Oxidative Stress Contributes to Sex Differences in Blood Pressure in Adult Growth-Restricted Offspring


Abstract—Numerous experimental studies suggest that oxidative stress contributes to the pathophysiology of hypertension and, importantly, that oxidative stress plays a more definitive role in mediating hypertension in males than in females. Intrauterine growth restriction induced by reduced uterine perfusion initiated at day 14 of gestation in the rat programs hypertension in adult male growth-restricted offspring; yet, female growth-restricted offspring are normotensive. The mechanisms mediating sex differences in blood pressure in adult growth-restricted offspring are not clear. Thus, this study tested the hypothesis that sex-specific differences in renal oxidative stress contribute to the regulation of blood pressure in adult growth-restricted offspring. A significant increase in blood pressure measured by telemetry in male growth-restricted offspring ($P<0.05$) was associated with a marked increase in renal markers of oxidative stress ($P<0.05$). Chronic treatment with the antioxidant Tempol had no effect on blood pressure in male control offspring, but it normalized blood pressure ($P<0.05$) and renal markers of oxidative stress ($P<0.05$) in male growth-restricted offspring relative to male control offspring. Renal markers of oxidative stress were not elevated in female growth-restricted offspring; however, renal activity of the antioxidant catalase was significantly elevated relative to female control offspring ($P<0.05$). Chronic treatment with Tempol did not significantly alter oxidative stress or blood pressure measured by telemetry in female offspring. Thus, these data suggest that sex differences in renal oxidative stress and antioxidant activity are present in adult growth-restricted offspring and that oxidative stress may play a more important role in modulating blood pressure in male but not female growth-restricted offspring. (Hypertension. 2012; 60:00-00.)

Key Words: intrauterine growth restriction ■ blood pressure ■ antioxidant

Intrauterine growth restriction (IUGR) is a marker of impaired fetal growth and can result from a wide range of etiologic factors, including maternal conditions, placental insufficiency, and fetal genomic pathology. Numerous studies indicate that growth restriction during late gestation is associated with the developmental programming of adult health and disease. Barker et al were the first to correlate adverse influences during fetal life with later cardiovascular risk, such as hypertension. Although numerous human and experimental studies address the theory of developmental programming of hypertension, the exact mechanism(s) remains unclear. However, insight from experimental studies indicates that hypertension programmed in response to adverse influences during critical periods of early life may involve activation of the renal nerves, the renin-angiotensin system, and oxidative stress. Thus, developmental programming of adult cardiovascular risk may involve multiple regulatory system interactions on target organs.

Men have higher blood pressure and exhibit greater cardiovascular (CV) and renal risk than age-matched women before menopause. Studies addressing sex differences in programmed CV and renal risk are limited, but many indicate that an inverse association between birth weight and blood pressure is present in both men and women. However, whether men and women differ in response to programmed CV and renal risk and whether age augments or attenuates this sex difference are not completely clear. Jones et al report that smaller size at birth is associated with higher blood pressure in boys but not girls during childhood. Hallan et al report that the effect of IUGR on renal function in young adulthood is weaker and less consistent in women compared with men. Jarvelin et al report an inverse association between birth weight and systolic blood pressure for young adult men regardless of adjustment for body mass index; however, this inverse association is significant only in young adult women when adjusted for current body mass index. Andersson et al report that size at birth, as a predictor of CV risk, is present in women at >60 years of age but not at 50 years of age. Thus, these studies indicate that human males exhibit greater programmed CV and renal risk compared with age-

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matched females, but the mechanisms responsible remain unknown.

Our laboratory uses a rat model of placental insufficiency that results in IUGR and sex differences in adult blood pressure.18 Adult male growth-restricted offspring are hypertensive, whereas adult female growth-restricted offspring are normotensive.18 Previous studies indicate that the renin nerves7,19 and the renin-angiotensin system7 play a critical role in the etiology and maintenance of hypertension in male growth-restricted offspring. However, the exact mechanisms that mediate sex differences in adult blood pressure in this model of IUGR are not clear.

