Developmental Programming of Hypertension
Insight From Animal Models of Nutritional Manipulation

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Low birth weight (LBW), defined as a birth weight of ≤2.5 kg at term, is a major health issue within the United States today. The risk for LBW is greater within the black population than the white, with a greater percentage of LBW occurring within the southern United States relative to other parts of the country.1 Infants born small for gestational age not only have a greater risk for survival at birth2–3 but, based on numerous epidemiological studies, face long-term consequences, such as increased risk for development of hypertension, cardiovascular disease, diabetes, and other health problems.4–6 Barker7 first proposed that an adverse environmental stimulus experienced during a critical period of fetal development leads to slow fetal growth and permanent structural and physiological changes in the fetus predisposing it to developmental programming of hypertension. Investigators using animal models to induce slow fetal growth are providing convincing evidence to support the concept of developmental programming of adult disease.8–17 Although there is compelling epidemiological and experimental data that suggest that cardiovascular diseases such as hypertension may be programmed in utero, the underlying pathophysiological mechanisms remain unclear. Investigators use unique animal models of nutritional manipulation to induce slow fetal growth to examine the mechanisms linking birth weight and chronic adult disease, such as hypertension. In this review, we discuss alterations in potential mechanistic pathways that evolve in response to fetal insult and lead to the development of hypertension, highlighting insight provided by animal models of nutritional manipulation.

Animal Models of Nutritional Manipulation During Fetal Life
Nutritional restriction is one of the most common experimental methods of fetal insult used for investigation into the mechanisms of programmed hypertension and was one of the first to demonstrate that exposure to an adverse environment in utero leads to marked structural and physiological alterations.18 Importantly, this method of fetal insult was also one of the first to demonstrate that timing of the insult is critical to the programming response, with a reduction in nephron number observed when the nutritional insult coincides with the nephrogenic period.8,13,18–21 Observations linking nutritional restriction during gestation with elevated blood pressure in offspring have been controversial.21–24 In the rat, variations in dietary nutrient and protein balance are reported to contribute to differing blood pressure responses in offspring,22 with postnatal influences such as excessive weight gain exacerbating the effect.25 In humans, childhood growth is also a strong determinant of adult blood pressure demonstrating the importance of the postnatal environment on adult disease.26 In the sheep model of nutrient restriction, birth weight rather than maternal diet may play a more critical role in the blood pressure response,23 with maternal body composition at the time of conception also critical to cardiovascular outcome.21 Animal models of reduced uteroplacental perfusion during late gestation also lead to an environment of undernutrition and hypertension in the intrauterine growth-restricted (IUGR) offspring.10,11 Contention exists with regard to the reproducibility of these models.27 However, consistent observations of IUGR are reported by numerous investigators10–12,28,29; moreover, similar phenotypic outcomes, such as a reduction in nephron number, have been observed in response to placental insufficiency, an observation that is not species specific.29–32 Overnutrition as a nutritional insult during fetal life also programs metabolic and cardiovascular dysfunction,33–35 implications critical because of the increased prevalence of obesity.36

Importantly, these models of nutritional manipulation demonstrate characteristics reflective of the human condition of LBW, including marked increases in blood pressure10–15,17,19,35,37,38 and reduced nephron number.13,15,18,28,29,39 In humans, nephron number is directly correlated with birth weight and inversely correlated with blood pressure.40 Thus, models of nutritional insult, whether induced by direct manipulation of the gestational diet or through a reduction in uteroplacental perfusion, serve as relevant pathophysiological models for investigation into the mechanisms linking birth weight and blood pressure and provide the basis for the discussion of potential mechanistic pathways presented in this review.
Mechanisms of Developmental Programming of Hypertension

Hormones
Hormones are known to play a critical role in the proper development and growth of fetal tissues, and it is well documented that alterations in the intrauterine hormonal environment can lead to long-term effects on fetal outcome and cardiovascular health. In experimental studies, inappropriate exposure to testosterone during gestation results in IUGR, impaired insulin sensitivity, and cardiovascular dysfunction, demonstrating a critical role for sex hormones in the developmental programming of adult cardiovascular disease.

