IN UTERO ORIGINS OF HYPERTENSION: MECHANISMS AND TARGETS FOR THERAPY

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Morton JS, Cooke C-L, Davidge ST. In Utero Origins of Hypertension: Mechanisms and Targets for Therapy. Physiol Rev 96: 549–603, 2016. Published February 17, 2016; doi:10.1152/physrev.00015.2015.—The developmental origins of health and disease theory is based on evidence that a suboptimal environment during fetal and neonatal development can significantly impact the evolution of adult-onset disease. Abundant evidence exists that a compromised prenatal (and early postnatal) environment leads to an increased risk of hypertension later in life. Hypertension is a silent, chronic, and progressive disease defined by elevated blood pressure (>140/90 mmHg) and is strongly correlated with cardiovascular morbidity/mortality. The pathophysiological mechanisms, however, are complex and poorly understood, and hypertension continues to be one of the most resilient health problems in modern society. Research into the programming of hypertension has proposed pharmacological treatment strategies to reverse and/or prevent disease. In addition, modifications to the lifestyle of pregnant women might impart far-reaching benefits to the health of their children. As more information is discovered, more successful management of hypertension can be expected to follow; however, while pregnancy complications such as fetal growth restriction, preeclampsia, preterm birth, etc., continue to occur, their offspring will be at increased risk for hypertension. This article reviews the current knowledge surrounding the developmental origins of hypertension, with a focus on mechanistic pathways and targets for therapeutic and pharmacologic interventions.

I. INTRODUCTION 549
II. MECHANISMS OF DEVELOPMENTAL … 552
III. THERAPEUTIC INTERVENTIONS 576
IV. SUMMARY AND CONCLUSIONS 581
V. APPENDIX: ANIMAL MODELS OF … 583

I. INTRODUCTION

The developmental origins of health and disease theory is based on evidence that the preconceptual, prenatal, and early postnatal environments have a significant impact on the long-term development of adult disease. While the underlying concept is much older, the publication of a landmark study by Dr. David Barker in 1989 demonstrating a correlation between low birth weight (LBW) and death from cardiovascular disease later in life (21) has led to the emergence of an ever-expanding body of literature in support of the developmental origins of cardiovascular disease hypothesis. In this manner, one of the largest bodies of evidence now available regarding the role of fetal programming is that a compromised prenatal (and early postnatal) environment leads to an increased risk of hypertension later in life. Hypertension is a silent, chronic, and progressive disease defined by elevated arterial blood pressure (>140/90 mmHg) that is strongly correlated with cardiovascular morbidity and mortality. Importantly, cardiovascular disease is one of the leading causes of death worldwide, of which hypertension is a major contributing factor. Despite extensive public health efforts in implementing a number of behavioral, lifestyle, and pharmacological interventions, hypertension continues to be one of the most common medical diagnoses and one of the most resilient health problems in modern society. Although essential hypertension often coincides with obesity, dyslipidemia, and coronary artery disease, the pathophysiological mechanisms are complex and remain poorly understood.

It is clear that developmental programming of noncommunicable diseases has the potential to greatly impact socioeconomic health; especially considering the disease burden of cardiovascular illnesses. For this reason, many researchers have dedicated their careers to determining the complex mechanisms behind programmed hypertension in the hope of developing more effective interventions. The developmental origins of adult hypertension theory may shed light on the etiology of this chronic disease with the hope of developing primary preventative strategies and/or more effective treatments for individuals who are at increased risk. The purpose of this article is to review the current knowledge surrounding developmental origins of hypertension, with a focus on mechanistic pathways and targets for therapeutic and/or pharmacologic intervention (see overview FIGURE 1).
A. Birth Weight and Hypertension

Early evidence from epidemiological studies has shown an inverse correlation between weight at birth and mortality from cardiovascular disease in certain geographical regions in England (21). These intriguing data were among the first to demonstrate an association between the environment in which a fetus develops and long-term human disease in adulthood, although examples from nature have been around for centuries (426). Population data from many different cultures have emerged corroborating and expanding the initial observations made by Barker et al. (21): for example, an inverse relationship between birth weight and blood pressure was found in 8-yr-old Brazilian children born at term (300), British school-aged children (428), Swedish men at the time of conscription to the military (30), and a cohort of French men born with LBW (456). Indeed, in one of the largest cohorts of Finnish men and women studied, those who developed hypertension were more likely to have been born with LBW (115). This study also demonstrated that adults with increased blood pressure were also more likely to have experienced accelerated postnatal growth, such that by 7 yr of age, body weight was found in 8-yr-old Brazilian children born at term (300), British school-aged children (428), Swedish men at the time of conscription to the military (30), and a cohort of French men born with LBW (456). Indeed, in one of the largest cohorts of Finnish men and women studied, those who developed hypertension were more likely to have been born with LBW (115). This study also demonstrated that adults with increased blood pressure were also more likely to have experienced accelerated postnatal growth, such that by 7 yr of age, body weight was not different from children born of appropriate weight (115). Indeed, an accelerated growth trajectory after birth has been reported as a major contributor to adult-onset disease (217, 218, 221); however, this is not always the case (76, 384, 430). Nevertheless, the importance of postnatal weight gain in children born small remains an important issue. In a cohort of middle-class children in the Netherlands, an inverse association between birth weight and systolic blood pressure was found, with a significant interaction noted between birth weight and body mass index (BMI) (410). The authors suggest that LBW in combination with a high current BMI is particularly detrimental in contributing to programmed hypertension (410). Accordingly, leaders in research, public health, and politics are stressing the importance of the “first 1,000 days of life” and the ability to either positively or negatively impact long-term health and wellness, a global problem not limited to the development of hypertension (36, 239).

B. Defining Birth Weight

Early studies on the programming of cardiovascular disease have commonly focused on newborn weight as an indicator of impaired fetal development. From a clinical perspective, different terms have been coined to define suboptimal prenatal growth, including LBW, intrauterine growth restriction (IUGR) or small for gestational age (SGA) for those born with a body weight below the expected growth curve and macrosomia and large for gestational age (LGA) for those born with a body weight above the normal range. The difference in these various designations is subtle: LBW babies are defined as those born weighing less than 2,500 g (which is the lower end of normal birth weight at term; 2,500-4,200 g). Further subcategories of LBW include very LBW (VLBW) when body weight is 1,500 g, and extremely LBW (ELBW) when body weight is <1,000 g. This convenient classification of fetal growth has been extensively implemented; however, it is also heavily criticized because it does
not take into consideration gestational age at birth. SGA takes this assessment one step further by correcting the birth weight based on the estimated gestational age (normally calculated using the date of the last normal menstrual period or ultrasound assessment of fetal anthropometrics early in pregnancy). Significantly more precise and labor intensive, this definition has become preferred in the post-ultrasound era but has also been criticized for not considering the unique genetic potential of each individual. For example, the usage of population specific growth charts (which take into consideration the ethnic diversity of a given area) may minimize misdiagnosis of SGA for IUGR (62, 280). Finally, IUGR is defined as fetuses that fail to reach their genetically determined potential. As appropriate as it may appear, this particular definition is impractical due to the challenge of assessing the genetic potential of a certain individual. Newer diagnostic tools allowing the longitudinal evaluation of fetal growth in a noninvasive fashion and the current development of fetal growth trajectories have been proposed as an alternative to assess growth potential and IUGR (127, 403). In this regard, premature infants represent an interesting group since they are usually born small but may be appropriate for their gestational age. The subtleties surrounding the definitions of “appropriate” growth can lead to inaccurate reporting or interchanging of terms in studies using human populations. Data in the developmental programming literature, therefore, should be interpreted with caution, especially in those which assume LBW to be a surrogate for a poor prenatal environment.

To illustrate these controversial issues, some studies indicate that the relationship between birth weight and adult hypertension is U-shaped, suggesting that LGA infants are at equally high risk of hypertension. Indeed, a population-based study of 15,600 children aged 3–6 yr in China demonstrated that higher weight percentiles at birth increased the risk of elevated blood pressure in both sexes (45). Additionally, children born to obese women are at increased risk of cardiometabolic disease regardless of their weight at birth (163). Animal studies in maternal high-fat models have demonstrated similar results (198, 353). Furthermore, in young Swedish men, the association between birth weight and risk of developing high blood pressure was significant only among men born preterm (182), which raises the question of whether the inverse correlation between birth weight and adult blood pressure is due to prematurity alone, which is also indicative of a compromised in utero environment. Indeed, in a large cohort of young adults who were born preterm, there was a strong association with high blood pressure and the need for antihypertensive medication, in particular for those born extremely premature (23–27 wk gestation), which was independent of birth weight (90). This observation has been corroborated by others, who speculate that prematurity may be a larger risk factor for hypertension than IUGR (389). However, conflicting results have also been reported. In a longitudinal population based study, Juonala et al. (187) found that in adults born premature, the risk of high blood pressure was greatest in those who were also small for gestational age. The underlying mechanisms leading to programmed hypertension have also shown controversial results regarding prematurity versus IUGR. For example, vascular stiffness was increased in 8-yr-old children born premature and IUGR, but not those who were born premature alone (73). On the other hand, renal programming in humans, including decreased kidney size (194) and enlarged glomeruli (393), appears to be more strongly correlated with premature birth rather than growth restriction. Thus the effect of prematurity on renal dysfunction may be due to an interruption of normal organogenesis rather than “programming” per se (2, 238). Because there is a lack of a robust animal model of prematurity, it is difficult to reconcile the controversial results relating to the contribution of IUGR versus prematurity to developmental programming of hypertension. This area warrants further investigation. Overall, the concept of newborn birth weight as a marker of adult cardiovascular risk should be expanded to include a variety of prenatal, postnatal, maternal, and fetal factors (including prematurity) and avoid the assumption that offspring who are seemingly “appropriately grown for gestational age” won’t manifest a hypertensive phenotype because of programming effects.

