with microhematocrit tubes (9 μL; Modulohm, Herlev, Denmark) to measure hematocrit values (7 minutes in hematocrit centrifuge). Immediately after this, litter size of the control group was reduced to match that of the IUS group. Not more than 2 litters from 1 mother were used in each experimental group. Because long-lasting consequences of the fetal environment might be influenced by gender, the present study was restricted to male offspring. The timing of experimental procedures is summarized in Figure 1.

**Arterial Reactivity**

Left and right segments of the common renal arteries were isolated from the 21-day-old male offspring of both groups and were studied in myographs as described by Sanders et al.

**Morphometric Analysis of the Arteries and Their Adrenergic Nerve Fibers**

Structural properties of renal arteries were determined according to the morphometric methods described by Stassen et al. Glyoxylic acid was used to visualize and quantify periarterial adrenergic nerve fibers in whole mount preparations.

**β-Adrenoceptor mRNA Expression**

Kidneys were rapidly removed and snap-frozen in liquid nitrogen and stored at -80°C until further processing. The determination of gene expression of β-adrenoceptor subtypes was performed as described by Fujinaga and Scott. Alterations in gene expression were determined by changes in the ratio of the intensity of the genes encoding β-adrenoceptor subtypes and the intensity of the housekeeping gene GAPDH.

**Morphometric Analysis of the Kidneys**

Kidneys of 21-day-old and 6-month-old rats were dissected and embedded in paraffin. Parallel transversal sections were stained with Jones methenamine silver. Glomerular properties were measured and the number of glomeruli within a known volume was calculated according to the formula: \( n = G/F \times A \times (D + T) \).

**Unilateral Nephrectomy**

Previous observations have shown that right renal arteries of young rats displayed an increased β-adrenergic–mediated arterial dilatation after surviving uterine stress, compared with control rats. For this reason, rats were anesthetized (ketamine, 40 mg/kg IP and xylazine 3 mg/kg SC) and subjected to left-sided nephrectomy via a paravertebral approach to investigate whether the remaining right kidney could compensate for the loss of the other one.

**Instrumentation and Renal Hemodynamics**

At 12 weeks old, rats were anesthetized with ketamine (40 mg/kg IP) and xylazine (3 mg/kg SC) and instrumented for blood pressure monitoring and the measurement of inulin (inutest; Laesovas Ge- sellschaft, Linz, Austria) and para-aminohippurate (PAH) (MSD West Point, Pa) clearances to evaluate renal hemodynamic function in conscious unrestrained animals. The procedure of instrumentation and the clearance techniques were performed as described by Fischer et al.

**Plasma Creatinine**

Plasma creatinine concentrations were measured using the standard Jaffé technique on a Hitack 747 analyzer (Boeringer Mannheim, Mannheim, Germany).

**Albumin Assay**

Rats were kept in metabolic cages for 24 hours. Urine was collected and kept at −20°C until further processing. Urine samples were centrifuged (1500 rpm, 10 minutes) and diluted in distilled water. Albumin concentrations were measured with the rat albumin enzyme immunoassay obtained from SPI-BIO (Massy Cedex, France).

**Data Analysis**

Concentration response curves were analyzed in terms of sensitivity (\( pD_2 = -\log EC_{50} \)) and maximal response by fitting individual concentration–response data to a sigmoid regression curve and interpolation (Graphpad Prism version 2.01; Graphpad Software Inc).

**Results**

**At Birth**

Bilateral ligation of the uterine arteries at day 17 of pregnancy resulted in significantly reduced birth weights (5.03±0.05 grams versus 5.35±0.04 grams, \( n = 137, n = 204, P < 0.001 \)) and litter size (5±1 versus 11±1, \( n = 22, n = 16, P < 0.001 \)). Hematocrit at birth was increased compared with control animals (0.44±0.01 versus 0.39±0.01, \( n = 77, n = 41, P < 0.001 \)).

**At 21 Days of Age**

At the age of 21 days, the differences in body weight were no longer present and IUS had not led to variations in organ weights, including kidney weight. However, both kidneys displayed an important decrease in glomerular density.

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**Figure 1.** Timing of experimental procedures.
(−31%) as a result of the prenatal insult. Glomerular area, circumference, and diameter were significantly increased (Table 1), suggesting that IUS induced glomerular hypertrophy. Morphometrical studies of the kidney showed similar results in left and right kidneys. Table 1 shows only the results observed in right kidneys for comparison with the remnant right kidney of nephrectomized animals at a later stage of life.