Models of developmental programming exhibit sex differences with severity of the fetal insult critical to the adult phenotypic outcome. Although severe protein restriction during gestation in the rat leads to hypertension and changes in renal structure in both male and female offspring, moderate protein restriction during gestation in the rat leads to marked increases in blood pressure and a reduction in nephron number in male but not female offspring. Therefore, female offspring appear to be protected from an unfavorable phenotypic outcome in response to a moderate undernutritional insult in utero. However, in models of programming induced by maternal diet-induced obesity, the prevalence for hypertension is greater or present only in female offspring, indicating that sex differences in the sensitivity to nutritional manipulation are insult specific. Sex differences in adult blood pressure are not observed in models of undernutrition programmed by placental insufficiency in the rat when assessed indirectly by the tail cuff in conscious, restrained animals. Conversely, sex differences in adult blood pressure are observed when measured directly by telemetry; blood pressure after puberty is stabilized to normotensive levels in female IUGR, yet is further increased in male IUGR. Castration abolishes hypertension in male IUGR; ovariectomy induces hypertension in female IUGR rats, an effect reverted by hormonal replacement therapy. Thus, sex differences in the blood pressure response to placental insufficiency in IUGR offspring indicate a potential role for sex hormones in mediating sex differences in the postnatal blood pressure response to fetal insult (Figure 1).

Sexual dimorphism is observed in human essential hypertension and in experimental models of hypertension, with a role for sex hormone involvement strongly indicated. Hypertension is less prevalent in premenopausal women as compared with age-matched men. However, after menopause, the risk of hypertension increases with age, suggesting that whereas the ovaries are functional, women have a lower risk for hypertension and cardiovascular disease than men. Experimental studies suggest that sex hormones play a mechanistic role in blood pressure control. Ovariectomy exacerbates the existing hypertension in female rats in some experimental models of hypertension, suggesting an important role for estradiol in blood pressure regulation. Androgens exacerbate hypertension in the male spontaneously hypertensive rat; castration reduces blood pressure in the male spontaneously hypertensive rat, thus indicating a role for androgens. Thus, sex hormones appear to contribute to sex differences in adult blood pressure regulation. Whether sex hormones are altered in LBW individuals is controversial. Moreover, few investigators have reported whether adult sex hormones are altered in response to fetal insult, nor have they examined the direct effect of sex hormones on postnatal hypertension in experimental models of developmental programming. Thus, the exact mechanism(s) by which sex hormones contribute to the developmental programming of blood pressure regulation has not been clearly elucidated but may involve modulation of systems critical to the long-term control of blood pressure regulation.

