Hypertensive Disorders of Pregnancy: Effects on Mother and Baby

Exposure to placental ischemia impairs postpartum maternal renal and cardiac function in rats

Nina D. Paauw,1 Jaap A. Joles,2 Frank T. Spradley,3 Bhavisha Bakrania,3 Zsuzsanna K. Zsengeller,4 Arie Frax,1 Marianne C. Verhaar,2 Joey P. Granger,3 and A. Titia Lely1

1Department of Obstetrics, Wilhelmina Children’s Hospital Birth Center, University Medical Center Utrecht, Utrecht, The Netherlands; 2Department of Nephrology and Hypertension, University Medical Center Utrecht, Utrecht, The Netherlands; 3Department of Physiology, University of Mississippi Medical Center, Jackson, Mississippi; and 4Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts

Submitted 1 December 2016; accepted in final form 12 February 2017

Preeclampsia (PE) is a pregnancy-specific disorder characterized by the development of de novo hypertension and proteinuria and/or other organ disturbances during the second half of pregnancy (33). Reduced uteroplacental perfusion is postulated to be the initiating event of PE, leading to placental ischemia and subsequent release of placental factors into the maternal circulation causing widespread endothelial dysfunction of the maternal vasculature (9). While symptoms of PE disappear soon after delivery of the placenta, these women appear to be at increased risk for future cardiovascular disease. Indeed, formerly preeclamptic women are reported to have a twofold increased risk for long-term cardiovascular disease (CVD) (4) and a 5- to 14-fold increased risk for end stage kidney disease (ESKD) (37, 38, 40).

An increased blood pressure has been reported in the first years after PE (17, 35), and epidemiologic studies suggest that formerly preeclamptic women develop hypertension 6–8 yr earlier compared with women having a history of normotensive pregnancy (11, 32). Cardiovascular disturbances in formerly preeclamptic women include endothelial dysfunction as measured by flow-mediated dilatation (39) and subclinical impairment of ventricular function, especially after early onset PE (<34 wk) (21, 22). So far, studies on glomerular filtration rate (GFR) and albuminuria after PE are sparse and include heterogeneous populations, with conflicting results (2, 20, 29). To what extent renal and cardiac hemodynamic abnormalities persist after PE or gradually develop during later life is unclear.

A current hypothesis is that cardiovascular disturbances observed after PE can be explained by risk factors that are already present before pregnancy (25). However, some human studies suggest that PE itself might contribute to this risk as common risk factors and familial factors could not fully explain the presence of cardiovascular disturbances in formerly preeclamptic women (7, 24, 36). Interpretation of these human studies is difficult due to confounding preexisting risk factors. Experimental models of PE might help us to determine whether exposure to PE affects long-term cardiovascular and renal function. Only a few studies have addressed this question in experimental models of PE. After both lipopolysaccharide (LPS) and soluble Fms-like tyrosine kinase-1 (sFlt-1) induced experimental PE, no changes in postpartum blood pressure were reported in rodents (5, 6, 34). However, rodents exposed to experimental PE were more sensitive to a second cardiovascular hit as compared with formerly normal pregnant rodents (23, 34). A limitation of these studies is that they used extrinsic stimuli to induce experimental PE, an approach not representative of placental ischemia. As yet, only one study investi-
gated postpartum effects of exposure to placental ischemia in rats and reported reduced endothelial-dependent mesenteric artery relaxation at 3 mo postpartum (3).

In this study, we hypothesized that PE, as elicited by placental ischemia in rats, results in increased maternal blood pressure, impaired endothelial function and reduced renal and cardiac function postpartum. We utilized the reduced uterine perfusion pressure (RUPP) model of PE in rats, which is characterized by the development of hypertension, endothelial dysfunction, proteinuria and a reduced GFR during pregnancy initiated by the placental ischemia (1, 9, 16). Rats were randomized to receive either a Sham or RUPP procedure, and 8 wk after delivery, blood pressure, renal and cardiac function and structure, and small artery vasorelaxation were assessed.