When the animals were 21 days old, renal arteries of the IUS group displayed an asymmetrically altered response to adrenoceptor stimulation. Relaxing responses to β-adrenoceptor stimulation with isoproterenol in left renal arteries were not affected by IUS (Figure 2A). However, responses and sensitivity of right renal arteries to isoproterenol were significantly increased as a result of IUS (Figure 2B; pD2, 7.02±0.14 versus 6.62±0.08, P<0.026). Experiments with forskolin showed that the augmented responses of the right renal artery to isoproterenol were accompanied by an increased sensitivity to direct activation of adenylyl cyclase (Figure 2C; pD2, 7.02±0.14 versus 6.62±0.08, P<0.026). Experiments with forskolin showed that the augmented responses of the right renal artery to isoproterenol were accompanied by an increased sensitivity to direct activation of adenylyl cyclase (Figure 2D; pD2, 6.37±0.19 versus 5.78±0.07, P<0.030). This was not observed in the left renal artery (Figure 2C). Compared with control, β2-adrenoceptor mRNA expression was significantly increased in the right kidneys after IUS (Table 2). However, IUS did not affect the expression of β2-adrenoceptors in the left kidneys, nor did it change the expression of β1-adrenoceptors.

Maximal contractile responses to a mixture of 125 mmol/L K+, 10 μmol/L phenylephrine, and 10 μmol/L 5-hydroxytryptamine (Tmax) and the media cross-sectional area (MCSA) were not significantly altered after IUS in left (Tmax, 4.01±0.44 versus 2.84±0.19 N/m, not significant [NS]; MCSA, 20.3±1.3 versus 17.2±1.2×103 μm2, NS) as well as right (Tmax, 3.65±0.45 versus 3.05±0.56 N/m, NS; MCSA, 23.7±2.0 versus 19.0±0.8×103 μm2, NS) renal artery segments.

IUS did not affect the contractile responses of renal arteries to stimulation of α1-adrenoceptors with phenylephrine (Figure 3A) and to sympathetic nerve stimulation (Figure 3B). With the use of glyoxylic acid, it could be observed that sympathetic nerve fiber density1 was larger in left renal arteries than in right renal arteries, but2 not modified at 21 days of age after IUS (Figure 4).

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**Table 1. Morphological Properties of Kidneys of Young and Adult Rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CON</th>
<th>IUS</th>
<th>CON-UNX</th>
<th>IUS-UNX</th>
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<tr>
<td>21 Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>0.27±0.01</td>
<td>0.27±0.03</td>
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<td>Glomerular</td>
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<td></td>
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<tr>
<td>Density (μm²)</td>
<td>522±41</td>
<td>360±29*</td>
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<td></td>
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<tr>
<td>Area (μm²)</td>
<td>2125±75</td>
<td>2645±130*</td>
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<tr>
<td>Circumference (μm)</td>
<td>176±3</td>
<td>196±5*</td>
<td></td>
<td></td>
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<tr>
<td>Diameter (μm)</td>
<td>56.1±1.0</td>
<td>62.3±1.5*</td>
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<tr>
<td>Adult (6 mo)</td>
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<tr>
<td>Kidney weight (g)</td>
<td>1.69±0.09</td>
<td>1.93±0.1</td>
<td>2.90±0.12</td>
<td>2.97±0.17</td>
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<tr>
<td>Glomerular</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density (μm²)</td>
<td>260±16</td>
<td>185±7*</td>
<td>165±11</td>
<td>129±7†</td>
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<tr>
<td>Area (μm²)</td>
<td>8316±268</td>
<td>9602±162*</td>
<td>11066±502</td>
<td>12779±433†</td>
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<td>Circumference (μm)</td>
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<td>370±4*</td>
<td>401±9</td>
<td>429±8†</td>
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<tr>
<td>Diameter (μm)</td>
<td>109±2</td>
<td>118±1*</td>
<td>128±3</td>
<td>137±3†</td>
</tr>
</tbody>
</table>

CON indicates control rats; CON-UNX, after unilateral nephrectomy at 21 days of age; IUS-UNX, rats that survived intrauterine stress and after unilateral nephrectomy at 21 days of age.

Values are mean±SEM.

*P<0.05, CON vs IUS. †P<0.05, CON-UNX vs IUS-UNX.
At 12 Weeks of Age
At the age of 12 weeks, all renal arteries failed to relax in response to isoproterenol, regardless of side or intervention (data not shown). However, the IUS-induced morphological features of the kidneys had major consequences for renal function at 12 weeks of age. The clearances of PAH (Figure 5A; +27%) and inulin (Figure 5B; +114%) and the filtration fraction (Figure 5C; filtration/perfusion ratio) were significantly increased. However, blood pressure was not affected (Figure 5D). Plasma creatinine concentrations were comparable with control animals, but urinary albumin concentrations were significantly increased, probably as a result of the renal hyperfiltration observed after IUS (Table 3).