C. Compromised In Utero Environments

Many epidemiological studies have demonstrated that a suboptimal prenatal environment causes a fetus to be at a higher risk of hypertension in adulthood. The Dutch Famine (1944–1945) was a tragic event in history that has provided a unique opportunity to study the relationship between exposure to prenatal famine and the development of health and disease in adulthood. Studies have utilized extensive perinatal databases to analyze the effect of maternal famine on long-term fetal outcomes. These studies discovered that maternal malnourishment could predispose offspring to adult-onset cardiovascular diseases (206). Specifically, blood pressure in offspring exposed to famine (in particular a low protein-to-carbohydrate ratio subset) in the third trimester had higher blood pressures as adults (337). Extensive studies in a variety of animal models have confirmed the correlations of maternal undernutrition demonstrated by the Dutch Famine data (reviewed in Ref. 213). Nonetheless, since the original concept of fetal programming became popularized, it has expanded to encompass many diverse pregnancy-related complications that can negatively affect the environment in which a fetus is developing. These include pathological situations such as preeclampsia, impaired placentation, nutritional deficits, hypoxia, toxin exposure, or systemic illness, to other conditions such as high altitude, maternal obesity, stress, corticosteroid exposure, and multiple pregnancies. Multiple pregnancies are unique because they may be further complicated by
twin-twin transfusion syndrome in monochorionic pregnancies (single placenta) or with discordant growth and selective IUGR in dichorionic twins (multiple placentas); however, these situations can also provide a useful matched control for study. Another maternal condition that should be considered as a potential state of compromised fetal development is advanced maternal age, an increasingly common phenomenon that is associated with increased obstetrical and perinatal complications, although its role in developmental programming remains to be determined. Therefore, multiple causes of a compromised in utero environment exist; however, human patients often carry multiple medical comorbidities and socioeconomic confounders. For this reason, animal models in which a single pathology can be controlled and studied provide a clear benefit for the advancement of knowledge.

D. Animal Models of Compromised In Utero Environments

Findings from epidemiological studies have guided basic biomedical research to explore numerous potential mechanistic pathways of early programming. The lengthy nature of this phenomenon, however, demands the implementation of alternative experimental models providing more reasonable timelines. In humans, for instance, the average length of gestation is ~280 ± 14 days, while in sheep it is ~147 ± 2 days and mice a mere 19 ± 0.2 days. Furthermore, differences in the anticipated life expectancy (up to 6–8 decades in humans, 1 decade for sheep, and a couple of years for most rodents) across species make animal models an important approach for understanding mechanisms and specific outcomes due to developmental contribution to adult chronic diseases, including hypertension. There are, however, limitations to the use of animal models. For instance, while the homogeneity of inbred animal strains reduces the variability of results allowing for a clearer interpretation of the effects of environmental manipulations on physiological outcomes, this precise factor fails to mimic the heterogeneity of the human population, thereby limiting the translation of findings, including development of early intervention strategies. For instance, the human population may respond differently to treatments dependent on their ethnicity or cultural background, underlying or concomitant pathologies, attrition rates, etc. Part of this heterogeneity, however, is the fact that humans often carry multiple comorbidities, thus providing a clear benefit of animal models in which a single pathology can be assessed in a controlled environment. Despite their limitations, animal models remain the only economical and feasible method to narrow the field of seemingly endless possible mechanisms and targets for therapeutic options. Indeed, even given the differences, consistencies are observed in the outcomes of fetal programming across multiple species. For example, in both humans and animal models affected by in utero programming, renal nephron numbers have been shown to be reduced (see sect. II, A1), the renin angiotensin system (RAS) has been observed to be impacted (see sect. II, A2 and B3) and DNA methylation patterns are altered (see sect. II, B), to name but a few of the mechanisms covered in this review.

Historically, animal models have been developed and refined for many years, and this continues to occur. An example of this is animal models of undernutrition. The first of such models was developed following the demonstration of a greater risk of adult cardiovascular disease in populations exposed to famine, specifically the Dutch Famine which occurred between 1944 and 1945. In the early stages of research, models used a global diet restriction to mimic this occurrence. Following further research, models have been expanded to include total calorie restriction as well as restriction of specific components such as proteins, fats, or micronutrients to further inform our knowledge of the relative importance of various dietary factors. The same can be said for models of overnutrition that have been developed to more accurately represent the “Western” style diets of modern times. It can be expected that all models of developmental programming will continue to undergo these transformations and refinements as we learn more and find new targets to investigate and that these models will continue to assist in the search for therapeutic targets.

The following sections of this review present a wealth of data collected from various human and animal studies of compromised in utero environments that have an impact on the development of hypertension in the offspring in later life. A detailed description of the animal models used to collect these data, along with tables and references, has been provided in the appendix (see sect. V).

II. MECHANISMS OF DEVELOPMENTAL ORIGINS OF HYPERTENSION

A. Programmed Renal Dysfunction

Although the control of systemic blood pressure has many regulators, the kidney plays a central role. Adult kidney function may be influenced by a variety of factors, beginning in the early embryonic stages of renal development. Normal renal development and nephron formation require an intricate interplay between growth factors, pro- and anti-apoptotic pathways, the RAS, oxygen, and nutrient supply. Indeed, much evidence exists that glomerular development specifically is highly sensitive to prenatal perturbations and that a compromised in utero development affects renal structure and function in both human and animal studies (reviewed in Ref. 362).

A basic understanding of renal embryology is necessary to appreciate how the environment during development might affect kidney function. In humans, the primordial kidney begins formation at approximately day 30 after conception,
and through the process of branching morphogenesis, the ureteric buds form scaffolding for the future nephrons. The process of nephrogenesis is complete by \(-36\) wk of gestation in humans (336). Thus a premature birth prior to completion of this process will affect a neonate’s nephron endowment. It is imperative to note, however, that the timing of renal development differs among species. In rats and mice, nephrogenesis continues until well after birth, and thus postnatal influences may play a significant role (116). The guinea pig, on the other hand, completes nephrogenesis prior to birth, similar to the human (108). These species differences must be considered when interpreting data collected from animal studies, particularly those focusing on the timing of intervention.

One possible etiology of adult hypertension may result from a prenatally acquired reduction in nephron number, leading to a decreased surface area for filtration, glomerular hypertrophy, and subsequently dysfunctional handling of salt and water. However, hormonal regulation of renal hemodynamics, especially via RAS, also factors into overall renal function. Therefore, prenatal (and early postnatal) environmental conditions have the potential to affect renal development, thus promoting the onset of hypertension in adulthood. Evidence in support of renal involvement in programmed hypertension, as well as potential underlying mechanisms, will be discussed in the following sections.

1. Renal structure and nephron number

A variety of human studies have demonstrated a correlation of renal structure with renal function and elevated blood pressure. The concept that nephron counts are related to hypertension is not new; indeed, in 1939, Hayman et al. (162) described an association between nephron number, kidney function, and hypertension. In 1988, after noting that in a variety of hypertensive rat strains nephron numbers were reduced, Brenner et al. (195) went on to hypothesize that decreased nephron number was an independent risk factor for hypertension (reviewed in Ref. 47). This theory was supported by autopsy studies in which subjects with a clinical history of hypertension had fewer but larger glomeruli, regardless of their cause of death (195). Indeed, follow-up studies sought to investigate a potential pathophysiological role of reduced nephron number. Hughson et al. (176) discovered a strong inverse relationship between nephron number and blood pressure in Caucasians of European descent. Interestingly, although the rate of death from hypertension and cardiovascular disease in African Americans was similar to Caucasians, the association with nephron number was weak (176). The explanation for this observation is not entirely clear but illustrates that there are likely multiple interrelated factors in addition to nephron number which determine blood pressure and subsequent risk of death from hypertension.

Since the popularization of the “Barker hypothesis,” the concept that renal structure and function may be related to birth weight has been investigated. Manalich et al. (244) performed autopsy studies on term neonates who died from nonrenal disease. Their data show that birth weight is positively correlated with nephron number and inversely correlated with nephron size (244). These data have been corroborated by Hughson et al. (175) who also found a direct relationship between total glomerular number and birth weight in both adults and children.