Numerous studies implicate oxidative stress in the development and maintenance of hypertension.20–22 However, in many experimental models of hypertension, oxidative stress plays a more important role in the regulation of blood pressure in males than in females.5,23 Several studies indicate that oxidative stress contributes to hypertension programmed by prenatal undernutrition,8,10,11 indicating a role for oxidative stress in the etiology of hypertension programmed by in utero insult. Many models of perinatally programmed hypertension exhibit sex differences in adult blood pressure11,24–27; however, the exact mechanisms that mediate the sex difference in adult blood pressure in response to prenatal insult are not known. Thus, the goal of this study was to test the hypothesis that sex-specific differences in renal oxidative stress contribute to the regulation of blood pressure in adult growth-restricted offspring.

Methods

Animals

All of the experimental procedures were conducted in accordance with National Institutes of Health Guidelines for the Care and Use of Laboratory Animals with approval by the animal care and use committee at the University of Mississippi Medical Center. Timed pregnant Sprague-Dawley rats were purchased from Harlan Laboratories, Inc (Indianapolis, IN) and housed in a temperature-controlled room (23°C) with a 12:12-hour light/dark cycle with food and water ad libitum. Drinking water was measured daily during the study of sex differences in oxidant status are the result of IUGR and not representative of a litter effect, only 1 male and 1 female offspring were selected per litter for study. All of the animals undergoing surgical procedures were anesthetized using 2% to 5% isoflurane by inhalation. All of the experimental end points were measured at 16 weeks of age to allow complete passage through puberty and into adulthood.

Reduced Uterine Perfusion in the Pregnant Rat

IUGR was induced by a reduction in uterine perfusion initiated at day 14 of gestation, as described previously.18 Pregnant rats for production of control offspring underwent a sham procedure. For more detail, see the online-only Data Supplement.

Chronic Administration of the Superoxide Dismutase Mimetic Tempol

Tempol was administered by drinking water (1 mmol/L) for 2 weeks starting at 14 weeks of age using a dose reported as effective to reduce oxidative status.28 Untreated animals received vehicle (tap water ad libitum). Drinking water was measured daily during the treatment period in all of the animals.

Mean Arterial Pressure in Conscious Offspring

Mean arterial pressure (MAP) was measured continuously in conscious, free-moving offspring using radiotelemetry (Data Sciences International, Minneapolis, MN), as described previously.7 For more detail, see the online-only Data Supplement.

Superoxide Anion Production Measurement

To determine basal and NADPH-stimulated renal and superoxide production in male and female rats, basal and NADPH-stimulated renal and superoxide production was measured using lucigenin chemiluminescence in the presence (NADPH stimulated) or absence (basal) of 0.1 mmol/L of NADPH. Briefly, renal basal superoxide anion production was measured via the lucigenin chemiluminescence method, as described previously by Park et al.23 For more detail, see the online-only Data Supplement.

F₂-isoprostanes

Twenty-four–hour urinary excretion of F₂-isoprostanes was measured according to a modified version of the procedure by Dobrian et al.24 with a competitive ELISA (Oxford Biomedical Research), as described previously,25 with values normalized to urinary creatinine levels (CR 01, Oxford Biomedical Research).

Renal Antioxidant Enzyme Protein Expression

Kidneys were flash frozen and stored at −80°C until use. Frozen tissue was homogenized in radioimmunoprecipitation assay buffer containing a protease inhibitor mixture (Roche Pharmaceuticals). Protein concentration was determined using Pierce BCA Protein Assay Kit (Pierce). Protein lysates were subjected to SDS-PAGE, transferred onto polyvinylidene fluoride membranes, and blocked with Odyssey Brand blocking buffer. Membranes were incubated with antibodies overnight, including anti-catalase (CAT), anti-manganese superoxide dismutase (SOD), anti-Cu/Zn SOD, anti-glutathione peroxidase (GPX; Meridian Life Science), and anti-α-tubulin (Neomarkers). Membranes were probed with the appropriate infrared dye secondary antibodies with bands visualized with an Odyssey infrared imaging system and quantification performed using the QuantiOne software. All of the samples were run in triplicate.