After Unilateral Nephrectomy
The renal clearance of PAH and inulin in control animals from which the left kidney had been removed was not significantly different from that of animals with both kidneys (Figure 5). However, when the left kidney was removed from animals that had experienced IUS, the remaining kidney failed to reach the same levels of renal blood flow (PAH) and glomerular filtration rate (inulin) as observed with both kidneys present (Figure 5). Blood pressure was not affected by IUS, but unilateral nephrectomy of the left kidney at 21 days of age caused a significant increase in blood pressure at 12 weeks after birth when compared with IUS alone (Figure 5). Plasma creatinine concentrations were significantly increased in nephrectomized animals after IUS, compared with IUS alone, indicating severe renal insufficiency (Table 3). In IUS animals, nephrectomy did not result in an additional significant increase in urinary albumin concentration.

At 6 Months of Age
The reduced density and increased size of the glomeruli, observed at 21 days of age, persisted throughout adulthood (Figure 6) and after nephrectomy (Table 1). However, despite the increased renal perfusion and filtration, we did not observe any histological signs of glomerular disease or glomerulosclerosis (histochemical staining with hematoxylin and eosin) 6 months after IUS with or without additional unilateral nephrectomy.

Discussion
The present study demonstrates a long-lasting alteration of the β-adrenergic system and a reduced glomerular density in the kidneys after IUS. The structural alterations persist in the adult animal and are accompanied by hyperfiltration and hyperperfusion of the glomeruli and by albuminuria, without changing blood pressure. An additional reduction of renal mass by unilateral nephrectomy increased blood pressure in animals that survived IUS.

IUS was induced by bilateral ligation of the uterine arteries at day 17 of pregnancy. The acutely reduced fetal supply...
of oxygen and nutrients resulted in a significant reduction of fetal survival and birth weight. Because birth weight seems to be only crude evidence for disturbed development during fetal life, we also determined hematocrit values at birth. Hematocrit was increased after IUS, indicating that the pups developed in a hypoxic environment during late fetal life or that the plasma volume was reduced as a result of changes in the water and electrolyte balance.

At the age of 21 days, we demonstrated that IUS resulted in an increased sensitivity and maximal relaxing response to isoproterenol in right renal arteries. The augmented responses of the right renal arteries were accompanied by increased \( \beta_2 \)-adrenoceptor mRNA expressions in right kidneys, implying that an increased number of \( \beta_2 \)-adrenoceptors is involved in the enhanced responses to isoproterenol. However, this only partially explains the elevated \( \beta_2 \)-adrenoceptor–mediated reactivity after IUS. Isoproterenol acts through \( \beta_2 \)-adrenoceptors to stimulate adenyl cyclase. During a period of intrauterine stress, humoral and neurogenic catecholamines might activate \( \beta_2 \)-adrenoceptor–mediated responses and redirect the cardiac output to tissues that are more vulnerable to hypoxia. Repeated administration of \( \beta_2 \)-adrenergic agonists has been shown to decrease neonatal G\,i expression and to enhance G\,s function. Direct stimulation of adenyl cyclase showed an increased sensitivity to forskolin, again solely in right renal arteries. These enhanced responses to forskolin suggest that adaptations along the signal transduction pathway contribute to the increased \( \beta_2 \)-adrenergic renal arterial responses. It has been shown that repeated treatment with isoproterenol increased the number of adenyl cyclase molecules during development and caused a shift from a Mn\,2\,+–stimulated adenyl cyclase activity to a forskolin-stimulated adenyl cyclase activity. These findings suggest that an increased release of norepinephrine during IUS throughout late gestation, as was reported for sheep, might continuously activate \( \beta_2 \)-adrenoceptors and alter postnatal development of \( \beta_2 \)-adrenoceptor regulation. However, these IUS-induced changes of \( \beta_2 \)-adrenoceptor mediated reactivity in the renal...
artery did not persist throughout adulthood. Why a negative fetal environment only affects the β-adrenergocceptor-mediated responsiveness of the right renal artery is not clear. Yet, it might have important consequence for the development of cardiovascular disease, because van Onna et al reported an asymmetry of renal blood flow in patients with moderate to severe hypertension.