**METHODS**

**Experimental animals.** All animal experiments were conducted in accordance with the National Institutes of Health (NIH, Bethesda, MD) Guide for the Care and Use of Laboratory Animals with all animal-use protocols approved by The University of Mississippi Medical Center’s Institutional Animal Care and Use Committee. Timed-pregnant Sprague-Dawley rats were purchased from Harlan Laboratories (barrier no 202A; Indianapolis, IN). Animals were maintained in the animal facility of the University of Mississippi Medical Center at a 12-12 h light-dark cycle. After operative procedures, rats were housed individually for 1 wk. During the postpartum experiments, animals were housed with two to three rats a cage. The rats had ad libitum access to Envigo 2020X (during pregnancy) and 8640 (postpartum) diets and tap water.

**Study protocol.** At gestational day (gd) 14, Sprague-Dawley rats were randomized to two groups: Sham (n = 24) or RUPP (n = 38). The RUPP procedure involved placing silver clips on the lower abdominal aorta and branches of the ovarian arteries to induce placental ischemia (16). Total viable and reabsorbed fetuses were also recorded on gd 14 before clip placement. The Sham procedure involved only opening the abdomen and externalization of the uterus. At day 18 of gestation 24-h urine was collected and at gd 19 a subgroup of animals underwent invasive blood pressure measurement and arterial blood sampling. After delivery, all pups were removed within 12 h after delivery to avoid effects caused by differences in litter size and lactation. Mothers (n = 3) of which we had no evidence that they had given birth were included in the analysis since rats with nonsurviving pups are most probably exposed to high levels of placental ischemic factors and therefore stand a good chance to have a long-term phenotype, even when exposure time to high amounts was shorter, and because we could not be certain surviving pups indeed did not give birth as we checked the rats once in 12 h and fragile pups might have been eaten by the mother. Some of the rats had a follow-up of 8 wk postpartum, at which time we collected 24-h urine samples and measured GFR and cardiac function. On the following day, blood pressure was measured and the rats were euthanized for tissue collection. Third-order mesenteric arteries were used to assess vasorelaxation, as detailed below.

**Mean arterial blood pressure.** On gd 19 (Sham n = 6; RUPP; n = 11) and at week 8 postpartum (Sham n = 11; RUPP n = 16), mean arterial blood pressure (MAP) and heart rates were measured (31). One to two days before the measurement, we inserted catheters under isoflurane anesthesia in the left carotid artery and exposed them at the nape (Butler Schein Animal Health, Dublin, OH). Catheters consisted of V1 tubing attached to V3 tubing (Scientific Commodities, Lake Havasu City, AZ). Approximately 2.5 cm of the V3 end of the catheter was inserted into the carotid. Catheters were filled with sterile heparin-saline (300 mg/ml; Pfizer, New York City, NY) and stoppered with a sterile nail. On the day of measurement, rats were placed in restrainers and catheters were connected to pressure transducers (MLT0699; ADInstruments, Colorado Springs, CO) coupled to a computerized data acquisition system (PowerLab; ADInstruments). Before recording was started, animals acclimatized to restraint for ~1 h. Means of a 30-min recording of blood pressure were used for analysis.

**Glomerular filtration rate.** At 8 wk postpartum, GFR was measured by a noninvasive transcatheter clearance measurement, as developed by Mannheim Pharma & Diagnostics (Mannheim, Germany) (Sham n = 8; RUPP n = 15). One to two days before measurements, jugular catheters were implanted during the same operative time as the carotid surgeries above. On the day of measurement, rats were briefly anesthetized with isoflurane to remove hair from the nape and to extend the jugular catheter and place the USB device and battery at the nape using doubled-sided adhesive tape. The device plus battery was immobilized by a jacket (Kent Scientific, Torrington, CT). Rats recovered from anesthesia for 15 to 20 min followed by a bolus dose of 3 mg/100 g body wt FITC-sinistrin in 0.2 ml sterile irrialtion saline (Baxter Healthcare, Deerfield, IL) via jugular catheter while rats moved freely in their cages. Determination of transdermally measured half time (t1/2) of FITC-sinistrin clearance was performed according to the one compartment model. GFR was calculated in units of milliliters per minute per 100 g [formula: GFR (ml·min⁻¹·100 g body wt⁻¹) = 31.26 (ml/100 g body wt/t1/2(FITC-s)[min]) multiplied by the body weight (27)].

