Renal Denervation Abolishes the Age-Dependent Increase in Blood Pressure in Female Intrauterine Growth-Restricted Rats at 12 Months of Age


Abstract—Perinatal insults program sex differences in blood pressure, with males more susceptible than females. Aging may augment developmental programming of chronic disease, but the mechanisms involved are not clear. We previously reported that female growth-restricted offspring are normotensive after puberty. Therefore, we tested the hypothesis that age increases susceptibility to hypertension in female growth-restricted offspring. Blood pressure remained similar at 6 months of age; however, blood pressure was significantly elevated in female growth-restricted offspring relative to control by 12 months of age (137±3 vs 117±4 mm Hg; P<0.01, respectively). Body weight did not differ at 6 or 12 months of age; however, total fat mass and visceral fat were significantly increased at 12 months in female growth-restricted offspring (P<0.05 vs control). Glomerular filtration rate remained normal, yet renal vascular resistance was increased at 12 months of age in female growth-restricted offspring (P<0.05 vs control). Plasma leptin, which can increase sympathetic nerve activity, did not differ at 6 months but was increased at 12 months of age in female growth-restricted offspring (P<0.05 vs control). Because of the age-dependent increase in blood pressure, Bilateral renal denervation abolished the elevated blood pressure in female growth-restricted offspring normalizing it relative to denervated female control offspring. Thus, these data indicate that age induces an increase in visceral fat and circulating leptin associated with a significant increase in blood pressure in female growth-restricted offspring, with the renal nerves serving as an underlying mechanism. (Hypertension. 2013;61:00-00.) • Online Data Supplement

Key Words: aging ■ cardiovascular risk ■ low birth weight ■ renal nerves ■ women’s health

Hypertension is the major cause of death worldwide, and it is a multifactorial disorder affected by both genetic and environmental factors. The Barker hypothesis suggests that events during fetal life impact adult health and program increased cardiovascular (CV) risk and hypertension.1 Adverse events, such as fetal undernutrition, are hypothesized to program adult chronic disease attributed to metabolic and endocrine changes, in addition to physiological and structural changes that occur in the undernourished organs.1 Low birth weight (LBW) serves as a crude marker indicative of undernutrition during fetal life, and numerous epidemiological and experimental studies demonstrate a clear relationship between birth weight and blood pressure.2,3 It is well established that blood pressure increases with age in men and women, with men exhibiting a higher blood pressure than women until postmenopause.4,5 Higher blood pressures are also observed in LBW boys relative to LBW girls during childhood,6 with men demonstrating a strong association between birth weight and blood pressure in young adulthood.7 The association between birth weight and blood pressure amplifies with age,8 and hypertension is reported in LBW women by age 60.9 Thus, age may serve as a secondary influence on the impact of poor fetal growth. Experimental models of fetal insult indicate that female offspring may be protected against programmed CV risk10-12 or exhibit a delay in the development of adverse CV function.13 Yet, whether birth weight indicative of a poor fetal environment impacts chronic health in later life in LBW women has not been extensively studied; moreover, the mechanisms involved have not yet been elucidated.

Our laboratory uses a rodent model of intrauterine growth restriction (IUGR) induced by placental insufficiency that results in a decrease in birth weight of offspring from the reduced uterine perfusion dams relative to control offspring from the sham-operated dams.12 Previously, we reported that before puberty at 8 weeks of age, male and female growth-restricted offspring have a significant increase in mean arterial pressure (MAP) relative to same-sex, age-matched controls.12
However, only male growth-restricted offspring demonstrate an increase in blood pressure after puberty, whereas blood pressure is normalized after puberty in female growth-restricted offspring relative to age-matched female controls. MAP remains normotensive in the female growth-restricted offspring at 4 months of age; yet, the impact of age-dependent changes on blood pressure in female growth-restricted offspring remains unknown.

