Long-Term Alteration in Maternal Blood Pressure and Renal Function After Pregnancy in Normal and Growth-Restricted Rats

Linda A. Gallo, Kate M. Denton, Karen M. Moritz, Marianne Tare, Helena C. Parkington, Meagan Davies, Melanie Tran, Andrew J. Jefferies, Mary E. Wlodek

Abstract—Intrauterine growth restriction is associated with increased risk of adult cardiorenal diseases. Small birth weight females are more likely to experience complications during their own pregnancy, including pregnancy-induced hypertension, preeclampsia, and gestational diabetes. We determined whether the physiological demand of pregnancy predisposes growth-restricted females to cardiovascular and renal dysfunction later in life. Late gestation bilateral uterine vessel ligation was performed in Wistar-Kyoto rats. At 4 months, restricted and control female offspring were mated with normal males and delivered naturally (ex-pregnant). Regardless of maternal birth weight, at 13 months, ex-pregnant females developed elevated mean arterial pressure (indwelling tail-artery catheter; +6 mm Hg), reduced effective renal blood flow (\(^{14}\text{C}-\text{PAH}\) clearance; \(-23\%)\)), and increased renal vascular resistance (\(+27\%)\) compared with age-matched virgins. Glomerular filtration rate (\(\text{H}^{-}\)-inulin clearance) was not different across groups. This adverse cardiorenal phenotype in ex-pregnant females was associated with elevated systemic (\(+57\%)\)) and altered intrarenal components of the renin-angiotensin system. After pregnancy at 13 months, coronary flow (Langendorff preparation) was halved in restricted females compared with controls, and together with reduced NO excretion, this may increase susceptibility to additional lifestyle challenges. Our results have implications for aging females who have been pregnant, suggesting long-term cardiovascular and renal alterations, with additional consequences for females who were small at birth. (Hypertension. 2012;60:00-00.) * Online Data Supplement

Key Words: pregnancy * growth restriction * maternal health * blood pressure * kidney function * Langendorff heart preparation

Uteroplacental insufficiency is the most common cause of intrauterine growth restriction and affects 7\% to 10\% of pregnancies in the Western world.\(^{1}\) Human studies worldwide and animal models have shown that suboptimal conditions in utero alter the development of key organ systems, including reductions in nephron and cardiomyocyte number.\(^{2,3,6,7}\) We and others have used a rat model to induce uteroplacental insufficiency, whereby uterine vessels are bilaterally ligated during late gestation resulting in offspring born lighter than sham controls.\(^{2,3,6,7}\) Although growth-restricted males go on to develop elevated blood pressure in adulthood, females appear somewhat protected up to \(\approx 18\) months, despite both sexes having similar nephron deficits.\(^{2,3,8,9}\) This sexual dimorphic response to disease development is commonly cited in the programming field, with females often presenting with less severe cardiovascular disease outcomes.\(^{10}\)

Disease risk associated with programmed changes in early life may be modulated by exposures after birth and throughout life. It has been suggested that a number of lifestyle factors, including high-salt/fat diets and aging, can increase or unmask disease outcomes in susceptible offspring.\(^{11}\) Low nephron endowment in growth-restricted females, for example, may be adequately compensated for until a postnatal stressor or “second hit” reveals a clinically relevant phenotype.\(^{2}\)

Pregnancy is associated with profound physiological demands that could constitute a second hit.\(^{12}\) In early pregnancy, reductions in peripheral vascular tone contribute to a small decrease in maternal blood pressure, but by late pregnancy, maternal blood volume expands by \(\approx 50\%)\) in humans and 30\% in rats. This low-resistance, hypervolemic state allows for increased uteroplacental blood flow and permits greater blood flow to the maternal kidneys, with glomerular filtration rate (GFR) reaching peak levels at midgestation.\(^{14}\) Associations have been noted between a woman’s own birth weight and her future pregnancy-related health, including pregnancy-induced hypertension, pre-

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From the Department of Physiology (L.A.G., M.Tr., A.J.J., M.E.W.), University of Melbourne, Parkville, Victoria, Australia; Department of Physiology (K.M.D., M.Ta., H.C.P., M.D.), Monash University, Clayton, Victoria, Australia; School of Biomedical Sciences (K.M.M.), University of Queensland, St Lucia, Queensland, Australia.

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Correspondence to Mary E. Wlodek, Department of Physiology, University of Melbourne, Parkville, Victoria 3010, Australia. E-mail m.wlodek@unimelb.edu.au

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eclampsia, and gestational diabetes. This, then, predisposes to adverse outcomes for her offspring, including intrauterine growth restriction and prematurity.