Renal CAT, GPX, and SOD Activity Assays

Renal CAT, GPX, and SOD activities in whole kidney tissue were analyzed according to manufacturer directions (Cayman Chemical Company). Activity data were normalized to protein concentration (protein assay kit, Pierce Product). For more detail, see the online-only Data Supplement.

Statistics

Graphpad PRISM version 5 and IBM® SPSS Statistics version 19 were used for all of the statistical analysis. Comparisons made between groups used ANOVA with adjustment for multiple comparisons. Bonferroni post hoc test was used for multiple comparisons. The general linear model (SPSS) univariate with 3-way interactions (3-way ANOVA) was used to calculate interactions related to birth weight, sex, and treatment. Statistical significance of the interaction was set with $P<0.05$ and $F$ values $>5$. The sample sizes for all of the experiments were calculated to attain a statistical power of $\simeq 0.85$.

Results

Body Weight and Water Consumption

Birth weight was significantly reduced ($P<0.05$) in growth-restricted offspring from reduced uterine perfusion dams as...
compared with control offspring from control or sham dams (Table). At 16 weeks of age, body weight did not differ on comparison of same-sex growth-restricted offspring to same-sex control offspring (Table). However, male offspring were heavier relative to counterpart female offspring within control and growth-restricted groups (Table). Water consumption did not differ between animals receiving vehicle (tap water) relative to animals receiving Tempol (Table).

Mean Arterial Pressure
As reported previously, IUGR leads to a marked increase in MAP at 16 weeks of age in adult male growth-restricted offspring (P<0.05) relative to control offspring that is not observed in adult female growth-restricted offspring (Figure 1A, available in the online-only Data Supplement). Chronic treatment with the antioxidant Tempol had no effect on MAP in male control offspring (Figure 1). However, chronic treatment with Tempol significantly reduced MAP in male growth-restricted offspring (P<0.05), normalizing blood pressure in Tempol-treated male growth-restricted offspring to levels comparable to untreated male control offspring (Figure 1). MAP was not significantly different between adult female control and adult female growth-restricted offspring in the untreated group; moreover, chronic treatment with the antioxidant Tempol did not significantly alter blood pressure in female offspring, control, or growth-restricted offspring (Figure 2).

Renal Markers of Oxidative Stress
Twenty-four–hour urinary excretion of F2-isoprostanes was significantly elevated in untreated male growth-restricted offspring compared with untreated male controls (P<0.05; Figure 3A). Chronic treatment with Tempol significantly reduced urinary F2-isoprostanes in male growth-restricted offspring (P<0.05) but had no significant effect in male controls (Figure 3A). Urinary excretion of F2-isoprostanes did not differ on comparison of untreated female control and untreated female growth-restricted offspring; chronic treatment with Tempol did not alter F2-isoprostane levels in female control or female growth-restricted offspring (Figure 3A). However, urinary excretion of F2-isoprostanes was significantly elevated in untreated male growth-restricted offspring relative to untreated female growth-restricted offspring (P<0.05; Figure 3A).

Renal basal superoxide anion production was significantly elevated in untreated male growth-restricted offspring as compared with untreated male controls (P<0.05; Figure 3B). Chronic treatment with Tempol significantly decreased renal basal superoxide anion production in male growth-restricted offspring (P<0.05) with no effect in male controls (Figure 3B).
Renal Antioxidant Enzyme Protein Expression

Renal protein expression of antioxidant enzymes CAT, GPX, manganese SOD, and copper/zinc SOD (Cu-Zn-SOD) did not differ on comparison of male growth-restricted offspring relative to male control in the untreated group (Figure 5). Tempol significantly increased renal CAT and GPX protein expression in male growth-restricted offspring relative to untreated counterparts ($P<0.05$; Figure 4). In female offspring, renal expression of the antioxidant enzymes CAT and GPX were significantly elevated in untreated growth-restricted offspring relative to untreated control ($P<0.05$; Figure 5). Chronic treatment with Tempol significantly reduced renal CAT and GPX protein expression in female growth-restricted offspring compared with their untreated counterparts ($P<0.05$; Figure 5). Renal expression of Cu-Zn-SOD and manganese-SOD did not differ on comparison of female control or growth-restricted offspring, treated or untreated.