**Cardiac ultrasound.** Echocardiographic data was undertaken by an experienced echographer (B. Bakrania) blinded to the group allocation using a Vevo-770 high-resolution in vivo imaging system and RMV71OB scan head for small rodents (VisualSonics, Toronto, ON, Canada) at 8 wk postpartum in Sham (n = 9) and RUPP (n = 19) rats. For the analysis, rats were anesthetized with ~2% isoflurane administered in 2 l/min O2. During anesthesia, rats were placed on a heating pad (37.5°C) with rectal temperature measured continuously and electrocardiograms and heart rate monitored. A total of four cardiac views were obtained per rat: parasternal long-axis, parasternal short-axis, four-chamber apical, and suprasternal views (focused on the aorta). Body weight indexing was used for cardiac structural and functional parameters. Functional parameters were calculated using the Vevo 770 imaging software.

**Mesenteric artery vasorelaxation.** After euthanasia, third-order mesenteric arteries were collected in 5 ml PSS containing the following (concentration in mmol/l): 118.3 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25 NaHCO₃, and 11.1 dextrose. Within 1 h after collection, the mesenteric arteries were cleaned of perivascular adipose tissue for vascular function studies. In short, vascular rings (1 per rat) of ~2.5 mm in length were mounted on a wire myograph (model 620M; Danish Myo Technology, Aarhus, Denmark) containing 5 ml PSS after which 4 mN of preload was placed on the rings. The integrity of the arterial rings (1 per rat) was tested with a bolus dose of phenylephrine (Phe) to produce vasoconstriction followed by a bolus of acetylcholine (ACh) to produce vasorelaxation. Arterial segments were washed, equilibrated for 15 min, and constricted with Phe (~10 μmol/l), and cumulative concentration-response curves were generated to ACh [1E-18M to 3E7M] (Sigma) and to sodium nitroprusside (SNP) [1E-10 to 3E-5] (Sigma). ACh response curves (Sham: n = 8; RUPP n = 8) and SNP response curves (Sham: n = 6; RUPP n = 9) were generated using the same ring.

**Quantification of sFlt-1 and albuminuria.** ELISA kits were used to measure plasma sFlt-1 (MVR100 R&D Systems with rat sFlt-1 cross reactivity) at gd 19 (Sham n = 9; RUPP n = 4) and 8 wk postpartum rats (Sham n = 12; RUPP n = 21). Urinary albumin concentration (mg/ml) was determined using the Exocell Neprhat kit (Philadelphia, PA) and multiplied by the total amount (ml) of urine excreted during 24 h at gd 19 and 8 wk postpartum.

**Histology.** Harvested kidneys and hearts were fixed in 10% formalin, embedded in paraffin, and sectioned. Kidney sections were stained with Periodic acid–Schiff and scored for glomerular and tubular-interstitial damage (14). To measure glomerular area, ×40 original magnification.
magnification images were acquired of 25 glomeruli. The surface of each glomerulus was outlined and total area in pixels was measured with ImageJ (NIH). Glomerular endothelial cells were stained with JG12 (mouse anti-JG12, BMS1104, 1:200; Bender Medsystems, Vienna, Austria). The endothelial (JG12) area in 25 glomeruli per kidney was determined using Adobe Photoshop CS5 Extended, version 12.0 (Adobe Systems, San Jose, CA). The JG12 area was corrected for glomerular area. Cardiac sections were stained with Sirius Red (SR) for collagen I and III content. Photographs were taken of 20 fields using a polarization filter, and percentage area fibrosis was calculated. Histological analysis of SR-stained sections was performed using Adobe Photoshop (Adobe Systems) and ImageJ (NIH). A technician blinded to the group allocation scored all the histology.