Human studies have also shown that pregnancy-induced hypertension and/or preeclampsia are associated with increased risk for chronic hypertension and subsequent kidney disease later in life. Maternal birth weights are rarely reported in follow-up pregnancy studies, and, thus, the contribution of a previous developmental insult compared with a spontaneous complication in the mother’s own pregnancy is unknown. We have demonstrated recently in a setting of low nephron endowment that 4-month–old growth-restricted female rats developed impaired glucose tolerance, altered sodium handling, and glomerular hypertrophy but remained normotensive during late pregnancy. Increased renal and uterine arterial stiffness, evident in growth-restricted virgin rats, was overcome during pregnancy, highlighting a profound degree of adaptation to match control rats and enhance fetal survival. These rapid, protective arterial adaptations may be transient, because the long-term consequences, postpregnancy, are unknown. We, therefore, consider pregnancy as a challenge that may accelerate and, hence, be a potential predictor of future ill health. The long-term maternal cardiovascular and renal outcomes associated with the aforementioned compensatory changes were a focus of the current study. We hypothesized that growth-restricted females that had been pregnant would develop hypertension and renal and cardiac dysfunction later in life compared with previously pregnant, normal birth weight mothers and growth-restricted virgin female rats.

Methods

Animal Procedures

All of the experiments were approved by the University of Melbourne Animal Ethics Committee before commencement. Wistar Kyoto rats housed in an environmentally controlled room had access to food and tap water ad libitum. Rats were mated and uteroplacental insufficiency (offspring termed “restricted”) or sham (offspring termed “control”) surgery performed at E18. Rats delivered at term (E22) and F1 control and restricted females were allocated to either virgin or ex-pregnant groups (1 per litter per group; n=12 per group). Those allocated to the ex-pregnant group were mated at 17 to 23 weeks of age with a normal male and gave birth naturally. Pups were weaned at day 35 and allocated to other studies. Ex-pregnant mothers and virgins were studied to 13 months, and a vaginal smear was performed postmortem to determine the stage of estrus cycle. Please see the online-only Data Supplement for body and organ weights, corrections for body weight, and absolute fetal weights. F1 litter size (5–6 restricted pups versus 8–9 control pups) at postnatal day 1 (P<0.05; please see Table S1 in the online-only Data Supplement). Restricted females were 32% to 38% lighter compared with controls at postnatal day 1 (P<0.05; Table S1). Regardless of pregnancy group allocation, restricted females remained lighter at all ages, except at 6 and 9 months, where ex-pregnant females only, and not virgins, were smaller than their control counterparts (P<0.05; Table S1). During F1 pregnancy, Restricted females gained 23% less weight than controls (P<0.05), and by 6 months, they were of similar weight to virgin counterparts, whereas controls remained 12% heavier (P<0.05; Table S1). There were no differences in F2 total litter size (9.3±0.3 versus 8.4±0.5) or day 1 body weight (4.58±0.05 versus 4.45±0.08 g) between control and restricted rats. Kidney weight (corrected for body weight) was reduced in restricted females compared with controls (P<0.05), but there were no differences in relative heart weights (Table S1).

Cardiovascular and Renal Function

Systolic blood pressure, measured by tail cuff, was not different between control and restricted groups before mating (4 months) or after pregnancy (Table). There were no differences in systolic blood pressure between virgin and ex-pregnant groups or across time (Table). At 13 months, mean arterial pressure, measured using an indwelling catheter, was elevated in ex-pregnant females compared with virgins, regardless of maternal birth weight (+6 mm Hg; P<0.05; Figure 1A).

Restricted females consumed 12% less food than controls (P<0.05), with no differences in water intake (Table). There were no differences in 24-hour urine output between groups (Table). Urinary sodium and chloride excretion were reduced in ex-pregnant females compared with virgins (P<0.05; Table). In restricted ex-pregnant females, total protein excretion was reduced (−42%) compared with control and virgin counterparts, and NOx excretion, corrected for food intake, was reduced (−40%) compared with virgins (P<0.05; Table). Plasma NOx concentrations, measured after a 24-hour fast were not different between groups (data not shown).