**Renal Antioxidant Enzyme Activity**

Activity of renal antioxidant enzymes CAT, GPX, and Cu-Zn-SOD did not differ on comparison of male growth-restricted offspring relative to male controls in the untreated group (Figure 6). Chronic treatment with the antioxidant Tempol significantly increased activity of renal CAT enzyme in male growth-restricted offspring relative to its untreated counterpart ($P<0.05$; Figure 6). Activity of the renal manganese-SOD enzyme was not measured because of technical difficulties with the assay. In female offspring, activity of renal CAT activity was significantly elevated in untreated growth-restricted offspring relative to untreated controls ($P<0.05$; Figure 6). Chronic treatment with Tempol significantly reduced activity of the renal CAT enzyme in female growth-restricted offspring relative to treated female controls and untreated female growth-restricted offspring ($P<0.05$; Figure 6). Activity of renal GPX and Cu-Zn-SOD did not differ on comparison of female control or female growth-restricted offspring, treated or untreated. The general linear model (IBM® SPSS) with 3-way interaction showed statistically significant effects of treatment ($P<0.03$; $F=5.17$) and sex ($P<0.02$; $F=5.88$) for CAT enzyme activity and an effect of sex ($P=0.000$ $F=41.00$) for GPX enzyme activity in growth-restricted offspring. No effects were observed for Cu-Zn-SOD enzyme activity.

**Heart Rate**

Heart rate (HR) averaged for a 24-hour period at 16 weeks of age was significantly elevated in adult male growth-restricted offspring compared with adult male control offspring ($P<0.05$; Figure 7). HR was significantly elevated in adult female offspring compared with age-matched male control and growth-restricted offspring ($P<0.05$; Figure 7). Chronic treatment with the antioxidant Tempol significantly reduced HR in adult male growth-restricted offspring ($P<0.05$), normalizing it relative to untreated male control offspring; chronic Tempol had no effect on HR in control male, female control, or female growth-restricted offspring (Figure 7).

**Discussion**

The major findings from this study are described here. First, hypertensive male growth-restricted offspring exhibited a marked increase in renal markers of oxidative stress compared with normotensive male control offspring. Second,
Chronic treatment with the SOD mimetic Tempol normalized blood pressure in male growth-restricted offspring relative to male control and, third, renal markers of oxidative stress in male growth-restricted offspring relative to untreated male animals. Fourth, hypertensive male growth-restricted offspring exhibited a marked increase in renal markers of oxidative stress compared with normotensive female control and growth-restricted offspring. Fifth, renal markers of oxidative stress did not differ on comparison of normotensive female growth-restricted offspring to normotensive female control. Sixth, and importantly, a significant increase in renal CAT protein expression and activity was observed in female growth-restricted offspring compared with female control. Thus, these findings suggest that sex differences in renal markers of oxidative stress are present in adult growth-restricted offspring and that oxidative stress may play a more important role in modulating blood pressure in male but not female growth-restricted offspring. Furthermore, upregulation of renal CAT in female growth-restricted offspring may serve as a counterregulatory mechanism against the increase in renal superoxide production and hypertension programmed by in utero insult.