**RESULTS**

**Pregnancy characteristics.** The pregnancy characteristics of the Sham and RUPP rats are presented in Table 1. The mean weight gain of the RUPP rats during pregnancy was significantly lower compared with the Sham rats indicating successful RUPP procedure. Placental ischemia elicited a PE-like maternal phenotype with high blood pressure, albuminuria, and increased plasma sFlt-1 (Fig. 1, A–C). Litter size was significantly reduced but mean weight of the pups at birth was not affected by the RUPP procedure.

**Postpartum blood pressure and GFR after placental ischemia.** At 8 wk postpartum, RUPP rats showed a significant reduction in GFR compared with Sham (Fig. 2A). No differences in mean arterial blood pressure were observed between groups (Fig. 1D). Furthermore, both systolic and diastolic
blood pressures were comparable between groups (systolic blood pressure: Sham 142 ± 11 vs. RUPP 143 ± 12 mmHg and diastolic blood pressure: Sham 110 ± 12 vs. RUPP 111 ± 12 mmHg) and heart rate did not significantly differ between groups during the blood pressure recordings (Sham 376 ± 27 vs. RUPP 406 ± 40 beats/min). Albuminuria was not different between groups (Fig. 1E) and plasma sFlt-1 had returned to normal values at 8 wk postpartum (Fig. 1F). RUPP and Sham rats had comparable body weights at termination (Sham 270 ± 13 vs. RUPP 266 ± 14 g).

Postpartum cardiac function and structure after placental ischemia. Cardiac echography revealed that RUPP rats had a significantly lower left ventricular ejection fraction at 8 wk postpartum compared with Sham rats (Fig. 2B). In line with this, we observed a trend toward a reduced fractional shortening (Fig. 2C). Structural features, cardiac output, and diastolic function parameters did not differ between groups (Table 2).

Postpartum mesenteric artery vasorelaxation after placental ischemia. Figure 3 shows the relaxation curves of the third-order mesenteric arteries in response to ACh (Fig. 3A) and SNP (Fig. 3B) in the Sham and RUPP rats at 8 wk postpartum. There were no significant differences at any of the concentrations for endothelial-dependent (ACh) or -independent (SNP) vasorelaxation between the groups (two-way ANOVA; ACh P = 0.254; SNP P = 0.758), although we did observe a trend toward a reduction in endothelial dependent vasorelaxation. Neither the AUC and logEC50 of the ACh relaxation curves (AUC: Sham 250 ± 31 vs. RUPP 291 ± 18; P = 0.274 and logEC50: Sham 7.5 ± 0.3 vs. RUPP 6.9 ± 0.14 –log [Ach, M]; P = 0.130) nor the AUC and logEC50 of the SNP relaxation curves (AUC: Sham 289 ± 24 vs. RUPP 281 ± 22 –log [SNP, M]; P = 0.803 and logEC50: Sham 7.3 ± 0.2 vs. RUPP 7.37 ± 0.2; P = 0.825) differed between the groups.

Postpartum morphology of kidneys and hearts after placental ischemia. At 8 wk postpartum no differences in kidney weight were observed between groups (Table 3). At this time point histological evaluation of the kidney also did not reveal any differences in glomerular and tubular-interstitial damage. In addition, mean glomerular area and the percentage of JG12 positive area within the glomerulus were similar. No fibrosis in the heart was visible in any rat after staining with SR.

**DISCUSSION**

In this study, exposure to placental ischemia elicited by the RUPP was accompanied by alterations in cardiac and renal function in the postpartum period characterized by a reduced GFR and reduced left ventricular ejection at 8 wk postpartum. The observed changes could not be explained by concurrent increases in blood pressure, vascular reactivity, and histological damage.