The Renin-Angiotensin System
The renin-angiotensin system (RAS) is a major regulator of blood pressure control and volume homeostasis. Numerous
studies indicate that the RAS plays an important role in the etiology of hypertension programmed by in utero insult\textsuperscript{15,49,50,68–72} (Figure 1). A critical role for the central RAS is indicated in hypertension programmed in response to maternal undernutrition in the rat with marked increases in angiotensin (Ang) type 1 receptor (AT\textsubscript{1}R) expression observed in areas of the brain critical to cardiovascular regulation\textsuperscript{73} (Figure 1). In the kidneys, temporal alterations in the RAS occur in response to fetal insult. A reduction in intrarenal renin and Ang II is observed at birth in response to maternal protein restriction in the rat\textsuperscript{15} followed by postnatal upregulation of the renal AT\textsubscript{1}R.\textsuperscript{70,72} In addition, inappropriate activation of the peripheral RAS, demonstrated by a marked increase in plasma renin activity, occurs after the development of hypertension.\textsuperscript{68,69} Importantly, hypertension is abolished by systemic blockade of the RAS,\textsuperscript{68,69,71} indicating that the RAS contributes to hypertension programmed in response to maternal nutrient restriction. In a model of undernutrition induced by placental insufficiency, temporal alterations in the renal RAS are also observed with renal angiotensinogen and renin mRNA expression suppressed at birth but markedly elevated in adulthood.\textsuperscript{74} Unlike models of maternal nutrient restriction, renal AT\textsubscript{1}R, Renin, and Ang II expression, as well as inappropriate activation of the peripheral RAS, are not observed,\textsuperscript{95,96} yet, the importance of the RAS is indicated as hypertension is abolished by RAS blockade.\textsuperscript{49} Although the contribution of the RAS to the development of hypertension is not clearly defined, it may involve an increased responsiveness to Ang II. Androgens can augment renal vascular responses to Ang II.\textsuperscript{75} Elevated levels of testosterone are observed in male IUGR programmed in response to placental insufficiency.\textsuperscript{49} Therefore, elevated levels of adult testosterone may be one mechanism by which sensitivity to Ang II is increased and may also contribute to the enhanced intrarenal renin and angiotensinogen mRNA expression observed in adult male IUGR.\textsuperscript{76} Modulation of the RAS by estradiol may also contribute to sex differences in hypertension programmed by fetal insult. Estradiol is reported to downregulate tissue angiotensin-converting enzyme (ACE)\textsuperscript{77} and AT\textsubscript{1}R mRNA expression,\textsuperscript{78} suggesting that estradiol may reduce Ang II, a potent vasoconstrictor peptide critical for blood pressure regulation.\textsuperscript{87,79} Estradiol may also alter the ACE2-dependent pathway,\textsuperscript{80} which generates Ang 1-7, a negative regulator of the vasoconstrictor effects of Ang II.\textsuperscript{79} Modulation of the RAS by estradiol may be one mechanism by which sex hormones play a protective role against an increase in blood pressure in adult female IUGR offspring in a model of undernutrition induced by placentational insufficiency. Hypertension is induced by ovariectomy in female IUGR but not female control offspring.\textsuperscript{50} ACE inhibition abolishes ovariectomy-induced hypertension,\textsuperscript{50} suggesting a critical role for the RAS. Normotensive adult female IUGR offspring exhibit a significant elevation in renal ACE2 mRNA expression that is decreased by ovariectomy with no effect observed in adult female control.\textsuperscript{50} Thus, loss of estradiol may decrease the vasoconstrictor effect provided by the ACE2 pathway leading to an increase in blood pressure in adult female IUGR after ovariectomy. Therefore, permanent alterations in the RAS occur in response to fetal insult and contribute to the development of hypertension; modulation of the RAS by sex hormones is one mechanism that may contribute to sex differences in programmed hypertension (Figure 1).

The Renal Nerves

Many known regulatory mechanisms control sodium balance, and alterations in sympathetic activity have sustained effects to reduce pressure natriuresis and result in long-term changes in arterial pressure.\textsuperscript{81,82} However, whether sympathetic function contributes to hypertension in LBW individuals is controversial.\textsuperscript{83–86} Circulating levels of catecholamines, neurotransmitters that serve as an indirect marker of sympathetic nerve outflow, are increased in response to fetal undernutrition in models of developmental programming induced by both gestational protein restriction\textsuperscript{97} and also placental insufficiency in the rat\textsuperscript{88} and sheep.\textsuperscript{89} The importance of the renal nerves in the etiology of hypertension programmed by in utero insult was demonstrated recently whereby renal denervation normalized arterial pressure in adult male IUGR offspring in a model of placental insufficiency with no significant effect on blood pressure in adult control offspring.\textsuperscript{90} Therefore, these findings indicate that the renal nerves play an important role in the etiology of hypertension programmed by fetal undernutrition induced by placental insufficiency (Figure 1). Increased sympathetic outflow including sustained increases in renal sympathetic nerve activity can also occur as a result of the actions of Ang II in regions of the brain critical for cardiovascular regulation.\textsuperscript{91} Thus, central activation of the RAS leading to an increase in renal sympathetic nerve activity may induce altered renal nerve development in IUGR offspring resulting in hypertension.