At 13 months, effective renal blood flow (corrected for kidney weight) was reduced by 23% in ex-pregnant females versus virgins (P<0.05; Figure 1B), but there was no difference in GFR (Figure 1C). Filtration fraction tended to be greater in ex-pregnant females, but this did not reach statistical significance (+22%; P=0.14; Figure 1D). Renal vascular resistance was 27% greater in ex-pregnant females compared with virgins (P<0.05; Figure 1E). The sole effect of fetal exposure to uteroaplacental insufficiency on renal function, independent of previous pregnancy effects, was a >2-fold increase in fractional sodium excretion (P<0.05;
Intrarenal and Plasma Measurements

Cortical and medulla angiotensin II content was greater in ex-pregnant females compared with virgins (31% and 68%, respectively), regardless of maternal birth weight (Figure 3A and 3B; P < 0.05). Renin activity was reduced in cortical (Figure 3C) but increased in medulla (Figure 3D) renal tissue in ex-pregnant control females compared with virgin and restricted counterparts (P < 0.05). Plasma renin activity was 57% greater in ex-pregnant females compared with virgins (Figure 3E; P < 0.05).

Plasma estradiol levels were 60% and 14% greater in restricted versus control females, from virgin and ex-pregnant groups, respectively (Table; P < 0.05). There was an even number of animals in proestrous/estrous and metestrous/diestrous within each group at postmortem, and plasma estradiol levels, on average, were similar when the data were split according to the stage of cycle (data not shown).

Discussion

The present study demonstrates that normal and small birth weight female rats, pregnant at 4 months of age, develop elevated blood pressure and reduced effective renal blood flow at 13 months. These findings were associated with increased renal vascular resistance, but GFR remained similar to that of age-matched virgins. Elevated plasma renin activity and renal angiotensin II content were associated with this postpregnancy phenotype in both control and restricted females. The sole effect of growth restriction on renal function, independent of pregnancy, was increased fractional sodium excretion, with plasma sodium remaining normal. Cardiac responsiveness to isoprenaline was suppressed in restricted and ex-pregnant rats. These data provide compelling insights into future health for aging females who have been pregnant previously.

Female offspring exposed to late gestation uteroplacental insufficiency were 32% to 38% smaller from postnatal day 1 and remained smaller throughout, and after, pregnancy. These restricted females also gained 23% less weight during pregnancy, which may be indicative of a blunted plasma volume expansion. Human studies have shown that low maternal
Birth weight was associated with small-for-date infants, attributed to low prepregnancy weight and inadequate gestational weight gain. We have also reported reduced body weight in male and female fetuses from growth restricted mothers exposed to environmental stress, but in the current study F2 birth weight and litter size from restricted mothers were unaffected. These results suggest that low maternal birth and prepregnancy weight and/or pregnancy weight gain, per se, irrespective of environmental factors, do not contribute to transgenerational whole body growth restriction in rat offspring. This may not discount the likelihood for disease transmission, because recent work using maternal protein restriction in rats reported perpetuated cardiorenal phenotypes in the absence of continued growth deficits. Consistent with our previous observation, restricted females consumed 12% less food versus controls (corrected for body weight). This might reflect an adaptive, protective behavior to match nutritional exposures and prevent the development of obesity that is often associated with a low weight at birth.

Regardless of maternal size at birth, females that were pregnant 9 months before developed elevated mean arterial pressure and systolic and diastolic blood pressures compared with age-matched virgins. Although this significant increase is only mild (~6 mm Hg), small increases in blood pressure may predict more severe cardiovascular events if left untreated. A recent study in humans found that long-term maternal blood pressure was ~2 mm Hg lower in parous women. It was suggested that, rather than an effect of pregnancy, per se, human pregnancy may reflect a subset of women with better cardiovascular function. Evidence suggests that women who experienced complicated pregnancies were more likely to develop chronic hypertension in later life. In our previous study, small birth weight females developed altered glucose control and sodium handling during late pregnancy, but controls showed normal adaptations. As such, the adverse cardiorenal phenotype observed in both control and restricted females cannot be explained by a compromised pregnancy 9 months before. The paucity of detailed, experimental knowledge on the long-term cardiovascular consequences after pregnancy implicates this as novel work and provides an impetus for follow-up. Although

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**Figure 1.** Blood pressure and renal function. Data show mean arterial pressure (A), effective renal blood flow (eRBF; B), glomerular filtration rate (GFR; C), filtration fraction (D), renal vascular resistance (E), and fractional sodium excretion (F). Values are expressed as mean±SEM; n=8 to 12 per group.
others have suggested that additional modifiers are necessary to unmask a hypertensive phenotype in susceptible females, we report comparable blood pressure between restricted and control females after a previous pregnancy. Indeed, an adverse diet or obesity superimposed on the physiological challenge of pregnancy may be necessary to reveal exacerbated hypertension in females that were born small.