Autopsy studies are limited in their ability to assess cause and effect, for which prospective cohorts are more effective. There are, however, few available methods to quantify nephron numbers in vivo. Silver et al. (382) studied the usefulness of ultrasound examination of kidney volume [which correlates with nephron number (25, 283)] in normal birth weight and IUGR infants. The results indicated that IUGR fetuses had renal volumes that were 31% less than control when matched for gestational age. The authors suggest, therefore, that decreased renal volume in IUGR fetuses, a surrogate for nephron number, may be one mechanism by which IUGR offspring are at risk of hypertension in later life (382).

Konje et al. (204) expanded this concept by performing serial ultrasounds for renal measurements in a cohort of 87 singleton fetuses from 22 to 38 wk gestation, in whom cord blood was collected at the time of birth. The results showed that kidney growth in multiple planes (antero-posterior, transverse, and circumference) was slower after 26 wk gestation in SGA fetuses, while at the time of birth, plasma renin activity was higher in the SGA compared with appropriate for gestational age (AGA) group. The authors performed ultrasound examination of kidney volume in SGA fetuses, a surrogate for nephron number, may be one mechanism by which IUGR offspring are at risk of hypertension in later life (382).

Studies that utilize animal models of programmed hypertension also support the theory that a compromised in utero environment and IUGR contribute to impaired nephrogenesis. Indeed, in utero-placental restricted rats, nephron endowment was reduced compared with control rats (434), findings that have been corroborated by others (363). Interestingly, Wlodek et al. (434) also showed that inducing growth restriction in the postnatal period in the rat (by reducing litter size at birth) also decreased nephron number and increased blood pressure in offspring at 22 wk of age. Furthermore, nephron deficit and hypertension could be reversed in placental restricted IUGR rat offspring by normalizing their environment during weaning (cross fostering with control dams) (434). These studies illustrate the asso-
An association between nephron number and hypertension, as well as highlighting that the critical window of renal development extends into the early neonatal period in the rat.

In a variety of other animal models of programmed hypertension, nephron number has been investigated. For example, in a placental embolization model of sheep IUGR, there was a 24% decreased nephron number in IUGR offspring compared with controls (454) [although this result could not be substantiated by another group (256)]. In a uterine artery ligation guinea pig model of fetal programming, offspring that were growth restricted (214) demonstrated a reduced number of glomeruli in the kidneys (48). In the low protein rat model of programmed hypertension, nephron number also appears to be reduced (214, 348). Siddique et al. (380) studied the differential effect of postnatal maternal dietary manipulation on renal morphology and blood pressure in adult offspring. Their intriguing data demonstrate that offspring born from a control prenatal environment, then reared by mothers fed a low-protein diet until weaning, were hypertensive but had normal nephron endowment. Rats whose mothers were on a low-protein diet during pregnancy and weaning had increased blood pressure and a decreased nephron number. However, offspring from low-protein dams who were reared by control dams during weaning had normal blood pressure and glomerular numbers (380). These data highlight the complexities of fetal programming of renal disease and hypertension, which is highly sensitive to both the type and timing of the insult (210).

Interestingly, in the maternal low-protein rat model, early postnatal overfeeding does not alter nephron number; however, it leads to an earlier development and more sustained hypertensive phenotype, only in males (43). Furthermore, vitamin A deficiency has been linked to IUGR and renal development (reviewed in Ref. 33) and in the maternal low-protein rat model, supplementation with retinoic acid (vitamin A derivative) improved nephron number in IUGR offspring (242). Thus overall there is a significant amount of data that supports the hypothesis that a suboptimal maternal diet or intrauterine environment programs renal nephron number and hence impacts the developmental programming of adult hypertension.

2. Renal RAS

The intrarenal molecular pathways that are affected in models of programmed hypertension are complex and have been intensely studied. In particular, the RAS is thought to be a significant contributor. Normal renal function requires a delicate balance of many components of the angiotensin pathway, since opposing physiological effects can result from altered receptor expression or activation. The classic RAS pathway begins with circulating renin, which acts on angiotensinogen to produce angiotensin I (ANG I), which in turn is converted into angiotensin II (ANG II) by angiotensin converting enzyme (ACE). ANG II is considered one primary effector of RAS and is known to exert its actions as a circulating hormone via angiotensin receptors, AT1 and AT2, which transmit biological signals often leading to opposite physiological effects [for example, vasoconstriction via AT1 and vasodilation via AT2 (295)]. In addition to its traditional role in stimulating aldosterone secretion from the adrenal gland, ANG II can also act in a paracrine and autocrine manner in a variety of organs other than the kidney (brain, adipose tissue, vasculature) (126). However, in recent years, an expanded view of RAS has emerged, including the discovery of ACE 2, which hydrolyzes ANG I to produce ANG-[1–9], which is subsequently converted into ANG-[1–7] by endopeptidases and ACE. ANG-[1–7] acts primarily via “Mas” receptors and plays a central role in balancing the vasoconstrictor and proliferative actions of ANG II with vasodilatory and antiproliferative effects (FIGURE 2) (315). Indeed, enhanced activity of the RAS pathway has been demonstrated in both humans and animal models of fetal programming. For example, maternal low-protein rat offspring have elevated circulating aldosterone levels by 4 wk of age (416). In an adult population from Hertfordshire, UK, adrenocorticotropic hormone (ACTH) stimulated aldosterone responses and blood pressure that were negatively correlated with birth weight (324). The following section reviews evidence for the role of RAS in the renal programming of hypertension.

In the maternal low-protein rat model, angiotensin infusion in 10-wk-old IUGR offspring demonstrated an exaggerated pressor response compared with control rats (252). Interestingly, although renal AT1a receptor expression was not affected in low-protein IUGR offspring, they demonstrated a significantly decreased expression of AT2 receptors compared with controls (252). The authors speculate that maternal exposure to a low-protein diet detrimentally programs the RAS, which may contribute to the impairment of renal development and elevation of blood pressure. This theory was supported by Sahajpal et al. (348) who assessed renal function following ANG II infusion in control and low protein IUGR rat offspring. Their results show that when challenged with ANG II, low-protein offspring responded with a greater decrease in glomerular filtration rate than controls. The authors speculate that blood pressure may be elevated in low protein rats to maintain glomerular filtration rate against a background of fewer nephrons, thus providing a physiological adaptation explanation which may link the observed finding of decreased nephron number with adult onset hypertension (348).

As was alluded to in the previous paragraph, much controversy exists in the literature regarding alterations in the various components of the RAS in animal models of programmed hypertension, in particular the maternal protein restricted rat models of IUGR. For example, IUGR rats exposed to a low-protein diet in utero had
decreased renal expression of AT1 and AT2 receptors on postnatal day 1, but increased expression at 4 mo of age (417). On the other hand, Cooke et al. (83) demonstrated that kidneys from female low-protein IUGR offspring had decreased AT2 receptor expression at embryonic day 19, with no difference noted in males, while AT1 receptor abundance was not different between groups. Furthermore, while AT2 receptor levels were increased in both male and female low protein offspring at postnatal day 21, AT1 was increased only in males (83). The sex of the offspring studied was not specified by Vehaskari et al. (417) and may partially explain the differing results (in addition to differences in dietary protocols). Thus there appears to be both sex-specific and temporal alterations of renal angiotensin receptors in models of programmed hypertension (TABLE 1).

In a rat model of placental insufficiency, the reduced utero placental perfusion (RUPP) model, adult-onset hypertension has been shown to be dependent on the RAS as blockade with enalapril during gestation prevents the development of elevated blood pressure in adult IUGR offspring. In addition, pressor responses to ANG II were enhanced in IUGR offspring, without evidence of altered angiotensin receptor expression (293). Furthermore, the mechanisms leading to hypertension in this model appeared to be sex-specific. Only male IUGR offspring develop sustained hypertension as they age, an effect which may be testosterone mediated since castration prevented both enhanced responses to ANG II and hypertension in this model (290). Conversely, female IUGR offspring appeared to be somewhat protected from programming effects by a beneficial effect of estrogen. Indeed, ovariectomy in IUGR offspring significantly increased blood pressure to a greater extent than in the controls, and this could be reversed with estrogen administration (289).