Table 2. In vivo structural and functional cardiac parameters measured by echocardiography in Sham and RUPP rats at 8 wk postpartum

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham (n = 9)*</th>
<th>RUPP (n = 19)*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight, g</td>
<td>0.85 ± 0.07</td>
<td>0.89 ± 0.07</td>
<td>0.185</td>
</tr>
<tr>
<td><strong>Structural</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>789 ± 166</td>
<td>812 ± 141</td>
<td>0.707</td>
</tr>
<tr>
<td>LV mass/body weight, mg/g</td>
<td>2.9 ± 0.7</td>
<td>3.0 ± 0.5</td>
<td>0.646</td>
</tr>
<tr>
<td>LVPWd, mm</td>
<td>1.7 ± 0.5</td>
<td>1.7 ± 0.4</td>
<td>0.886</td>
</tr>
<tr>
<td>LVPWs, mm</td>
<td>2.6 ± 0.6</td>
<td>2.3 ± 0.5</td>
<td>0.274</td>
</tr>
<tr>
<td>LVAWd, mm</td>
<td>1.5 ± 0.3</td>
<td>1.6 ± 0.3</td>
<td>0.408</td>
</tr>
<tr>
<td>LVAWs, mm</td>
<td>2.5 ± 0.2</td>
<td>2.5 ± 0.3</td>
<td>0.741</td>
</tr>
<tr>
<td>LVDDd, mm</td>
<td>7.25 ± 0.64</td>
<td>7.12 ± 0.61</td>
<td>0.624</td>
</tr>
<tr>
<td>LVDDs, mm</td>
<td>4.34 ± 0.86</td>
<td>4.63 ± 0.54</td>
<td>0.278</td>
</tr>
<tr>
<td>LV VOLDd, μl</td>
<td>278.8 ± 51.9</td>
<td>273.6 ± 36.7</td>
<td>0.761</td>
</tr>
<tr>
<td>LV VOLSs, μl</td>
<td>89.3 ± 38.3</td>
<td>100.9 ± 26.0</td>
<td>0.355</td>
</tr>
<tr>
<td><strong>Functional</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>337 ± 19</td>
<td>342 ± 23</td>
<td>0.613</td>
</tr>
<tr>
<td>CO, ml</td>
<td>63.7 ± 8.9</td>
<td>59.1 ± 11.1</td>
<td>0.300</td>
</tr>
<tr>
<td>Mean aortic velocity, mm/s</td>
<td>743 ± 167</td>
<td>982 ± 364</td>
<td>0.115</td>
</tr>
<tr>
<td>Peak aortic velocity, mm/s</td>
<td>1338 ± 332</td>
<td>1735 ± 593</td>
<td>0.115</td>
</tr>
<tr>
<td>Peak aortic gradient</td>
<td>7.6 ± 3.6</td>
<td>10.5 ± 5.8</td>
<td>0.235</td>
</tr>
<tr>
<td>MV E, mm/s</td>
<td>904 (741–927)</td>
<td>861 (602–912)</td>
<td>0.667</td>
</tr>
<tr>
<td>MV A, mm/s</td>
<td>659 (604–807)</td>
<td>653 (508–737)</td>
<td>1.000</td>
</tr>
<tr>
<td>MV E/A</td>
<td>1.3 ± 0.2</td>
<td>1.3 ± 0.1</td>
<td>0.957</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>19.2 ± 5.3</td>
<td>22.0 ± 6.3</td>
<td>0.318</td>
</tr>
<tr>
<td>IVCT, ms</td>
<td>21.1 ± 7.2</td>
<td>23.9 ± 7.3</td>
<td>0.415</td>
</tr>
</tbody>
</table>