Experimental models of hypertension, such as obesity-induced, deoxycorticosterone acetate salt and the spontaneously hypertensive rat, indicate a link between increased oxidative stress and hypertension. However, recent studies indicate that oxidative stress may have differential effects on blood pressure in males versus females in experimental models of hypertension. Sex differences in adult CV risk are observed in models of developmental programming of hypertension induced via prenatal protein restriction, uteroplacental insufficiency, and fetal glucocorticoid exposure. However, whether sex differences in oxidative stress and antioxidant capacity contribute to the sex-specific programming of hypertension in these models of in utero insult is not clear. Previously, we noted a key role for testosterone in mediating hypertension in adult male growth-restricted offspring. Plasma levels of testosterone are elevated 2-fold in adult male growth-restricted offspring relative to control offspring and castration abolishes hypertension in adult male growth-restricted offspring. Testosterone induces oxidative stress in the male spontaneously hypertensive rat. Furthermore, male sponta-
neously hypertensive rats exhibit higher levels of oxidative stress in microvessels as compared with female spontaneously hypertensive rats. In the current study, only male growth-restricted offspring exhibited an increase in renal production of superoxide and urinary excretion of F2-isoprostanes. Thus, the increase in renal superoxide production and urinary excretion of F2-isoprostanes in adult male growth-restricted offspring may be mediated via increased plasma levels of testosterone programmed by IUGR.

We reported previously that male and female growth-restricted offspring develop hypertension as early as 4 to 6 weeks of age and that female growth-restricted offspring become normotensive after puberty. Ovariectomy induces hypertension in adult female growth-restricted offspring, whereas estrogen replacement reverses this effect, indicating that estrogen is protective against hypertension programmed by IUGR. Numerous studies indicate that estrogen exhibits antioxidant properties. Activity of the antioxidant enzyme CAT is strongly regulated by estrogen in female rats; furthermore, total SOD activity is greater in females rats compared with males. GPX is increased by estrogen treatment in female rats and in humans; serum GPX levels and activity are higher in women compared with men. In the current study, renal protein expressions of CAT and GPX were significantly increased in female growth-restricted offspring relative to female control. Importantly, female growth-restricted offspring exhibited a significant increase in renal CAT activity relative to female control. Increased reactive oxygen species (ROS) production and hypertension in angiotensinogen transgenic mice are prevented by overexpression of CAT. Therefore, compensatory upregulation of antioxidant capacity may contribute to normalization of blood pressure in adult female growth-restricted offspring and contribute to sex differences in hypertension programmed in response to IUGR. Furthermore, upregulation of antioxidant capacity by estrogen may be one mechanism by which estrogen is protective against hypertension induced by IUGR in adult female growth-restricted rats.

Figure 5. Protein expression of renal antioxidant enzymes catalase (CAT), glutathione peroxidase (GPX), manganese superoxide dismutase (Mn-SOD), and copper-zinc SOD (Cu-Zn SOD) in adult female control and intrauterine growth-restricted (IUGR) offspring treated with the SOD mimetic Tempol (1 mmol/L) or vehicle (tap water ad libitum) from 14 weeks to 16 weeks of age. Visualization of the protein of interest and tubulin are from the same blot. Results are expressed in arbitrary units for the protein normalized to tubulin. *P<0.05 vs female control. †P<0.05 vs untreated female counterpart. Data values represent mean±SE. □, control; ■, IUGR.
The exact mechanisms by which ROS contribute to elevations in blood pressure are not clear. Experimental studies indicate that ROS may increase blood pressure via activation of the sympathetic nervous system.\textsuperscript{43–46} Whether ROS activate peripheral sympathetic nervous system activity\textsuperscript{46} or peripheral and central sympathetic nervous system activity is not clear.\textsuperscript{43} Moreover, the acute actions of systemic Tempol to reduce blood pressure in several experimental models of hypertension are associated with a reduction in renal sympathetic nerve activity\textsuperscript{43–46} that occurs in the absence of a reduction in vascular superoxide production.\textsuperscript{44} Thus, the mechanism by which Tempol reduces blood pressure acutely may involve different sites and mechanisms of action that may be specific to the experimental model. The marked increase in blood pressure and HR in male growth-restricted offspring was normalized relative to male control offspring by chronic treatment with Tempol. In addition, the reduction in blood pressure after chronic treatment with Tempol was associated with a marked reduction in renal superoxide production, suggesting that the drop in blood pressure in male growth-restricted offspring is associated with a decrease in ROS within the kidney. However, the antihypertensive effect of Tempol on male growth-restricted offspring in this study may also be attributed to influences mediated via the sympathetic nervous system. The renal nerves play a key role in hypertension induced by IUGR in male growth-restricted offspring.\textsuperscript{7,19} Thus, further studies will determine whether the reduction in MAP in male growth-restricted offspring after chronic treatment with Tempol involves direct inhibition of sympathetic nerve activity. In addition, further studies may be needed to determine whether the mechanism by which chronic treatment with Tempol leads to a reduction in MAP in male growth-restricted offspring involves downregulation of inflammation.\textsuperscript{47} Adult male growth-restricted offspring exhibit a marked increase in circulating inflammatory cytokines indicative of chronic inflammation.\textsuperscript{49} Thus, the mechanisms by which the antioxidant Tempol acts to reduce blood pressure may be complex and multifactorial.