It is well established that aging is a critical mediator of increased risk of hypertension. Aging is generally associated with a reduction in lean body mass and an increase in adipose tissue, particularly central body fat. Insulin resistance, dyslipidemia, and enhanced sympathetic activity result from an elevation in the adipose-derived chemokine leptin. Moreover, the sympathetic nervous system is thought to contribute to obesity-related hypertension. A diminution in kidney function, such as a decreased ability of kidney to excrete sodium and progressive decrease in functioning nephron units, may also contribute to age-related increases in blood pressure. We previously reported that the ovarian hormones play a protective role against the development of high blood pressure in young adult female growth-restricted offspring. Age-related changes in CV risk in women are often associated with passage in menopause; yet, whether the increase in CV risk after menopause in women is attributed to aging per se, or to menopause itself, is unknown. Thus, this study tested the hypothesis that age-dependent changes, up to 1 year of age, serves as a secondary hit to program an increase in blood pressure in female growth-restricted offspring. Moreover, this study examined whether age-induced changes in adiposity accompanied the increase in blood pressure in female growth-restricted offspring and if the renal nerves, indicative of sympathetic involvement in obesity-related hypertension, served as a potential underlying mechanism.

Methods

Please see online-only Data Supplement for extended Methods.

Animals

All experimental procedures were in accordance with National Institutes of Health guidelines 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 Inc (Indianapolis, IN). Offspring from 24 control (sham) pregnant and 27 offspring reduced uterine perfusion pregnant rats were randomly assigned to groups studied at 6 or 12 months of age. The experimental protocol included one group subjected to body composition measurements and 24-hour metabolic studies conducted 1 week before the experimental end point at 6 months of age. The experimental end point involved measurement of renal and systemic hemodynamic parameters and harvest of plasma, serum, and tissues at 6 months of age. A second group followed the same experimental protocol but was studied at 12 months of age. A third group was studied at 12 months of age but was subjected to either sham or bilateral renal denervation (RDV) as described below. In the third group, 2 weeks after sham denervation or bilateral RDV, MAP was measured via telemetry in a subgroup and via carotid catheter in all animals in the conscious state.

Reduced Uterine Perfusion in the Pregnant Rat

Reduced uteroplacental perfusion was used for induction of IUGR on day 14 of gestation as previously described.

Body Composition

Total fat mass and total lean mass were determined in conscious animals using an Echo-MRI-700 (Echo Medical Systems, Houston, TX). Results are expressed as gram weight.

Bilateral RDV

Bilateral RDV was performed as previously described.

Measurement of MAP and Heart Rate by Radiotelemetry

Implantation of the radiotelemetry probe and collection of data were performed as previously described.

Measurement of Blood Pressure and Renal Hemodynamics

MAP, glomerular filtration rate, effective renal plasma flow, and calculation of renal vascular resistance (RVR), renal blood flow, and filtration fraction were performed in conscious animals as previously described.

Leptin

Plasma leptin levels were measured using a commercially available kit (Leptin Quantikine ELISA Kit, R&D system, MN).

Measure of Renal Catecholamine Content

Renal norepinephrine was measured by high-performance liquid chromatography via electrochemical detection.

Statistics

Data are presented as mean values±SEM, with n representing the number of female offspring from different mothers per group. Data were analyzed for significance using the Student t test for comparison between 2 groups for birth weight and renal hemodynamic data (Figures 1A and 4). Pearson R correlation coefficient was used to calculate the relationship between body weight and fat mass. Two-way randomized ANOVA was used for the remainder of studied data (Figures 1C, 2, 3, and 5 and renal norepinephrine as well as food intake). Post hoc testing was performed using the Bonferroni test (GraphPad Prism 5.0). Differences were reported as significant only when P<0.05.

Results

Birth Weight, Body Weight, and Growth Rates

Birth weight was significantly reduced in female growth-restricted offspring compared with female control (P<0.05; Figure 1A). However, female-growth-restricted offspring no longer exhibited a significant reduction in body weight relative to the age-matched control by 2 months of age (Figure 1B). Body weight did not differ at 6 months of age in animals studied at 6 months and 12 months of age (Figure 1C). Body weight remained similar at 12 months of age, but the body weight of female control and growth-restricted offspring was significantly greater at 12 months of age relative to the 6-month body weights (P<0.05; Figure 1C).