In a sheep model of IUGR and programmed hypertension, renal dysfunction is also thought to be RAS dependent. Offspring from betamethasone-treated pregnant sheep developed increased blood pressure, an effect which could be prevented by candesartan (AT1 receptor blocker). Furthermore, an increase in serum ACE and decrease in ACE 2 activity was demonstrated in betamethasone-treated offspring. These data suggest that antenatal steroid treatment may cause a chronic alteration of ACE and ACE 2, which may contribute to the higher blood pressure in this model of fetal programming-induced hypertension (372). Similar studies have also found that plasma renin activity is increased in offspring from betamethasone-treated pregnant
Table 1. Summary of changes in renal expression of components of the RAS in a variety of animal models of developmental programming of cardiovascular disease

<table>
<thead>
<tr>
<th>Model</th>
<th>Species</th>
<th>Gestational Age Studied</th>
<th>Sex</th>
<th>Findings</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal LP diet GD 12-birth</td>
<td>Rat</td>
<td>PDO0</td>
<td>NS</td>
<td>↔ AT1a protein, ↓ AT1b, increased AT2 RNA, ↓ AT2 protein</td>
<td>417</td>
</tr>
<tr>
<td>Maternal LP diet GD 0-birth</td>
<td>Rat</td>
<td>PDO0</td>
<td>NS</td>
<td>↓ renin RNA, ↓ ANG II</td>
<td>435</td>
</tr>
<tr>
<td>Maternal LP diet GD 0-birth</td>
<td>Rat</td>
<td>4 weeks, mixed</td>
<td></td>
<td>↔ AT1, ↓ AT2</td>
<td>252</td>
</tr>
<tr>
<td>Maternal LP diet GD O-weening (PD21)</td>
<td>Rat</td>
<td>E19, male</td>
<td></td>
<td>↑ renin RNA, ↑ ACE RNA, ↑ AT1 RNA</td>
<td>83</td>
</tr>
<tr>
<td>Maternal LP diet GD O-weening</td>
<td>Mice</td>
<td>PD 30, mixed</td>
<td>male</td>
<td>↑ renin, ↔ AT1, ↔ AT2, ↔ ACE protein</td>
<td>173</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>female</td>
<td>↔ AT1a, ↔ AT1b</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone GD 14.5–16.5</td>
<td>Rat</td>
<td>E16.5, E20.5, 6 months</td>
<td>NS</td>
<td>↓ AT2, ↑ AT1a, ↑ AT1b</td>
<td>383</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↑ ACE RNA, ↑ renin RNA, ↔ AT1a, ↔ AT1b</td>
<td>437</td>
</tr>
<tr>
<td>Dexamethasone GD 26–28</td>
<td>Sheep</td>
<td>E130, NS</td>
<td></td>
<td>↑ AT1 RNA, ↑ AT2 RNA, ↑ angiotensinogen</td>
<td>262</td>
</tr>
<tr>
<td>Cortisol GD 26–28</td>
<td>Sheep</td>
<td>E130, NS</td>
<td></td>
<td>↑ AT1, ↔ AT2 RNA</td>
<td>262</td>
</tr>
<tr>
<td>RUPP</td>
<td>Rat</td>
<td>newborn, 6 weeks, 16</td>
<td>NS</td>
<td>↓ renin RNA, ↓ angiotensinogen, ↔ AT1</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>weeks</td>
<td></td>
<td>↔ renin RNA, ↔ angiotensinogen, ↔ AT1</td>
<td>150</td>
</tr>
<tr>
<td>Bilateral uterine artery ligation, GD 18</td>
<td>Rat</td>
<td>6 months, 18 months, 3</td>
<td>male</td>
<td>↑ AT1a RNA, ↑ AT1b</td>
<td>263, 433</td>
</tr>
<tr>
<td></td>
<td></td>
<td>months</td>
<td>female</td>
<td>↔ AT1a, ↔ AT1b</td>
<td>263, 433</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18 months</td>
<td>↔ AT1a, ↔ AT1b</td>
<td>263, 433</td>
</tr>
<tr>
<td>Prenatal caffeine deficiency</td>
<td>Rat</td>
<td>fetal, PD 24, male, E21</td>
<td>male</td>
<td>↓ AT2 RNA</td>
<td>13</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>female</td>
<td>↔ AT1a, ↔ AT2 RNA</td>
<td>142</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>female</td>
<td>↔ AT1, ↔ AT2</td>
<td>142</td>
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<td>↔ AT1, ↔ AT2</td>
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<td>↑ AT1 RNA and protein, ↔ AT2 RNA and protein</td>
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NS, not specified; LP, low protein; GD, gestational day; PD, postnatal day; E, embryonic day. [Modified from Moritz et al. (259).]
Thus it is clear that regardless of the animal model studied, there appears to be an integral role of the RAS in developmental programming of hypertension, which is not limited to the kidney, but also involved detrimental systemic effects in the vasculature and adipose tissue.

3. Pre- and postnatal dietary mismatch and renal function

Epidemiologic evidence exists that the detrimental effects of being born small are exacerbated if the growth trajectory is accelerated after birth (3). Less is known, however, about the effects of postnatal catch up growth on renal structure and function. Boubred et al. (43) modified the maternal low-protein diet to include a postnatal overfed group. Their data demonstrate that blood pressure was increased in male but not female overfed groups (IUGR and control birth weight), but this effect could not be fully explained by a decreased nephron number observed in IUGR males (43). Thus early postnatal overfeeding may act as a “second hit” contributing to renal dysfunction and hypertension, especially in males. In a sheep model, Cleal et al. (78) found that sensitivity to ANG II, renal function, and blood pressure were most affected in animals that were exposed to a dietary mismatch (for example, control diet in utero/undernourished postnatally, or undernourished in utero/control diet after birth) (78). These data support the concept that blood pressure and kidney (and vascular) function may be predetermined by the intrauterine nutritional environment, with the expectation that it will be similar to the postnatal nutritional environment. If these predictions are not met, the adult renal function may be maladapted and at greater risk of cardiovascular disease.

In summary, the kidney is highly sensitive to in utero developmental perturbations, which makes it a central player in fetal programming of hypertension. Multiple mechanisms contribute to renal programming, including reduced nephron number and an abnormally activated RAS, although the contribution of each varies depending on animal model and sex. Nevertheless, targeting renal function for monitoring and therapies aimed at preserving renal function may benefit those neonates born from a suboptimal intrauterine environment.

B. Vascular Dysfunction

Control of blood volume by the kidney is one of two primary mechanisms that closely regulates systemic blood pressure; the second is the control of systemic vascular resistance. Peripheral vascular resistance is determined by the patency of blood vessels which are small in diameter and provide the majority of resistance to the flow of blood (326). Children born SGA or LBW have been shown to have impaired vascular function and increased vascular resistance both in early postnatal life (138, 249, 279) and on into their late childhood, teenage years (183, 185, 250, 370, 391), and early adulthood (314). The patency of blood vessels is a product of both passive (physical aspects of the blood vessel) and active (vascular reactivity) properties. Both of these components of the vascular phenotype have, therefore, been extensively investigated in the development of hypertension following a compromised pregnancy.

1. Vascular remodeling

Many studies have demonstrated remodeling of the vasculature following a compromised pregnancy. Umbilical arteries, vessels of a fetal origin, from pregnancies compromised by growth restriction in utero, have been shown to have increased wall stiffness associated with decreased expression of insulin-like growth factor I (IGF-I), a regulator of elastin synthesis (50). Increased vascular stiffness was found to continue into childhood; in lower birth weight twins from twin-twin transfusion syndrome, arterial distensibility was reduced up to 14 mo of age (72). Children born with a LBW had decreased arterial elasticity at 5–8 yr of age (334) and increased carotid wall thickness at 3–6 yr of age (88, 89). Furthermore, children born SGA were also shown to have increased vascular wall intima-media thickness in both the aorta and carotid arteries (368, 390, 421).

In line with observations in human vessels, an increased intima thickness was also observed in aortae of adult offspring from Japanese macaques, Western sheep, Sprague-Dawley and Wistar rats, and chicks exposed to adverse pregnancy conditions (58, 117, 200, 305, 350, 401). The mechanisms were further investigated in Sprague-Dawley rat offspring in which the aorta was shown to have increased smooth muscle cell number, decreased endothelial cell volume, and increased vascular stiffness following a maternal high-fat diet (15). In sheep, aortic vascular remodeling was associated with increased collagen (100) and reduced elastin content that was vascular bed specific as no changes were observed in the superior mesenteric artery (401). An increased occurrence of aortic internal elastic lamina defects was also observed in offspring of bilateral uterine artery ligation rats (299). Vascular remodeling has been observed in the pulmonary arteries, where maternal nutrient restriction led to increased smooth muscle thickness following acute (240) or chronic (375, 445) exposure of the offspring to hypoxia. Furthermore, the mesenteric...
arcade smooth muscle content was increased in a maternal undernutrition model (200). One opposing study found that the smooth muscle content of mesenteric arteries was reduced following maternal protein restriction in rats (328); this might, however, be due to a lack of protein available for normal vascular tissue synthesis.

Taken together, these data provide a wealth of evidence that a compromised in utero environment leads to changes in the development of the systemic vasculature causing the formation of stiffer, less distensible blood vessels via alterations in the collagen/elastin balance, thereby contributing to the later development of hypertension.