In conclusion, hypertension in adult male growth-restricted offspring was associated with a marked increase in renal markers of oxidative stress. Female growth-restricted offspring did not demonstrate an increase in renal markers of oxidative stress. Chronic treatment with the antioxidant Tempol abolished hypertension and reduced renal markers of oxidative stress in adult male growth-restricted offspring. Female growth-restricted offspring showed elevated renal CAT activity. Thus, data from this study suggest that IUGR induced by placental insufficiency programs an increase in renal oxidative stress that contributes to the etiology of hypertension in male growth-restricted offspring. Testosterone may exacerbate renal superoxide production, whereas estrogen may counteract the increase in renal superoxide production via upregulation of key antioxidant enzymes. Therefore, sex-specific programming of renal superoxide production and antioxidant capacity may contribute to sex differences in hypertension programmed by IUGR.
Perspectives

Hypertension is a major risk factor for CV disease and is the primary cause of death in men and women worldwide. Although the efforts to prevent and/or find a cure for hypertension are great, the number of patients with hypertension is increasing. New paradigms suggest an association between the pathophysiology of hypertension and birth weight. Insight into the mechanisms linking birth weight with blood pressure in later life may help or prevent CV disease by targeting populations at risk. Whether sex differences in CV risk exist in low birth weight individuals is not yet clear. However, experimental studies indicate that sex hormones may be an important determinant of oxidative status and CV risk in models of IUGR. CV risk increases with age. Thus, further studies are need to clarify whether sex hormones, in particular when impacted by aging, alter CV risk in low birth weight individuals.

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Disclosures

None.

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### Novelty and Significance

**What Is New?**

- Investigation into mechanisms that mediate sex differences in models of hypertension programmed by fetal insult are very limited. We reported previously that female growth-restricted offspring are protected against marked increases in blood pressure in adulthood, whereas male growth-restricted offspring are hypertensive.

**What Is Significant?**

- Numerous experimental models of hypertension report that oxidative stress plays a more important role in blood pressure regulation in males compared with females. Thus, oxidative stress may be one mechanism that contributes to sex differences in adult blood pressure in models of prenatal programmed hypertension.

### Summary

Thus, this study indicates that sex-specific programming of renal oxidative stress contributes to sex differences in the developmental programming of adult blood pressure in growth-restricted offspring. Insight from this study highlights the sex-specific programming of oxidant status in male growth-restricted offspring relative to female growth-restricted offspring.
Oxidative Stress Contributes to Sex Differences in Blood Pressure in Adult Growth-Restricted Offspring
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OXIDATIVE STRESS CONTRIBUTES TO SEX DIFFERENCES IN BLOOD PRESSURE IN ADULT GROWTH RESTRICTED OFFSPRING.

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Running title: Sex differences, IUGR, oxidative stress

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EXPANDED MATERIALS AND METHODS
Reduced uterine perfusion in the pregnant rat. IUGR was induced via a reduction in uterine perfusion as previously described. Briefly, a silver clip (0.203-mm ID) was placed around the lower abdominal aorta above the iliac bifurcation and on each branch of the ovarian artery (0.100-mm ID) in pregnant rats at 14 days of gestation. Pregnant rats used for the production of control offspring underwent a sham procedure (Control Pregnant). All animals exposed to surgical procedures were anesthetized with isoflurane at 2% (W.A. Butler Co) delivered by vaporizer (Ohio Medical Products).