Plasma Leptin and Body Fat

No difference in plasma leptin was observed in the group studied at 6 months of age. However, circulating levels of leptin were significantly increased in female growth-restricted offspring compared with female control at 12 months of age (P<0.05; Figure 2A). The increase in circulating leptin was associated with a marked increase in total body fat mass and visceral fat in...
the female growth-restricted offspring relative to female control (P<0.05) at 12 months of age (Figure 2B and 2C). Moreover, there was a positive correlation (Pearson R, 0.7562; P<0.007) between body weight and fat mass in female growth-restricted offspring at 12 months of age relative to age-matched control counterparts. However, lean body mass (Figure 2D) did not differ on comparison of female growth-restricted offspring relative with female control offspring at 12 months of age.

MAP and Renal Hemodynamic Parameters

MAP when measured via arterial catheter in conscious, chronically instrumented rats was similar on comparison of female growth-restricted offspring to female control offspring at 6 months of age. Yet, blood pressure was significantly elevated in female growth-restricted offspring relative to their age-matched controls at 12 months of age when measured via catheter (Figure 3). To determine the mechanism underlying the age-dependent increase in blood pressure in female growth-restricted offspring at 12 months of age, renal hemodynamic parameters were measured. Glomerular filtration rate (2.18±0.06 and 2.58±0.28 mL/min, control vs IUGR, respectively) and glomerular filtration rate adjusted for kidney weight (Figure 4A) did not differ at 12 months of age (Figure 4A). Filtration fraction was also not altered (Figure 4B). However, renal blood flow (Figure 4C) and effective renal plasma flow were significantly reduced in female growth-restricted offspring relative to female control (14.92±0.31 and 8.14±0.46 mL/min, control vs IUGR; P<0.05, respectively). In addition, the significant increase in blood pressure in female growth-restricted offspring at 12 months of age was associated with a marked increase in RVR (Figure 4D).

Effect of Bilateral RDV on Blood Pressure, Heart Rate, and Renal Catecholamine Content

Bilateral RDV initiated 2 weeks before 12 months of age in the 12-month study group abolished the age-dependent increase in blood pressure in female growth-restricted offspring normalizing it relative to MAP in the denervated female control offspring (P<0.05). This was evident regardless of whether MAP was measured via 3 days continuous collection by radiotelemetry (Figure 5A) or in conscious, chronically instrumented animals (P<0.05; Figure 5B). Verification of RDV was confirmed by analysis of renal catecholamine levels. Renal norepinephrine content was significantly reduced by bilateral RDV in control and growth-restricted offspring relative to their sham denervated counterpart (213.9±18.9 vs 30.2±5.9, and 182.9±5.9 vs 29.9±7.3 pg/mg kidney tissue; control sham vs control RDV, and IUGR sham vs IUGR RDV, respectively; P<0.05; sham vs RDV counterpart). Heart rate was not significantly different between female growth-restricted relative to age-matched female sham or female RDV (348.5±9.5, 355.6±7.5, 359.2±6.5, and 359.1±7.0 beats per minute; control sham, control RDV, IUGR sham, and IUGR RDV, respectively).

Effect of Age on Uterine Weight

Uterine weight was not altered in female growth-restricted rats at 6 (1.69±0.20 and 1.78±0.20 g/grams body weight; control vs IUGR, respectively) or 12 (2.83±0.43 and 2.34±0.22 g/grams body weight; control vs IUGR, respectively) months of age.

Discussion

This study reported numerous novel findings. First, we reported that IUGR leads to age-dependent changes at 12 months of age that were associated with an increase in total body fat mass, circulating levels of leptin, and increased blood pressure in female growth-restricted offspring relative to female control. Second, we demonstrated that the increase
in blood pressure in female growth-restricted offspring at 12 months of age was not associated with a marked reduction in glomerular filtration rate or changes in the 24-hour excretion of urinary sodium. However, renal blood flow was significantly decreased and RVR was increased at 12 months of age in female growth-restricted offspring. Third, bilateral RDV abolished the increase in blood pressure in the female growth-restricted offspring normalizing blood pressure relative to renal denervated female control offspring, indicating a role for the renal nerves as an underlying mechanism in the age-dependent increase in blood pressure in female growth-restricted offspring.