2. Angiogenic factors

The formation of vascular tissue is regulated by various angiogenic factors. In animal models of programming, numbers of microvascular blood vessels were decreased in various vascular beds from fetal sheep and rats (57, 107, 275, 308, 342). Furthermore, blood from umbilical cords of IUGR pregnancies (385) and capillaries in IUGR rats (308) have both been shown to have a reduced angiogenic capacity. Other studies have shown that factors involved in the stimulation of vascular growth and proliferation, such as vascular endothelial growth factor (VEGF) (42, 117, 277, 422, 438), placental growth factor (PIGF) (42), and IGF-I (50, 370, 453) are reduced in offspring following a compromised pregnancy, suggesting that this might be a cause of the reduced angiogenic capacity. A study by Sesso et al. (370) found that levels of matrix metalloproteinase (MMP)-2 and -9, gelatinases capable of breaking down vascular collagen, were elevated in children born SGA when measured at 8–13 yr of age (370) while others showed that these proteases were reduced in utero (385, 401). A loss of proliferative factors combined with increased breakdown of existing vascular structural factors may lead to the observed reduction in vascularity. Both decreased volume and elasticity of the vasculature would contribute to increased peripheral resistance in growth-restricted offspring.

3. Vasoconstrictor mechanisms

The other aspect of vascular resistance is vascular responsiveness to agonists. The vascular response to a given agonist may be assessed both in terms of sensitivity and efficacy. If the vascular sensitivity is altered, the vessel may respond to a lower (or higher) than normal concentration of agonist, and hence may become hyper- (or hypo-) reactive to physiological levels of that agonist. Alternatively, pathology may reside in the downstream mediators of a vascular response. In this situation, normal vascular reactivity of specific receptors may lead to heightened (or suppressed) constrictor or dilator effects. Various alterations in vascular function have been shown following a suboptimal in utero environment (FIGURE 3), and the outcome may be dependent on the vascular bed studied, period of insult, or sex of the offspring to name but a few of the potential variables.

Increased reactivity to vasoconstrictors may cause blood vessels to have an increased basal tone, and therefore reduced lumen diameter, in the presence of normal levels of agonist. Indeed, increased myogenic tone has been observed in mesenteric (166, 223) and uterine (165, 415) arteries in offspring from Sprague-Dawley rats exposed to a high sucrose diet, undernutrition, or hypoxia during pregnancy. A sex-specific effect was seen in offspring of rats exposed to cocaine in utero whereby septal coronary arteries from female offspring displayed increased myogenic tone while males had decreased tone (444). This was thought to be due to a differential effect on Ca2+ sensitization.

A) ADRENERGIC MECHANISMS. The sympathetic branch of the autonomic nervous system relies primarily on the neurotransmitter norepinephrine (NE; also known as noradrenaline), which is released from the synapses of sympathetic neurons. Adrenoceptors, of which there are α and β subtypes, are present in both vascular and cardiac smooth muscle and respond to released or circulating catecholamines including NE and epinephrine (adrenaline). A sucrose-enriched maternal diet was shown to increase heart rate variability, an indication of increased autonomic control of the heart and, to some extent, increased peripheral sympathetic tone (95), and increased renal levels of NE in the adult (3 mo of age) hypertensive offspring (353), effects which were also observed following early postnatal stress in rats (231). Interestingly, in adult female, but not male, offspring of maternal nutrient-restricted sheep, the sympathoadrenal (catecholamine) response increased following exposure to stress (310).

Renal denervation, resulting in a >90% reduction in renal NE, has been shown to normalize hypertension in offspring of the rat RUPP model, suggesting a role of increased adrenergic nerve activity on kidney function and modulation of blood pressure (5, 180, 291). This series of studies further demonstrated that hypertension only persisted into adulthood in males, and both the early development and maintenance of hypertension was dependent on renal innervation. Indeed, following maternal exposure to cocaine, a stimulant known to increase blood pressure, only male rat offspring demonstrated increased blood pressure responses to NE administration at 3 mo of age (440). Interestingly, the sensitivity of renal arteries to the α1-adrenergic receptor (AR) agonist phenylephrine (PE) was decreased only in female offspring exposed to hypoxia-induced growth restriction in rats (418), suggesting that this might have a protective effect against the development of hypertension in this sex. Sympathetic innervation was increased in the tibial arteries following exposure to hypoxia in utero in rats.
and plasma levels of the adrenergic neurotransmitter NE were increased in IUGR fetal sheep (93) and chicken eggs incubated in hypoxic conditions (227). Interestingly, in the sheep study, blood pressure was reduced only in response to pan-α-AR inhibition but not to α1-AR inhibition (93). Paradoxically, femoral responses to sympathetic nerve stimulation were decreased in the offspring of hypoxic rat dams (335), suggesting a potentially desensitizing effect of sympathetic hyperinnervation, while in contrast, fetal femoral artery responses to PE ex vivo were shown to be in-

(335), and plasma levels of the adrenergic neurotransmitter NE were increased in IUGR fetal sheep (93) and chicken eggs incubated in hypoxic conditions (227). Interestingly, in the sheep study, blood pressure was reduced only in response to pan-α-AR inhibition but not to α1-AR inhibition.
increased in a similar model (431). Overall, models of in utero programming, therefore, often demonstrate increased sympathetic innervation, particularly of the kidney, leading to increased circulating levels of the neurotransmitter NE. However, the increased agonist levels may induce receptor desensitization in some cases.

Many studies have shown increased reactivity of mesenteric arteries to adrenergic agonists such as NE or the specific α1-AR agonist PE following a compromised pregnancy. The mesenteric arcade is known to be particularly important in vascular resistance given its ability to accommodate ~25% of the total blood volume (149, 341). As such, blood pressure may be particularly sensitive to changes in the reactivity of this vascular bed. For example, maternal obesity in mice (352), maternal Protein restriction in mice (332), maternal dexamethasone treatment in rats (285), chicken eggs exposed to hypoxic conditions (257), endothelial nitric oxide synthase (eNOS) knockout mice (230), and maternal cocaine exposure in rats (440) have all been shown to produce offspring with increased mesenteric artery sensitivity to adrenergic agonists. While α-ARs cause vasoconstriction, β-ARs mediate vasodilation. Response to the β-AR agonist isoprenaline (also known as isoproterenol) has also been shown to be reduced in IUGR offspring of eNOS knockout mice (230), further contributing to a constrictive phenotype. Other models of maternal hypoxia (44) or protein restriction in rats (46, 58, 358, 406) have not observed an alteration in mesenteric AR function, a discrepancy which might be due to the type and/or timing of the in utero insult experienced by the developing fetus.

The carotid artery is a conduit artery important in the supply of blood to the brain, an organ which has been shown to be spared in growth-restricted fetuses (reviewed in Ref. 243). Thus it is suggested that function of this artery is preserved in compromised pregnancies. Indeed, decreased carotid contractility to PE, allowing for a less constrictive phenotype, has been observed in male but not female offspring following maternal exposure to either a high-fat diet (54) or nicotine (122) and in fetal offspring (sex not reported) of rats exposed to maternal undernutrition (59) or hypoxia (431). Other studies, however, have shown increased reactivity of the carotid artery to NE or PE following maternal hypercholesterolemia (209) or in eNOS knockout mice (75, 77, 86, 230) that occurred in both male and female offspring. Effects of in utero programming on the carotid β-AR function has also been shown to be variable with either no change (54) or decreased (230) responsiveness to isoprenaline observed. This suggests a high degree of variability in the effect of poor pregnancy conditions on the outcome of carotid artery AR function, which may be dependent both on the in utero insult experienced and the sex of the fetus.

The aorta is a primary conduit artery that is important in the progression of cardiovascular pathologies such as atherosclerosis (reviewed in Ref. 80). A maternal high-fat diet in mice (68), AT1-Ab positive rats (452), and maternal hypoxia in rats (167) have all been demonstrated to produce offspring with decreased aortic responsiveness to adrenergic agonists. In one study this was shown to be sex dependent, occurring in females but not males (67). Other studies did not find altered reactivity in either sex (15, 58, 331) or found enhanced reactivity in both sexes (366) following compromised pregnancies.

These data demonstrate the high degree of variability that is observed across programming models. While, in general, adrenergic induced vasoconstriction is increased, there may be differential effects in different vascular beds (mesenteric > carotid > aorta), sexes (male ≥ female), or models.

b) Vascular RAS. As previously described, the RAS is known to be altered by conditions in utero and may have a considerable bearing on the ability of the kidney to regulate chronic alterations in blood pressure. The RAS may also have a direct impact on vascular function and, thereby, acute regulation of blood pressure. Indeed, male IUGR offspring of the RUPP model had an increased hypertensive response to ANG II treatment that was attenuated by a Rho kinase inhibitor, suggesting a vascular origin of the hypertensive phenotype (292). Furthermore, hypertension in IUGR rat or sheep offspring was prevented by treatment with the ACE inhibitor enalapril or its active metabolite enalaprilat (57, 150, 208) or by AT1 antagonism using losartan or candesartan (307, 373, 374).