In isolated hearts, basal left ventricular function and heart rate were not different between control and restricted females, similar to observations in male offspring after intrauterine hypoxia. Although ventricular function was unaltered, basal coronary flow was higher in ex-pregnant controls only compared with virgin counterparts. This may reflect adaptation of the coronary vasculature to pregnancy that is sustained in the long term or a postpregnancy alteration that is absent in restricted females. Whether the reduced coronary flow in ex-pregnant restricted females renders them more vulnerable to coronary events, despite values similar to virgins, awaits determination. Global ischemia did not reveal any differences in the sensitivity of the myocardium to infarct. Some early life insults, such as prenatal hypoxia, are similarly associated with no changes in basal cardiac function, with differences seen only when the hearts are stressed. Compared with virgin controls, the responses evoked by isoprenaline were smaller in hearts from restricted and ex-pregnant rats, and they seemed to be resilient to the effects of ischemia-reperfusion injury. β-Adrenoceptor signaling is reported to be altered in offspring exposed to prenatal protein restriction in a sex-dependent manner; males exhibit the altered cardiovascular phenotype. The underlying mechanisms for reduced

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**Figure 2.** Left ventricular function. Coronary flow (A), isoprenaline-induced change in heart rate (B), +dP/dt (C), −dP/dt (D), and left ventricular myocardial infarct size (E). Values are expressed as mean ± SEM; n = 6 to 10 per group. α P < 0.05 vs control counterpart; β P < 0.05 vs virgin counterpart; γ P < 0.05 vs 2.0 µg/mL within group. PI indicates postischemia. For B through D, ○, virgin control; □, virgin restricted; ●, ex-pregnant control; ■, ex-pregnant restricted.
responsiveness to isoprenaline in the hearts of restricted and ex-pregnant females await elucidation.

Independent of pregnancy effects, the sole effect of growth restriction on renal function was increased fractional sodium excretion in both virgin and ex-pregnant groups. This is consistent with observations in low birth weight infants, but animal studies often show no difference. Underlying mechanisms for this alteration in tubular sodium handling are unclear but suggest sodium loss because of tubular necrosis. Importantly, there were no differences in GFR, a gold-standard measure of renal function, between control and restricted females. During pregnancy at 4 months of age, we recently reported reduced nephron endowment with glomerular hypertrophy in growth-restricted females. Despite this, total protein excretion was reduced in restricted ex-pregnant females compared with both control and virgin counterparts. This is an interesting finding given that glomerular hypertrophy can often predict future glomerular damage and proteinuria. As we suggested previously, increased individual glomerular size during pregnancy is likely to reflect “perfect adaptation” for optimal renal function. The current findings further support this as an acute, protective response during pregnancy that does not progress into the “vicious cycle” of eventual renal damage and systemic hypertension.

Independent of maternal birth weight, ex-pregnant females had reduced urinary sodium and chloride (\(\text{Na}^+ / \text{Cl}^-\)) excretion compared with virgins but no differences in potassium excretion at 12 months of age. Although this might suggest sustained retention of \(\text{Na}^+ / \text{Cl}^-\) after pregnancy, plasma concentrations were unaffected, and systolic blood pressure was normal at this age. There were no differences in food or water intake or urine flow rate that could explain reduced \(\text{Na}^+ / \text{Cl}^-\) excretion after pregnancy, such that the physiological relevance remains unknown. At 13 months, ex-pregnant females had reduced effective renal blood flow and concomitant increase in renal vascular resistance. However, GFR was maintained at the expense of elevated filtration fraction. This suggests that the increased renal resistance and reduced blood flow after pregnancy are predominantly postglomerular effects. Fractional excretion of sodium was not different between virgin and ex-pregnant groups, but this does not rule out alterations.
in total urinary excretions that were not measured at this later age. Alterations in renal hemodynamics and systemic blood pressure at 13 months are contrary to work by Baylis and Renneke, showing no change at 11 months after 5 consecutive gestations in rats. Despite a similar age of study (~1 year), differences between studies may be attributed to a 9-month follow-up in the current study compared with a shorter, 2-month follow-up after the final gestation in the former.