Sex-specific programming of high blood pressure after fetal insult is observed in many models of developmental programming.\(^{10-12,22}\) Specifically, male offspring of late gestation diabetic dams,\(^{22}\) moderate protein-restricted dams,\(^{11}\) and dams that undergo uteroplacental insufficiency via total ligation of the uterine vessels at day 18 gestation\(^{10}\) or reduced uterine perfusion at day 14 gestation\(^{12}\) exhibit a marked increase in blood pressure, whereas female littermates remain normotensive. Previously, our laboratory demonstrated a protective role for estradiol at 4 months of age against programmed increases in blood pressure in female growth-restricted offspring of reduced uterine perfusion dams.\(^{14}\) The importance of estradiol is also demonstrated in female offspring exposed to a severe reduction (6%) in maternal protein intake during fetal life.\(^{23}\) Characteristic changes in rodent ovarian hormonal cycles indicative of perimenopause occur around 18 months of age.\(^{24}\) In females, rats exposed to uteroplacental insufficiency induced by bilateral uterine ligation at day 18 of gestation, no significant increase in blood pressure was observed at 18 months of age.\(^{10}\) However, the method for collection of arterial pressure in the aforementioned study involved tail cuff and tail arterial catheter.\(^{10}\) Whether an increase in adiposity occurs in this study is not reported.\(^{10}\) Thus, this model of uteroplacental insufficiency differs in the timing and severity of fetal insult and may be protected against age-dependent increases in arterial pressure.

As previously reported, female growth-restricted offspring are normotensive at 4 months of age.\(^{14}\) In the current study, we report that blood pressure remained normotensive in female growth-restricted offspring relative to age-matched female control offspring at 6 months of age, with hypertension present by 12 months. These findings indicate that the
age-dependent increase in blood pressure in female growth-restricted offspring at 12 months of age did not develop as a direct consequence of placental insufficiency per se, but rather evolved in response to an additional influence(s) that impacted the effect of IUGR in female offspring. Hypertension can be induced in young adult female growth-restricted offspring by removal of the ovarian hormones.\textsuperscript{14} However, the aims of this study were to examine the effect of age before the onset of changes in ovarian hormone status, which is present by 18 months of age.\textsuperscript{24} Uterine weight, a crude indicator of ovarian hormone status,\textsuperscript{25,26} was not altered in female growth-restricted offspring relative to age-matched female control at 12 months of age. Thus, these findings suggest that a change in ovarian status may not be a causative factor that contributes to the age-dependent increase in blood pressure in female growth-restricted offspring. Obesity is well recognized as a major risk factor for increased blood pressure\textsuperscript{17}; recent studies also implicate the importance of adiposity.\textsuperscript{27} Programming of increased adiposity is reported in models of maternal undernutrition.\textsuperscript{28,29} In the current study, hypertension in female growth-restricted offspring was associated with an increase in total and visceral fat mass at 12 months of age. Thus, age-dependent increases in adiposity may be a causative factor in the development of hypertension in female growth-restricted offspring at 12 months of age.

Adiposity has a significant influence on blood pressure with leptin indicated to be an important mediator acting through the sympathetic nervous system.\textsuperscript{19} Increased body fat is associated with an increase in plasma leptin in women.\textsuperscript{30} Obesity is also linked to an increase in sympathetic neural discharge in obese women.\textsuperscript{31,32} Moreover, age can impact sympathetic neural activity and blood pressure in women independent of ovarian hormonal status.\textsuperscript{33} Although hypertension may be programmed by events that occur during fetal life,\textsuperscript{34} sympathetic overactivity may be influenced by an increase in adiposity\textsuperscript{35} and plasma leptin\textsuperscript{36} that develop with age. In female growth-restricted offspring at 1 year of age, significant increases in visceral adiposity were associated with an increase in circulating levels of leptin. Leptin, a circulating hormone produced by adipose tissue, acts on the hypothalamus to increase renal sympathetic nerve activity and blood pressure.\textsuperscript{37} Thus, one aim of this study was to delineate the importance of the renal nerves in mediating the age-dependent increase in blood pressure in female growth-restricted offspring. Bilateral RDV normalized blood pressure in the female growth-restricted offspring relative to renal denervated female control offspring resulting in a decrease in MAP. Thus, findings from this study indicate that the renal nerves contribute to the development of age-dependent hypertension after IUGR in the female rat.