Sympathetic hyperinnervation and vascular smooth muscle hyperplasia may be involved in the development of hypertension in experimental animals and possibly humans. Previous studies from our own group in the chicken embryo demonstrated that chronic moderate hypoxia results in a sympathetic hyperinnervation of the arterial system. Additionally, in the rat renal vasculature, α1-adrenoceptors mediate the action of sympathetic stimulation and an increased renal vascular α1-adrenergic responsiveness is associated with hypertension in adult spontaneously hypertensive rats. Therefore, we evaluated the effects of IUS for the α1-adrenoceptor and sympathetic nerve-mediated responses in renal arteries. Our results presented no IUS-induced differences in response to phenylephrine. Although the rats in this study developed in a hypoxic fetal environment as indicated by the high hematocrit percentages, fluorescent histochemical staining showed that renal arteries were barely innervated by catecholamine-containing nerves and their densities were not persistently altered as a result of IUS. Maximal contractile responses to nerve stimulation did not significantly differ between renal arteries of rats that survived IUS and control. Although chronic moderate hypoxia results in sympathetic hyperinnervation in the chicken embryo, the model of placental insufficiency used in our experiments involves an acute reduced fetal supply of oxygen and nutrients. The combination of both stimuli might have different consequences for vascular development than the separate interventions. Moreover, the innervation of rat arteries develops, unlike in the chicken embryo, during the second and third postnatal weeks. The intrauterine intervention occurred before the appearance of the sympathetic innervation and therefore might not affect nerve densities of renal arteries. Alternatively, after 21 days of postnatal life, arterial innervation might have recovered from IUS-induced changes of catecholamine-containing nerves.

IUS did not lead to differences in body weight or organ weights at the age of 21 days. To reach the same body weight as control animals after 21 days, the IUS animals, which had a reduced birth weight, must have had an accelerated weight gain during this early postnatal period. Unlike in humans, nephrogenesis in rat kidneys continues during the first 2 weeks of postnatal life. Although little is known about the way catch-up growth modifies the effects of low birth weight, it is possible that catch-up growth during this critical period of renal development might have additional consequences for adult renal function and blood pressure.

Morphometric analysis of the kidneys displayed a significant decrease in glomerular density as a result of IUS, whereas glomerular area, circumference, and diameter were significantly increased. These findings suggest that an IUS-induced decrease in glomerular density is a stimulus for the occurrence of glomerular hypertrophy. The reduced glomerular density and the accelerated postnatal growth may result in an excessive demand on the limited number of nephrons. Previous animal and human studies have reported a lower-than-desirable number of functional nephrons as a result of disturbances during fetal growth and described a compensatory increase in glomerular diameter in rats subjected to intrauterine food restriction. It was also postulated that fewer nephrons at birth results in the development of hypertension in the adult and a study in patients with <50% loss of renal mass reported that a higher mean planar glomerular area increases the risk for hypertension when there is only mild loss of renal mass. Even though we found a reduced glomerular density (~31%) and glomerular hypertrophy, these morphological features did not lead to hypertension in the adult rat. A more thorough analysis of blood pressure regulation at a later time point, by, for instance, continuous 24-hour recording of blood pressure, heart rate, and their variabilities may be required to fully appreciate the functional consequences of the structural alterations. However, a strong reduction of renal mass, induced by unilateral nephrectomy at 21 days of age, resulted in a significantly increased blood pressure. A moderate deficiency in glomeruli alone did not result in hypertension, but several lines of observations suggest that individuals with a relatively deficient nephron endowment at birth are predisposed to hypertension when subjected to dietary or other stresses. Yet, systemic hypertension is not required for glomerular capillary hyperfiltration and hypertension. IUS did not affect blood pressure in our experiments, but we did find a significant increase in glomerular filtration rate, renal blood flow, and filtration fraction 12 weeks after IUS, whereas plasma creatinine values were normal. These findings suggest that IUS resulted in glomerular hypertrophy and hyperfiltration to compensate for the decreased glomerular density and to achieve an adequate renal function, as indicated by the normal plasma creatinine concentrations. Urinary albumin concentrations were significantly increased as a result of IUS. The albuminuria is most likely the result of the increased intraglomerular pressure associated with hyperfiltration and predicts an increased risk for cardiovascular disease and renal functional abnormalities.

A further reduction in the number of glomeruli by unilateral nephrectomy at 21 days of age resulted in an increased blood pressure after IUS and renal insufficiency, as indicated by the high plasma creatinine values and the severe albuminuria. Progressive albuminuria and glomerulosclerosis eventually occur in most experimental models of renal disease characterized by glomerular hyperfiltration and hypertension. However, we did not observe any histological signs of glomerular disease in rats that experienced IUS or additional unilateral nephrectomy. The time point of 6 months might be too early to observe visible signs of glomerular deficiencies as a result of kidney dysfunction.

**Perspectives**

Alterations of kidney development by IUS and possibly catch-up growth induced glomerular hemodynamic changes that resulted in glomerular hyperfiltration, an adaptation seen in response to a reduction in functional nephron number, to achieve an adequate renal function. Hyperfiltration alone was not sufficient to induce an increased blood pressure, but
lifestyle factors, such as high salt intake or obesity, coupled with a low nephron number at birth, might make animals, which experienced IUS, more susceptible to hypertension.

Acknowledgments
We thank Dr Paul Schiffrs and Jet Bost for their contributions to this study by their plasma PAH/inulin measurements.

References
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Hypertension. 2004;43:1283-1289; originally published online April 26, 2004; doi: 10.1161/01.HYP.0000127787.85259.1f

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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