Because systemic blood pressure and renal vascular resistance are elevated in NO-deficient states, we measured urinary and plasma NOx levels. Urinary NOx excretion was reduced in ex-pregnant restricted females compared with virgin counterparts that may explain, at least in part, the postpregnancy blood pressure elevation and altered renal hemodynamics in this group. Lack of reduction in urinary NOx excretion in ex-pregnant controls, together with normal plasma NOx, suggests that other mechanisms may be involved. Although unlikely to alter our interpretation, it is worth noting that fasted plasma and 24-hour excretions were determined at 12 months of age, whereas renal hemodynamic and systemic blood pressure alterations after pregnancy were observed at 13 months.

Plasma renin activity is often used as a marker of the systemic RAS, and high levels have been linked with adverse cardiac and renal outcomes. Intrarenal components of the RAS also play a critical role in blood pressure and sodium regulation. The role of the RAS during pregnancy has been extensively studied, whereas postpregnancy investigations have been limited to the immediate postpartum period of complicated pregnancies. Currently, we demonstrate that elevated blood pressure and reduced effective renal blood flow after pregnancy were associated with a 57% increase in plasma renin activity. Cortical and medulla angiotensin II levels were also elevated in ex-pregnant females, regardless of maternal birth weight. Interestingly, renin activity was reduced in cortical tissue but elevated in medulla (~250-fold lower concentration than cortex) of ex-pregnant controls. The reduction in cortical renin levels may be a compensatory measure for elevated systemic renin, albeit not present in restricted females.

Alterations in plasma estradiol levels were not associated with postpregnancy cardiorenal effects. Levels were increased in virgin and ex-pregnant restricted offspring. This might suggest a compensatory response to reduced estradiol sensitivity, preventing the development of hypertension and renal dysfunction in restricted females. Others have shown previously that RAS blockade protects against ovariectomy-induced hypertension in postpubertal growth-restricted rat offspring. The cardiorenal protective effects of estrogen involve the angiotensin-converting enzyme/angiotensin-converting enzyme 2 and angiotensin II type 1/angiotensin II type 2 receptor pathways. Ojeda et al showed elevated renal angiotensin-converting enzyme 2 mRNA expression in growth-restricted female rats, and ovariectomy resulted in decreased expression in growth-restricted but not control offspring. These data, together with the present findings, highlight the complexity of pathways involved in RAS regulation between growth-restricted and control offspring, providing an impetus for future investigation.

**Perspectives**

The current results have implications for all aging females who have been pregnant, suggesting consequences for cardiovascular and renal health. Our data indicate that a normal, healthy pregnancy can induce long-term alterations in cardiovascular and renal function later in life that are absent in nonparous females. Further investigation into components of the RAS may help tailor therapeutic strategies to minimize the contribution of a previous pregnancy to later life hypertension and renal dysfunction in our aging population. Our findings indicate that low maternal birth weight was not associated with overt cardiorenal alterations after pregnancy versus normal birth weight controls. However, reduced coronary flow and renal NOx excretion and alterations in intrarenal renin activity may predispose restricted females to increased disease risk, particularly in settings of additional lifestyle challenges.

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**Disclosures**

None.

**References**


Pregnancy results in a long-term increase in blood pressure and reduced renal blood flow that might be mediated by changes in RAS activity. Maternal low birth weight does not exacerbate this phenotype but is associated with alterations in renal excretion, intrarenal RAS, and coronary flow.

What Is Relevant?
- Women who have been pregnant may be at increased risk of developing hypertension later in life compared with never-pregnant women.

Summary
Independent of maternal birth weight, pregnancy is associated with increased blood pressure and reduced renal blood flow later in life. Mechanisms may be pregnancy specific, and future studies should focus on tailoring hypertension therapies that consider a woman’s parity.
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LONG-TERM ALTERATION IN MATERNAL BLOOD PRESSURE AND RENAL FUNCTION AFTER PREGNANCY IN NORMAL AND GROWTH RESTRICTED RATS

Linda A. Gallo1, Kate M. Denton2, Karen M. Moritz3, Marianne Tare2, Helena C. Parkington2, Meagan Davies2, Melanie Tran1, Andrew J. Jefferies1 and Mary E. Wlodek1

1Department of Physiology, The University of Melbourne, Parkville VIC Australia; 2Department of Physiology, Monash University, Clayton VIC Australia; 3School of Biomedical Sciences, University of Queensland, St Lucia QLD Australia.