Alterations in vascular angiotensin signaling pathways have been observed in many models of developmental programming and in many vascular beds. As briefly described earlier in this review (FIGURE 2), under normal conditions the precursor ANG I is converted to ANG II by ACE and the AT1 receptor is responsible for the mediation of ANG II vasoconstrictor effects. Both ANG I and ANG II can be broken down further by ACE 2 to ANG-[1–9] and ANG-[1–7], respectively. ANG-[1–9] is also further degraded by ACE to ANG-[1–7] which mediates vasodilation via the Mas receptor. In the maternal low-protein model, increased blood pressure in the offspring was associated with increased carotid artery vascular AT1 receptor responsiveness to ANG II (57, 450). Increased involvement of the AT1 receptor in ANG II-mediated vasoconstriction has been corroborated in various models and vascular beds including mesenteric arteries of maternal high-sucrose-diet offspring (223), renal expression in maternal betamethasone-exposed offspring (154, 372), coronary arteries of maternal dexamethasone-exposed offspring (329), aortas of maternal nicotine-exposed offspring in which the specific AT1a receptor was identified (439, 443), and the pulmonary vasculature of mice exposed to hypoxia in utero in which the AT1b recep-
Developmental Programming of Hypertension

Correspondingly, the vasodilator effects of ANG-[1–7] may also be reduced in programmed offspring. Indeed, Chappell and co-workers demonstrated that renal AT2 receptor expression and ACE 2 activity were decreased (154, 372) while in the aorta of offspring exposed to postnatal stress, the involvement of the AT2 receptor in ANG II-mediated responses was abolished (232). Levels of cerebrospinal fluid ANG-[1–7] endopeptidase, an enzyme which breaks down ANG-[1–7] to ANG-[1–4], were also shown to be increased in offspring of betamethasone-treated sheep with correspondingly decreased levels of the ANG-[1–7] protein (247, 248) and decreased expression of the Mas receptor (246). This series of experiments also noted a decreased baroreflex sensitivity which has been linked to decreased cerebral ANG-[1–7] expression and may further contribute to the hypertensive phenotype in IUGR offspring (247, 373).

Interestingly, Xiao and co-workers (441, 443) found a protective effect of estrogen in the programming of ANG II pathways whereby pressor responses to ANG II were increased in males but decreased in females, with the female effect being reversed by ovariectomy. A sex-specific effect was also found by Fox et al. (122) who demonstrated that renal ANG levels were increased only in male offspring of nicotine-exposed mice, and by Chiossi et al. (75) who demonstrated increased carotid artery constriction to ANG II primarily in male offspring of eNOS knockout mice. This sex difference was also observed in the development of hypertension in response to ANG II treatment, which was observed only in male and not female offspring of hypoxic (441) or RUPP (292) pregnancies, an effect which could be also observed in females following ovariectomy. It seems likely, therefore, that the RAS is partly protected in female offspring of compromised pregnancies and might mediate aspects of the cardioprotection observed in some models of developmental programming of cardiovascular disease.

The RAS pathway has been well characterized in programming models and shows a more defined phenotype than the adrenergic pathways. A compromised in utero environment shifts the balance between the vasodilator (ACE/ANG-[1–7]/AT2/Mas) and the vasoconstrictor (ACE/ANG II/AT1) aspects in favor of a constrictive phenotype. Due to a larger number of studies, there is also more evidence supporting a clear sex dichotomy whereby males are affected to a greater extent than females, particularly at an early age.

c) Endothelin Mechanisms. Endothelin (ET) is one of the most potent vasoconstrictors and, as such, has been touted as a major player in many cardiovascular pathologies such as systemic hypertension, pulmonary arterial hypertension, and dilated cardiomyopathy (reviewed in Ref. 273). In keeping with these observations, the vascular pathologies associated with in utero programming may also be associated with alterations in ET signaling. In a model of maternal undernutrition, pulmonary endothelin-1 (ET-1) levels were shown to be increased (446), potentially due to alterations in the epigenetic profile. In line with this finding, aortic contractility to ET-1 was also enhanced in female, but not male, offspring born to dams with gestational diabetes (366). In other vascular beds, the results have been variable: femoral artery contractility was unaltered in fetal offspring of hypoxic rats (431), mesenteric artery responsiveness to ET was shown either to be reduced in offspring of sheep treated with dexamethasone (367), unaltered in rats exposed to hypoxia in utero (44), or increased in chicks incubated in hypoxic conditions (257) while carotid artery responsiveness was also unaltered in offspring exposed to maternal undernutrition (59) or hypoxia (431). Interestingly, in the rat hypoxia model, expression of both ET-1 and its inactive precursor bigET-1 were increased in mesenteric arteries from both sexes while responses to bigET-1 (and systolic blood pressure) were increased only in males. Furthermore, this increased blood pressure could be reduced by treatment with tezosentan, an ET receptor inhibitor (44).

While research to date has provided highly variable answers as to the involvement of ET in the pathophysiology of the in utero origins of hypertension, it is possible that the impact of a compromised pregnancy may occur at either the level of epigenetic programming of bigET-1 gene expression and/or in conversion of the inactive precursor to the active ET isoform. Further investigation of this pathway in the programming of hypertension is warranted.

d) OTHER CONSTRICCTOR MECHANISMS. Although abundant evidence exists that vasoconstrictor pathways are increased in
models of programmed hypertension (described above), other constrictor mechanisms have shown contrary results. For example, a high-fat diet given to mouse offspring post-weaning was shown to interact with a maternal high-fat diet to reduce aortic responses to the thromboxane analog U46619 (68). Aortic sensitivity to U46619 was also reduced in rats following maternal undernutrition (58), while mesenteric artery sensitivity was reduced in adult rat offspring of the maternal protein restriction model (3 mo of age); however, this was shown to be normalized in later life (5.5 mo of age) (46). In mouse offspring of the maternal high-fat diet model, a sex difference was observed in decreased responsiveness to the thromboxane analog in carotid arteries (54) and aorta (67) with only females, and not males, demonstrating reduced responses.

With respect to vascular responsiveness to serotonin (also known as 5-hydroxytryptamine, 5-HT), maternal hypercholesterolemia did not alter carotid artery function in either male or female offspring (209). In offspring of maternal high-fat diet mice, however, a sex difference was observed in that males, but not females, had decreased carotid responsiveness to serotonin (54). Interestingly, the precise opposite effect was found in offspring of maternal protein restricted rats; females, but not males, had reduced mesenteric artery sensitivity to serotonin (358). Pregnancy at high altitude also demonstrated decreased pulmonary artery sensitivity to serotonin in fetal sheep that was secondary to a decreased involvement of the 5-HT_1B/D receptors (146); however, another study showed increased pulmonary artery responses to serotonin that were found to be due to increased Rho-kinase involvement (37). Interestingly, only female offspring demonstrated enhanced aortic contractility to serotonin in a rat model of maternal gestational diabetes (366). Given the heterogeneity of the literature to date, further studies are required to determine the involvement, or otherwise, of the serotonin or thromboxane pathways in the programming of hypertension.

The application of a high potassium solution (KCl) is used to determine the non-receptor-mediated constrictor capacity of a vascular bed. In both male and female offspring of maternal high-fat diet mice, aortic constrictor capacity was reduced (67). Maternal undernutrition (58), maternal hypoxia (167, 184), and pregnancy at high altitude (174) were also found to reduce maximal responses to KCl in the aorta, carotid, and pulmonary arteries of rat offspring. A maternal low-protein diet in mice (331, 332) or rats (406, 450), maternal dexamethasone treatment in mice (331), chicken eggs incubated in hypoxic conditions (227, 257), offspring of eNOS knockout mice (229), and maternal cocaine exposure (444), however, had no effect on aortic, carotid, mesenteric, femoral, or coronary artery constrictor capacity. In contrast, other studies demonstrated increased constrictor responses to KCl in mesenteric arteries of only male offspring from dams treated with dexamethasone (285), in aorta of offspring from dams exposed to hypoxia (58), in pulmonary arteries of offspring from pregnancies at high altitude (37), and in carotid arteries of offspring of eNOS knockout mice (229). Changes in KCl responses may reflect vascular remodeling, which has an impact on the total constrictor capacity. In previous sections we detailed the vascular remodeling observed in offspring of both human and animal pregnancies which were complicated in utero, and these changes resulted in increased vascular wall thickness. Since altered intima-media thickness was thought to result from increased smooth muscle cell number, this would be in line with studies which found an increased responsiveness to KCl but not with those demonstrating a decreased responsiveness. An increased number of smooth muscle cells, however, might not necessarily reflect improved constrictor capacity if the components of the cell were disrupted or malformed. Indeed, some of the vascular remodeling work has shown that an increased number of defects were observed in the elastic lamina layers (299). Furthermore, vascular stiffness was shown to be increased as a result of increased collagen content, and this could hinder the flexibility of the vessel both in terms of vasodilation and vasoconstriction; therefore, both increased and decreased responses to KCl could be equally indicative of vascular dysfunction following a compromised pregnancy.

While the current literature reflects a generalized hyperconstrictive phenotype in models of programming of hypertension, there exists a complete range of results in almost every vascular bed and model studied. Of the factors that may influence the observed results, sex and age appear to have the most influential roles. The age at which a study is conducted may capture distinct stages in the development of disease in these offspring. Several studies have described an early progression of a pathological phenotype compared with control animals, and this is further compounded by a differential progression in females and males. Additional studies would be valuable in elucidating this potential “early aging” phenomenon and its relation to sex.