Development of hypertension via the action of the renal nerves may involve alterations in tubular sodium reabsorption, RVR, or renin release.\textsuperscript{37} Hypertension induced by prenatal exposure to glucocorticoids is associated with an increase in renal sodium transporter abundance in male offspring through a mechanism that involves the renal nerves.\textsuperscript{38} Despite the elevated blood pressure at 12 months of age in female growth-restricted offspring, 24-hour urinary excretion of sodium did not differ on comparison with female control offspring (Table S1 in the online-only Data Supplement). These findings suggest that female growth-restricted offspring at 12 months of age are able to maintain sodium balance at the expense of an increase in blood pressure. The age-dependent increase in blood pressure in female growth-restricted offspring at 12 months of age was associated with significant increase in RVR. Whether the effect of bilateral RDV on blood pressure was attributable to a reduction in vasomotor tone leading to an overall decrease in total peripheral resistance is not yet known. Moreover, the importance of renin in the age-dependent development of
be influenced to a greater degree by changes in body mass than increase in blood pressure that occurs with age in women may fail to program adult hypertension in female rats. Few epidemiological studies have examined sex differences in the developmental programming of CV risk. Fewer studies have studied the impact of aging. Findings from this study indicate that age impacts programmed CV risk in female growth-restricted offspring, implicating a need for further investigation in the effects of age on CV health in LBW women. The influence of adiposity in middle-age after LBW may denote a potential confounding factor on later chronic health in LBW women. Moreover, the importance of the renal nerves in mediating the increase in blood pressure that accompanies the age-dependent increase in adiposity in female growth-restricted offspring in this study advocates investigation in the importance of a sympathetic component with regard to future therapeutic interventions in LBW women.

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**Disclosures**

None.

**References**

Novelty and Significance

What Is New?
- Few studies have investigated sex differences in programmed cardiovascular risk; fewer studies have investigated the age-dependent changes in programmed cardiovascular risk.

What Is Relevant?
- Aging was associated with a significant increase in adiposity, circulating leptin, and increased blood pressure after intrauterine growth restriction in female rats. Age-dependent increases in blood pressure in female growth-restricted rats did not develop as a direct consequence of the fetal insult, but rather was attributed to an additional influence that did not impact cardiovascular health in female control counterparts. An age-related increase in adiposity leading to an increase in renal sympathetic nerve activity may serve as an underlying mechanism in the age-dependent increase in blood pressure that follows intrauterine growth restriction in female rats.

Summary
Age-dependent changes may serve as a secondary influence in the developmental programming of adult blood pressure, and insight from this study highlights the importance for further studies investigating the impact of age on modulating cardiovascular risk in low birth weight women.
Renal Denervation Abolishes the Age-Dependent Increase in Blood Pressure in Female Intrauterine Growth-Restricted Rats at 12 Months of Age

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Renal denervation abolishes the age-dependent increase in blood pressure in female intrauterine growth-restricted rats at 12 months of age

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EXTENDED MATERIALS AND METHODS

Animals. At day 14 of gestation rats destined for reduced uterine perfusion were clipped as described below. Rats were housed in a temperature-controlled room (23°C) with a 12:12-hour light/dark cycle with food and water available ad libitum. All dams were allowed to deliver at term with birth weight recorded within 12 hours of delivery. At 48 hours of age offspring in the control and reduced uterine perfusion litters were culled to 8 offspring per dam to ensure equal nutrient access for all offspring. The ratio of male to female offspring remained equivalent after culling when possible; however, only female offspring were used in this study. Offspring were weighed three times weekly until weaning at 3 weeks of age; after weaning, rats were weighed every two weeks. Rats were anesthetized with isoflurane for all surgical procedures with maintenance at approximately 2%.