Short title: Cardiorenal post-pregnancy effects

Corresponding Author:
A/Prof Mary Wlodek
Department of Physiology,
The University of Melbourne,
Parkville, Victoria 3010,
Australia
Telephone: +61 3 8344 8801
Fax: +61 3 8344 5818
email: m.wlodek@unimelb.edu.au
Expanded Methods

Animal Procedures
Body weights from Control and Restricted females were measured at postnatal days 1, 7, 14 and 35 and months 2, 3, 4, 6, 9, 12 and 13, as well as at mating and delivery in ex-pregnant groups. Systolic blood pressure was measured in the morning by tail-cuff prior to mating at 4 months and at 6, 9 and 12 months (1;2). At 12 months, animals were placed in metabolic cages for 24 hr to obtain measurements of food and water intake and urine production, after training (2;3). Electrolyte (Rapidchem 744 Analyzer, Bayer HealthCare LLC, Norwood, MA, USA) and total protein (Cobas Integra 400, Roche Diagnostics, Burgess Hill, West Sussex, UK) concentrations were determined and excretions calculated.

Blood pressure was measured at 13 months using an indwelling tail-artery catheter in conscious rats (2). Rats were anaesthetised and ³H-inulin (5.58 µCi.mL⁻¹) and ¹⁴C-PAH (1.7 µCi.mL⁻¹) infused intravenously for estimation of GFR and effective renal blood flow (eRBF), respectively (4). The heart and kidneys were excised and weighed using a three-decimal place precision balance.

The Isolated Heart (Langendorff) Preparation
The heart was mounted via the aorta, on a Langendorff apparatus (ADInstruments, BellaVista, NSW, Australia). A latex balloon, filled with 0.9% saline was inserted into the left ventricle and diastolic pressure was set at 5 mmHg. The balloon was connected to a blood pressure transducer (MLT0699, ADInstruments) and bridge amplifier. Ventricular pressure, heart rate and coronary flow were acquired using a PowerLab 16/30 and displayed and analysed using Labchart 7.0 (ADInstruments). The heart was perfused at a constant pressure of 60 mmHg at 36°C. The hearts were allowed to equilibrate for 20 min. Physiological saline solution (PSS) contained (in mM): 118 NaCl, 4.7 KCl, 25 NaHCO₃, 11 glucose, 1.2 KH₂PO₄, 1.2 MgSO₄, 1.2 CaCl₂, pre-warmed and gassed with 95% O₂ and 5% CO₂. Isoprenaline (in 200 µl warmed PSS, with 200 µl PSS chase, 2x10 sec) was applied through a fine tube into the inflow. Isoprenaline was delivered in increasing concentrations (0.6 & 2 µg/ml) with full recovery from each concentration before the next concentration was administered.

Ischemia/reperfusion
On recovery from the last dose of isoprenaline the flow of PSS into the heart was stopped for 20 min. Then reperfusion was resumed for 1 hr, with a single dose of isoprenaline (2 µg/ml) administered at 30 min following commencement of reperfusion. At the end of the experiment, the heart was blotted and weighed. The heart was then dissected into atria and left ventricle with septum, and each component was weighed. The left ventricle was cut into 2 mm slices and alternate slices were placed in 2,3,5-triphenyltetrazolium (TTZ 10 mg/ml) and incubated at 37°C for 15 min (5). The slices were stored in 4% paraformaldehyde and photographed within 24 hr and infarct area was determined.

Plasma Intrarenal RAS Analyses
Blood samples (0.5ml) were collected into chilled tubes containing BAL-EDTA or heparin at completion of the renal function experiment and the left kidney was divided into cortex and inner medulla. Plasma renin activity and tissue levels of renin and AngII were determined via radioimmunoassay (Prosearch International, Malvern, Victoria, Australia) (6) and plasma estradiol levels were determined (Cayman enzyme immunoassay kit).


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<td>239 ± 3</td>
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</table>

| Organ weight (% BW)             |        |        |             |             |
| Whole heart                     | 0.446 ± 0.015 | 0.442 ± 0.010 | 0.438 ± 0.009 | 0.442 ± 0.008 |
| Left ventricle and septum       | 0.306 ± 0.005 | 0.295 ± 0.006 | 0.307 ± 0.005 | 0.304 ± 0.004 |
| Kidney                          | 0.615 ± 0.010 | 0.587 ± 0.012* | 0.638 ± 0.009 | 0.593 ± 0.010* |

Table S1. Body and organ weights relative to body weight (% BW). Values are expressed as means ± SEM; n=10-12/group. * P<0.05 vs Control; † P<0.05 vs virgin. ‡ P<0.05 vs Control counterpart; § P<0.05 vs virgin counterpart. --- indicates not applicable.