4. Vasodilator mechanisms

A fine balance exists whereby changes in either the vasoconstrictor or vasodilator capacity can lead to an imbalance in the tone of a vascular bed, potentially leading to the development of a hypertensive phenotype. Impaired endothelial cell function is an early phenotype and is often a precursor to hypertension. The total vasodilator capacity in response to the endothelial-dependent agent acetylcholine (ACh), its synthetic analog methylcholine (MCh), or bradykinin has been shown to be reduced in rodent mesenteric arteries from offspring of dams fed either a high-fat (352) or nutrient-restricted diet (46, 123, 271, 328, 405, 406). In mouse offspring from nutrient restricted dams, this effect occurred only in males (332), while in a study of rat offspring from low-protein-fed dams, mesenteric artery responses were reduced in females only following ovariectomy (271), sug-
gesting a protective effect of estrogen. In line with this hypothesis, a female specific increase in mesenteric ACh-induced vasodilation was observed in offspring of mice fed a high-fat diet (34). Conversely, renal artery vasodilation to MCh was reduced in only female offspring of rats exposed to hypoxia in utero (418), demonstrating the vascular bed specificity of these protective effects.

Vasodilation to ACh was also reduced in aortas from programmed offspring of Japanese macaques (117), mice (68, 331, 333), or rats (15, 226, 442, 452). In a mouse model of high-fat diet-induced programming, aortic endothelial dysfunction was further exacerbated by the consumption of a high-fat diet in the postnatal period (68), demonstrating an extended window of timing for developmental influences. Rat offspring of dams with gestational diabetes demonstrated aortic dilation to ACh that was impaired in females but was enhanced in males (366). Furthermore, a male specific increase was observed in aortic dilation in offspring of C57Bl/6 mice fed a high-fat diet (67), suggesting that the protective effect of female hormones in the programming of endothelial function occurred only in resistance arteries such as the mesenteric and not in larger conduit arteries. This was confirmed to some extent by the observation of reduced vasodilation in resistance-sized femoral arteries of hypoxic rat offspring (135) while no changes were observed in ACh-induced vasodilation of other conduit arteries such as the renal arteries of Japanese macaques (117), carotid arteries of hypercholesterolemic mice (209), aorta of nutrient-restricted rats (58, 406), and aortas or pulmonary arteries of hypoxic rats (58, 167, 184, 266).

The effects of in utero programming on total vasodilator capacity (as assessed by dilation responses to ACh) have shown primarily reduced endothelial-dependent responses in small resistance-sized arteries. However, the specific mechanisms underlying the development of vascular endothelial dysfunction in animal models of programming have also been investigated in some detail. There are three primary vasodilator pathways, namely, nitric oxide (NO), endothelium-derived hyperpolarization (EDH), and prostaglandin mediated, and the evidence regarding alterations in these pathways is as follows.

A) NITRIC OXIDE. One of the major vasodilator pathways that has been shown to be affected by developmental programming is the NO pathway. The primary enzyme responsible for the production of vascular NO is nitric oxide synthase (NOS), which catalyzes the production of NO from L-arginine (FIGURE 4). There are three isoforms of NOS, namely, eNOS (also known as NOS III), neuronal NOS (nNOS, also known as NOS I), and inducible NOS (iNOS, also known as NOS II). NO and L-citrulline are produced through the oxidation of L-arginine in a reaction that requires the presence of flavins (flavin adenine dinucleotide, FAD; and flavin mononucleotide, FMN), heme, reduced nicotinamide ade-

Homozygous offspring of eNOS knockout mice are born IUGR and have delayed development with increased postnatal mortality (297). Several groups have investigated the effect of in utero programming using the eNOS knockout mouse strain and have observed effects in either heterozygous or homozygous offspring to determine the relative influence of maternal (eNOS knockout intrauterine environment) versus paternal (normal intrauterine environment) genetics. With the use of heterozygous offspring, the maternal eNOS knockout intrauterine environment was found to be critical for the development of hypertension, both basal and stress-induced, in offspring (414), demonstrating a role for the NO pathway in the development of cardiovascular disease. In the eNOS knockout model, expression of the endothelial isoform of NOS is abolished, but this does not preclude upregulation of either the neuronal [also expressed in the endothelium (18, 60, 61)] or inducible isoforms. As would be predicted by a reduction in NOS, however, ACh-mediated vasodilation was shown to be reduced in carotid arteries of both male and female offspring of homozygous and heterozygous eNOS knockout mice when studied up to 21 wk of age (75, 230). Furthermore, reduced basal NO production was found to be responsible for increased vasoconstriction in this vascular bed in heterozygous offspring (86), and vascular dysfunction was found to extend to the mesenteric arcade (230). In addition, the eNOS knockout mouse has been useful in determining other influences on the hypertensive outcome. For instance, Clark et al. (77) determined that cross-fostering of heterozygous offspring onto mothers with the opposite genotype could reverse the carotid vascular effects, demonstrating the importance of the postnatal environment in compounding the development of cardiovascular disorders and providing an extended window of opportunity for therapeutic intervention. Interestingly, in the eNOS knockout mouse, maternal parity [increased parity reducing vascular dysfunction (229)] and generational [persistent hypertension and vascular dysfunction in second generation offspring (87)] effects have also been observed. However, these trans-generational effects on aortic vascular dysfunction were not confirmed in another model of in utero programming, the rat high-fat-diet model, in either male or female F2 offspring (14).

In vascular tissues from animal models of in utero programming, eNOS expression has been shown to be reduced in the aorta of offspring of rats fed a protein-restricted diet (405), high-salt diet (305), or zinc-restricted diet (404); rabbits...
exposed to hypoxia (71); mice exposed to nicotine (122); or pulmonary arteries of a maternal hyperthermic sheep model (342). As a result, basal production of NO has been shown to be decreased in the aorta (124, 331) and pulmonary arteries (342). In other studies, eNOS expression was unaltered: aortic or renal beds from offspring of Japanese macaques fed a high-fat diet (117), in cerebral (208) or in mesenteric arteries of rats exposed to maternal hypoxia (432). Expression levels alone, however, do not provide information on the functionality of the enzyme. Altered phosphorylation of eNOS can directly regulate the balance of NO and superoxide production (69) and, thereby, impact NO bioavailability. Depending on the amino acid involved, phosphorylation of eNOS may have either positive [Ser1177 (human and rodent)/Ser1179 (bovine)] or negative (Thr495) effects on its enzymatic activity (120). In human full-term IUGR complicated pregnancies, phosphorylation of eNOS at Ser1177, eNOS activity, l-arginine transport, and NO synthesis have all been shown to be reduced in umbilical vein endothelial cells without any reduction of eNOS protein abundance (63). In rat offspring exposed to maternal cocaine, eNOS protein expression was unaltered, but phosphorylation at Ser1179 was decreased leading to decreased NO-mediated vasodilation of mesenteric arteries (440). Furthermore, ANG II-stimulated production of reactive oxygen species (ROS) was increased while that of NO was decreased in the offspring of sheep exposed to beta-methasone (154), which could be due to the uncoupling of eNOS leading to the generation of ROS and scavenging of NO (FIGURE 4).

FIGURE 4. Schematic representation of eNOS uncoupling. A: normal functioning of the eNOS enzyme in catalyzing the production of nitric oxide (NO) plus L-citrulline (L-cit) from molecular oxygen (O_2) and L-arginine (l-arg). This reaction requires the presence of the flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) to facilitate the transfer of electrons from oxidized nicotinamide adenine dinucleotide phosphate (NADPH) to donation to the iron of the haem and tetrahydrobiopterin (BH4) molecules also associated with the eNOS enzyme. Calmodulin is activated by calcium and is required to control the flow of electrons during the catalytic activity of eNOS. BH4 donates an electron and proton to the cycle which facilitates the conversion of O_2 plus l-arg to NO plus l-cit. B: in the presence of increased oxidative stress and peroxynitrite (OONO·), BH4 is oxidized to the BH_3 radical and then to BH_2, reducing its availability to eNOS. This uncoupling of eNOS from its cofactor results in the donation of an electron to molecular oxygen leading to the production of the superoxide (O_2·) radical and preventing the production of NO. O_2· may also be produced by NADPH oxidase and, in a reaction with NO, enhance OONO· production thereby further contributing to the uncoupling of eNOS and BH_4 and reduction of NO bioavailability.
tine in mesenteric and uterine arteries (165, 166) was decreased in the offspring of hypoxic rat dams. In addition, renal artery NO-mediated vasodilation was decreased only in female offspring (418). In contradiction, the contribution of NO to bradykinin-induced vasodilation was shown to be increased in the fetal coronary arteries of offspring of nutrient-restricted sheep (379). Taken together, these results indicate that, in general, either reduced expression or activity of the NOS enzyme may result in reduced production of NO in programmed offspring.