Mean arterial pressure in conscious offspring. Mean arterial pressure (MAP) was measured continuously in conscious, free moving offspring using radio telemetry (Data Sciences International, Minneapolis, MN) from 12 to 16 weeks of age as previously described. Briefly, at 10 week of age in animals under isoflurane anesthesia a flexible catheter attached to a radio transmitter was inserted in the abdominal aorta just below the renal arteries. The transmitter was secured to the abdominal wall muscle and the transmitter remained in the abdominal cavity for the duration of the experiment. After surgery, offspring were housed in individual cages positioned over a RLA-3000 radio-telemetry receiver. Measure of MAP was initiated 2 weeks after surgery to allow full recovery. Data were recorded every 10 minutes with 10-second sampling, 24 hours a day.

Superoxide anion production measurement. To determine basal and NADPH-stimulated renal and superoxide production in male and female rats, basal and NADPH-stimulated renal and superoxide production was measured using lucigenin chemiluminescence in the presence (NADPH stimulated) or absence (basal) of 0.1 mmol/L NADPH. Briefly, renal basal superoxide anion production was measured via the lucigenin chemiluminescence method as previously described by Schiffrin et. al. Briefly, kidneys were homogenized (1:8 wt/vol) in RIPA buffer (PBS, 1% Nonidet P-40, 0.5% sodium deoxicholate, and 0.1% SDS (Sigma Aldrich), and protease inhibitor cocktail (Roche) and then the samples were centrifuged at 12,000 g for 20 min at 4°C. The homogenate supernatant was incubated with lucigenin for chemiluminescent detection using a Berthold luminometer. The results are reported as relative light units per mg protein (RLU/mg protein) in the kidney homogenate. For measurement of NADPH oxidase-dependant superoxide anion production, we also used the lucigenin chemiluminescence method; however, kidney cortex homogenate supernatant was processed as previously described by Gorin et al. Specifically, 100 µl of homogenates were added to 900 µl of 50 mM phosphate buffer, pH 7.0, containing 1 mM EGTA, 150 mM sucrose, 5 µM lucigenin, and 100 µM NADPH. Photon emissions in terms of relative light units were measured every 20 or 30 seconds for 10 minutes in a Berthold luminometer. There was no measurable activity in the absence of NADPH. A buffer blank (less than 5% of the signal) was subtracted from each reading. Superoxide production was expressed as relative chemiluminescence (light) units (RLU)/mg protein. Protein content was measured using the Bio-Rad protein assay. (All reagents from Sigma Aldrich).

Renal Catalase, Glutathione Peroxidase, and Superoxide Dismutase Activity Assays. Whole kidney tissue was homogenized in the buffer outlined in the activity assay protocol provided by the manufacturer (Cayman Chemical Company). Homogenate protein concentration was determined using the BCA protein assay kit (Pierce Product #23225). Homogenates were serially diluted and activity assays were performed to determine the optimal dilution for the
standard curve of each assay. Total catalase and glutathione peroxidase was measured by commercially available enzyme activity assay kits (Cayman Chemical Company Product #707002 and #703102, respectively). The homogenates for the superoxide dismutase kit were centrifuged to separate cytosolic (CuZn-SOD) fractions from mitochondrial (Mn-SOD) fractions. Only the cytosolic (CuZn-SOD) portion was assayed. All assays were carried out using a Bio-Tek plate reader. Activity data was normalized to protein concentrations obtained from the BCA protein assay.

Supplemental References


Figure S1. Mean arterial pressure in untreated male and untreated female control and growth-restricted (IUGR) offspring at 16 weeks of age measured by radio telemetry in conscious, free moving animals. *P<0.05 versus male control offspring. †P<0.05 versus male IUGR offspring. Data values represent mean±SE.