Oxidative Stress

A decrease in bioavailability of antioxidants leading to an increase in oxidative stress is indicated to play an important role in essential and experimental hypertension.\textsuperscript{92,93} Oxidative stress is increased in LBW children,\textsuperscript{94} suggesting that alterations in oxidant status may contribute to hypertension programmed in response to fetal insult. In experimental models of in utero exposure to undernutrition, hypertension is abolished by administration of the superoxide dismutase mimetic Tempol\textsuperscript{95} or the lipid peroxidation inhibitor lazaroïd,\textsuperscript{96} implicating an important role for oxidative stress (Figure 1). Ang II stimulates oxidative stress through its AT\textsubscript{1}R.\textsuperscript{97} Thus, upregulation of renal AT\textsubscript{1}R and inappropriate activation of the RAS may serve as stimuli for increased oxidative stress in the developmental programming of hypertension.

Nephron Number

A reduction in nephron number is a phenotypic outcome commonly observed in the fetal response to gestational insult in experimental models of undernutrition induced by maternal nutrient restriction\textsuperscript{8,14,15,20,21,99} and placental insufficiency.\textsuperscript{29–32} A reduction in nephron number leading to a reduction in renal excretory function is suggested to serve as a mechanism in the developmental programming of hypertension. Whether a reduced nephron complement could lead to hyper-
Fetal programming of hypertension. Potential renal mechanisms.

- ↓ Nephron number
- ↑ Tubular Na⁺ reabsorption
- ↓ Pressure natriuresis
- ↓ GFR

Figure 2. Potential renal mechanisms whereby developmental programming in response to in utero insult leads to the development of hypertension.

Tension was demonstrated in a study whereby removal of 1 kidney during the nephrogenic period in the rat led to adult hypertension. However, recent findings indicate that a reduction in nephron number is not always critical to the developmental programming of hypertension. A maternal diet rich in fat does not alter the nephron number in offspring. Furthermore, an increase in glomerular volume is often observed in conjunction with a reduced nephron number. Glomerular filtration rate is not decreased in a rat models of maternal protein restriction or placental insufficiency. Thus, compensatory hyperfiltration may occur in response to fewer nephrons at birth, resulting in preservation of the glomerular filtration rate, suggesting that factors other than a reduced nephron complement contribute to the etiology of hypertension programmed by in utero insult. Numerous factors including intrarenal sodium transport defects and abnormalities in the extrarenal control systems that regulate kidney function can mediate a reduction in renal sodium excretory function observed in hypertension. Thus, a sodium retaining defect not mediated by alterations in the filtered load of sodium may be the main contributor to a sodium retaining defect not mediated by alterations in extrarenal regulatory systems critical to renal programming.

Susceptibility to Renal Disease

Progression of renal injury and disease is closely linked to hypertension. It is well established that the kidney exhibits increased vulnerability to fetal insult as evidenced by the association of reduced nephron number with IUGR in animal models of feto-nutrient manipulation. However, recent studies indicate that susceptibility to renal disease is greater in LBW individuals with implications for a gender bias and a protective role for estradiol in women. Hypertension associated with an increase in urinary albuminuria, glomerulosclerosis, and histological determinants of renal damage is observed in offspring of nutrient-restricted dams. Hypertension, proteinuria, and glomerulosclerosis are reduced by long-term postnatal L-arginine supplementation; however, evidence of renal damage persists. Therefore, these studies indicate that increased susceptibility to renal damage is not just the result of hypertension but may have an origin in fetal life. Furthermore, a reduction in nephron number at birth in conjunction with an increase in glomerular size and compensatory hyperfiltration may contribute to an increased susceptibility to renal injury and enhance the later development of hypertension in LBW individuals (Figure 1).

Perspectives

Insight provided by animal models of nutritional manipulation during fetal life suggests that slow fetal growth leads to alterations in the normal regulatory systems involved in the long-term control of blood pressure regulation. The pathogenesis of hypertension programmed by in utero insult is multifactorial and may involve intrinsic intrarenal defects or alterations in extrarenal regulatory systems critical to renal sodium excretory function. Moreover, a role for sex steroids is also demonstrated. Understanding the complexity of the fetal programming of adult disease may lead to preventive measures and early detection of cardiovascular risk in LBW individuals.

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Disclosures

None.

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