Data are means ± SD or medians (25–75 percentile). LV, left ventricle; LVPWd and LVPWs, left ventricular posterior wall during end diastole and end systole; LVAWd and LVAWs, left ventricular anterior wall during end diastole and end systole; LVDDd and LVDDs, left ventricular inner diameter end diastole and end systole; LV VOLD and LV VOLS, left ventricular volume during diastole and systole HR, heart rate; CO, cardiac output; MV E, peak mitral valve (MV) early filling; MV A, peak MV active filling; MV E/A, MV early-to-active filling ratio; IVRT, isovolumetric relaxation time; IVCT, isovolumetric contraction time. *Total number of rats in each group, all parameters were successful in at least 70% of the animals.
This appears to be the first study to show that the maternal renal and cardiac function in the postpartum period is affected in an animal model for PE, induced by placental ischemia. One study assessed postpartum effects of exposure to placental ischemia in RUPP rats but only reported on vascular function showing reduced endothelial dependent mesenteric artery relaxation at 3 mo postpartum (3). Other studies addressing the question of post-PE cardiovascular function mainly looked at blood pressure and proteinuria and were performed in models for experimental PE based on the administration of very high levels of single molecules that are associated with the development of PE. These models have the limitation that they only mimic some of the consequences of the placental ischemia syndrome without placental ischemia, the primary causal event. In rats exposed to LPS-induced PE, no baseline differences were observed in blood pressure and proteinuria at 9 wk postpartum. Similarly, no differences in blood pressure were found 6 mo after sFlt-1-induced PE (6, 34). In line with this, we did not observe a difference in blood pressure postpartum after placental ischemia at 8 wk postpartum in this study. However, our assessment of more sensitive parameters for cardiovascular function revealed slightly compromised function of heart and kidney after exposure to placental ischemia.

The presence of reduced renal and cardiac function 8 wk after exposure to placental ischemia implies that placental factors released during pregnancy can play a role in the development of cardiovascular and renal disease in the long term. In the RUPP model, it was shown that placental ischemia induces several functional and structural abnormalities in heart and kidney during pregnancy including a reduction in GFR by ~40% (1), a reduced cardiac index (28), and fibrosis in the kidney (10). In our study, there was no evidence of histological damage postpartum, which suggests resolution of myocardial fibrosis after discontinuation of exposure to placental ischemia. We could also not identify signs of glomerular damage by assessing the intraglomerular endothelium with JG12 staining (19). Moreover, the absence of differences in glomerular area suggests that at 8 wk postpartum there was no glomerular stress or loss of nephrons (8, 12). Therefore, the functional alterations might be either the result a slower recovery of function compared with the histological changes or ongoing disturbances in regulatory systems. Slow recovery of some initial disturbances observed after PE is reported in humans, such as for proteinuria which resolves in almost all patients within 2-yr time period (2). On the other hand, the report of Brennan et al. (3) on vascular dysfunction after RUPP pregnancy suggests ongoing or even permanent target organ damage and/or dysregulation as they show that reduced mesenteric artery relaxation at 3 mo, but not 1 mo postpartum following RUPP pregnancy.

Functional disturbances that might lead to ongoing impairment of postpartum cardiovascular and renal function after placental ischemia derive from human are increased sensitivity of renin angiotensin-aldosterone system (RAAS) and increased sensitivity of blood pressure to salt (13, 15, 18, 26, 30), but this has not yet been investigated in this animal model. Previous animal studies do suggest that the risk of cardiovascular dysfunction after PE might mainly result from enhanced responsiveness to a second hit administered after apparent resolution of PE. Pruthi et al. (23) showed increased smooth muscle cell proliferation and increased fibrosis after carotid damage in the sFlt-1 mouse model, and van de Graaf et al. (34) showed an increased hypertensive response after angiotensin II infusion in the pregnant LPS rat model. Future studies should elucidate whether there are specific factors that aggravate the observed target organ dysfunction after placental ischemia as this might uncover important targets for secondary prevention to reduce the cardiovascular burden in formerly preeclamptic women. In addition, aging studies are required to reveal whether the observed mild disturbances in cardiac and renal function lead to clinical features of hypertension and cardiovascular disease in the long term.