Reduced uterine perfusion in the pregnant rat. Intrauterine growth restriction was induced at day 14 of gestation as previously described (1). Briefly, a silver clip was placed around the lower abdominal aorta (0.203-mm ID) above the iliac bifurcation and at each branch of the ovarian artery (0.100-mm ID); the sham procedure involved visualization of the uterine horn (control).

Bilateral renal denervation. Bilateral renal denervation, performed as previously described (2), involved a midline abdominal incision followed by gentle isolation and stripping of the nervous and connective tissue surrounding the renal artery and veins. Vessels were then coated with 10% phenol to destroy the sympathetic nerves. Sham-operated rats received a similar midline abdominal incision, but the renal nerves were left intact. Animals were allowed to recover for 2 weeks prior to measurement of blood pressure.

Measurement of mean arterial pressure and heart rate by radiotelemetry. As previously described (2), a flexible catheter attached to a radio transmitter (Data Sciences, Minneapolis, MN) was inserted in the abdominal aorta just below the renal arteries after the sham or RDV procedure. The transmitter was secured to the abdominal muscle and remained in the abdominal cavity for the duration of the experiment. After surgery, rats were housed in individual cages positioned over an RLA-3000 radiotelemetry receiver. Rats received food and water ad libitum. Blood pressure measurements were obtained after 2 weeks of surgery and were recorded every 10 min for 24 h for 3 days.

Measurement of blood pressure and renal hemodynamics. As previously described (1), offspring under isoflurane anesthesia were surgically instrumented with a flexible catheter (PE 50 tubing) in the right jugular vein for infusion and, in the right carotid artery for measurement of arterial pressure; the bladder was instrumented with a flexible catheter (PE-90 tubing) for collection of urine. All catheters were tunneled to the nape of the neck and exteriorized. Mean arterial pressure (MAP) was monitored in conscious animals following 24 hours of recovery. MAP was determined via connection of the arterial catheter to a pressure transducer and a data acquisition system with a computer for continuous recording (ADInstruments, PowerLab 16/30; with software, L). Calculation of glomerular filtration rate (GFR) and effective renal plasma flow (eRPF) were determined utilizing $^{125}$iodothalamate (Questcor Pharmaceuticals) and para-aminohippuric acid (PAH) (Sigma- Aldrich) in plasma and urine, respectively. Renal blood flow (RBF), renal vascular resistance (RVR) and filtration fraction (FF) were calculated: RBF=ERPF/(1-hematocrit), RVR=(MAP/ERPF)(1-hematocrit) and FF=(GFR/ERPF), respectively. Data were collected for 20 minutes after a 60 minute stabilization period.

Measure of renal catecholamine content. Renal norepinephrine was measured by HPLC via electrochemical detection. An internal standard (dehydroxybenzylamine/DHBA) was included with each extraction to monitor recovery and aid in quantitation.
24 hour food and water intake, urinary albumin, Na+ and K+ excretion. Urine samples were collected from 24 hour metabolic studies. The NephRat for Quantitation of Rat Urinary Albumin kit (Exocell, PA) was used to determined total urinary albumin excretion. Urinary excretion of Na+ and K+ were determined using an Easylite Electrolyte Analyzer (Medica Corporation, MA). Food intake per gram body weight was also measure via 24 hour metabolic study.

RESULTS
24 hour metabolic studies. Food and water intake did not differ at 6 or 12 months of age upon comparison of control to growth-restricted offspring (Table S1). Twenty-four hour urine volume or urinary excretion of Na+ and K+ as well as kidney weight were not significantly different between female growth-restricted relative to age-matched female control (Table S1). Urinary excretion of albumin was similar and within non-pathophysiological levels (3.5 ± 1.6 and 1.2 ± 0.8 mg/day; control versus IUGR, respectively).

REFERENCES
**Table S1.** Food and water intake, urine volume, sodium (Na⁺) and potassium (K⁺) excretion collected during 24-hour metabolic studies in control and growth-restricted (IUGR) offspring at 6 and 12 months of age.

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