While the strength of this study is the observation of subclinical damage after placental ischemia in an animal model without confounding by preexisting factors, the use of the RUPP model in healthy rats can also be viewed as a limitation. First, PE might elicit different long-term sequelae in response to placental ischemia/PE in subjects with a predisposition for cardiovascular disease compared with the healthy baseline of SD rats used for the RUPP procedure (25). Additionally, in RUPP rats the clips remain in situ during the postpartum

Table 3. Macroscopic and microscopic features of kidney in Sham and RUPP rats at 8 wk postpartum

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 12)</th>
<th>RUPP (n = 21)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left kidney weight, g</td>
<td>0.72 ± 0.08</td>
<td>0.70 ± 0.04</td>
<td>0.443</td>
</tr>
<tr>
<td>Right kidney weight, g</td>
<td>0.73 ± 0.07</td>
<td>0.75 ± 0.06</td>
<td>0.526</td>
</tr>
<tr>
<td>Glomerular area, pixels × 10⁷</td>
<td>243 ± 27</td>
<td>241 ± 28</td>
<td>0.885</td>
</tr>
<tr>
<td>Glomerular damage, %glomeruli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>77.1 ± 7.5</td>
<td>77.1 ± 6.3</td>
<td>0.921</td>
</tr>
<tr>
<td>Partial</td>
<td>22.7 ± 7.4</td>
<td>22.1 ± 6.5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.2 ± 0.6</td>
<td>0.2 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Tubulo-interstitial infiltrate score</td>
<td>0.03 (0.00-0.06)</td>
<td>0.00 (0.00-0.06)</td>
<td>0.765</td>
</tr>
<tr>
<td>Tubulo-interstitial fibrosis score</td>
<td>0.11 (0.05-0.15)</td>
<td>0.10 (0.00-0.17)</td>
<td>0.765</td>
</tr>
<tr>
<td>Tubulo-interstitial atrophy score</td>
<td>0.06 (0.00-0.12)</td>
<td>0.06 (0.00-0.10)</td>
<td>0.866</td>
</tr>
<tr>
<td>JG12+, %glomerular area</td>
<td>23 ± 4</td>
<td>25 ± 7</td>
<td>0.518</td>
</tr>
</tbody>
</table>

Data are means ± SD or medians (25–75 percentile).
period, which theoretically could influence long-term cardiovascular function. The persistent effect of clips is unlikely to be an issue because of the limited flow postpartum through the ovarian artery. Furthermore, it is unlikely that aorta flow is increased in view of the stable maternal weight. Finally, the aorta clip is not likely to influence GFR and blood pressure values since the clip is placed on the lower abdominal aorta (caudal to the kidney arteries) and previous studies showed that in the nonpregnant state an abdominal clip of this size did not influence blood pressure (1). Another limitation of this study is that we cannot predict whether decreased GFR and ejection fraction, which were measured once in a terminal setting, will indeed contribute to the increased risk for cardiovascular and renal disease in later life. In general, it remains to be investigated whether a finding in an animal model for PE reflects the human situation.

**Perspectives and Significance**

In summary, we show alterations in heart and kidney function in the postpartum period after exposure to placental ischemia in an animal model for PE. These alterations occurred in the absence of differences in blood pressure, vascular reactivity, and histological damage. Our results suggest that exposure to placental ischemia during pregnancy affects the long-term cardiovascular health status of the mother.

**ACKNOWLEDGMENTS**

We thank M. Arany, K. Cockrell, K. den Ouden, R. C. Kleisen, and E. van Veen for technical support during the experiments.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the American Heart Association.

**GRANTS**

Research reported in this publication was supported by the Dutch Kidney Foundation (150KK65 to NDP and KJPB 11.026 to A. T. Lely); National Institute of General Medical Sciences Grant P20-GM-104357; National Heart, Lung, and Blood Institute Grants P01-HL-051971, R01-HL-108618, and HL36279; and American Heart Association Grant AHA-13POST16240000.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