Despite the observation of reduced production of NO or involvement in vasodilation, many studies have demonstrated no changes in the smooth muscle sensitivity to NO. For example, sodium nitroprusside (SNP), an NO donor, responses were unaltered in rodent mesenteric arteries of offspring from dams fed either a high-fat (54, 352) or nutrient-restricted (123, 271) diet. In the rodent aorta, aqueous NO responses were unaltered in offspring of the maternal high-fat diet (15), and SNP responses were unaltered in the maternal low-protein-diet mouse model (331), mice exposed to maternal dexamethasone (331), and rats exposed to maternal hypoxia (58, 266). In pulmonary arteries, SNP-induced responses were unaltered in the hypoxia rat model (184). Other investigators have observed increased smooth muscle sensitivity to SNP in the aorta of maternal undernutrition offspring (58) and mesenteric arteries of offspring from dams exposed to protein restriction in utero (332). In some cases, the downstream signal transduction mechanisms of the smooth muscle may also become involved. Indeed, expression of the smooth muscle mediators of NO-induced vasodilation, namely, the enzyme soluble guanylate cyclase (sGC) and its product cGMP, were both found to be decreased in protein restricted rat offspring, sex not reported (208). However, reduced sGC was not observed in female rat mesenteric arteries in a similar model (328). SNP-induced vasodilation has also been shown to be reduced in some studies, e.g., in aorta, carotid, cerebral, and mesenteric arteries from the offspring of rodent models of maternal protein restriction (46, 57, 208, 332), and in the aorta of rats exposed to hypoxia in utero (167). This suggests that while the mechanism of reduced NO-mediated vasodilation may reside primarily in the endothelium and disruption of the eNOS/NO pathways, there may be a further compounding effect of disruption of downstream signal transduction mechanisms.

The contribution of NO to basal vascular tone in programming models is also controversial. In the rat nutrient restriction model, the pan NOS inhibitor Nω-nitro-l-arginine methyl ester hydrochloride (l-NAME) did not alter the increased myogenic tone observed in uterine arteries (415) nor cause increased vascular tone in carotid arteries (59), suggesting that basal NO-mediated vasodilation had little involvement in vascular tone in these offspring. The involvement of vascular NO in modulating blood pressure may, however, be heightened in some models of in utero programming. Studies have demonstrated that infusion of l-NAME increased systemic blood pressure to a greater extent in female mouse offspring from protein restricted dams than their controls, an effect which was not observed in males (332). Interestingly, the same study demonstrated that inhibition of 11β-HSD (a model of fetal exposure to the stress hormone cortisol) in the mother led to the opposite effect with increased pressor responses to l-NAME in male but not female offspring (332), consistent with both model- and sex-specific programming effects. Offspring of dexamethasone-treated sheep also demonstrated an increased pressor response to another NOS inhibitor, l-NAME, nitroarginine (l-NNA) (367), although in this study results were not discriminated by sex.

Disruption of the eNOS/NO pathway, therefore, has been shown to be instrumental in causing a hypertensive phenotype via vascular endothelial or smooth muscle dysfunction, leading to increased constrictor and decreased dilator effects in many of the programming models. It is important to note, however, the conflicting evidence for NO involvement in models of developmental programming. In the case of upregulation of the NO pathway, this might be attributed to a compensatory effect in response to increased blood pressure, a mechanism which might lead to greater vascular dilation and reduced peripheral resistance. It is possible, however, that failure to upregulate NO or for NO to adequately offset the effects of renal function programming (for example) might lead to subsequent progression of cardiovascular disease. The time course of the development of vascular dysfunction and hypertension, therefore, requires further investigation.

In summary, although NO is a critical modulator of function in many vascular beds, the effects of developmental programming on this particular pathway are complex (including effects on NO bioavailability that are addressed in sect. II). A greater knowledge of the progression of vascular pathology during fetal and postnatal life might be instrumental in identifying therapeutic options for the treatment or prevention of hypertension.

b) ENDOTHELIUM-DERIVED HYPERPOLARIZATION. While less research has been performed on the EDH pathways (FIGURE 3), a study in offspring of nutrient-restricted sheep has shown that EDH-mediated vasodilation was absent in coronary artery responses to bradykinin (379). A maternal high-fat diet has also been demonstrated to reduce the involvement and expression of large calcium-activated potassium channels (BKca) in the vasodilation of mesenteric arteries from offspring of rats fed a high-sucrose diet throughout gestation (223). The primary activator of BKca is epoxyeicosatrienoic acid (EET); however, changes in the production of EET from arachidonic acid have not been addressed in models of developmental programming. The
involvement of other potassium channels, such as the voltage-gated potassium channel (K_V), was reduced in the pulmonary arteries from rat offspring of nutrient restricted dams, while the K_Ca and inward-rectifying potassium channels (K_IR) were unaltered (240). In other studies, EDH contribution to vasodilation was maintained in rat offspring of hypoxic dams while the involvement of myoendothelial gap junctions increased in females but not males (265, 266). There is, therefore, much less evidence on which to determine the effect of in utero programming on EDH pathways and whether these might be instrumental in mediating hypertension, or indeed maintaining vascular function, leaving it open as an interesting option for potential therapeutic development.

C) Prostaglandins. The involvement of prostaglandins (FIGURE 3) in mediating vasodilation is often relatively minor (266, 432); however, developmental programming has been shown to further reduce its involvement in mesenteric artery vasodilation in male offspring of protein-restricted (405) or vascular tone in male offspring of hypoxic (166) rats. This may be a result of reduced receptor expression since a study by Van Huyen et al. (413) demonstrated reduced vasodilation that was a result of reduced prostacyclin receptor expression. Correspondingly, pulmonary reactivity to the vasoconstrictor prostaglandin F_2 alpha was found to be decreased in offspring of hypoxic rat dams (184), in line with the previously mentioned decreased reactivity to thromboxane in aorta (58, 67, 68), carotid (54) and mesenteric arteries (46), and suggesting a systemic alteration in prostaglandin receptors. In growth-restricted fetuses from twin pregnancies in uterine artery-ligated sheep, however, blood levels of the vasodilatory and pyretic prostaglandin E_2 were increased (32). Since anti-inflammatory treatment with sulfasalazine was unable to ameliorate this increase, it is possible that this was a result of a compensatory vascular increase in the production of prostaglandins rather than an excess inflammatory response. Conversely, a study by Williams et al. (432) found that mesenteric arteries in adult offspring of hypoxic dams had an increased prostaglandin-mediated vasoconstrictor response. Furthermore, acute hypoxia (gestational day 19–21) combined with cyclooxygenase inhibition caused right ventricle hypertrophy, pulmonary artery remodeling, and increased vascular eNOS expression (445), suggesting that a reduction of prostaglandins in utero might lead to the development of pulmonary hypertension.

C. Oxidative Stress

An area that has engendered particular attention in the programming of cardiovascular disease is the development of both cellular and systemic oxidative stress and its subsequent effect on vascular endothelial function. Oxidative stress is a strong contender as a main contributor to reduced endothelial function in models of programmed hypertension. Increased levels of ROS cause increased scavenging of NO to produce peroxynitrite, thereby reducing the bioavailability of NO for vasodilation. Furthermore, peroxynitrite can cause uncoupling of eNOS leading to further production of superoxide and scavenging of NO (FIGURE 4). Peroxynitrite acts on the cofactor for eNOS enzymatic activity, BH_4, causing its conversion to the BH_3 radical and then BH_2. This loss of BH_4 impacts the ability of eNOS to convert O_2 plus L-arginine to NO plus L-citrulline and instead leads to the production of superoxide. Total antioxidant capacity has been shown to be reduced in the offspring of women with obesity (346), while markers of oxidative stress, oxidized LDL, and malondialdehyde, were increased in human IUGR twins (241). In animals, liver glutathione levels, an indicator of antioxidant status, were decreased in rat offspring of nutrient restricted dams (57). Interestingly, both reduced antioxidant (glutathione) expression and enhanced production of markers of ROS observed in whole blood of nutrient restricted rat offspring were resolved by adulthood (57). This series of experiments also demonstrated increased aortic superoxide production at 9–12 wk of age (450), suggesting that tissue specific oxidative stress may continue throughout adulthood. Endogenous antioxidants, such as SOD, catalyze the breakdown of superoxide to maintain the tissue oxidant balance. There are several isoforms of SOD, namely, copper/zinc (CuZnSOD) and manganese (MnSOD) which are found in the cytosol and mitochondria, respectively, and a third extracellular iron isoform (FeSOD). In some studies, endogenous SOD activity was shown to be reduced in aortas of nicotine-exposed rat offspring (specific SOD isoform not defined) (442) and ROS were increased in offspring of a maternal high-salt diet (305), confirming a general reduction in antioxidant capacity and correspondingly increased ROS production in other models of programming. The lack of antioxidant capacity may also explain increased ANG II-stimulated ROS production in renal (154) and coronary arteries (329) of offspring from betamethasone-exposed sheep and increased NADPH-induced aortic production of ROS in male, but not female, offspring of maternally diabetic rats (192).

An area that has not been investigated in much detail is the relative importance of the SOD isoforms. Since the mitochondria are a key potential source of ROS generation, via the electron transport chain, MnSOD may play an important role in the regulation of oxidative stress in the offspring of complicated pregnancies. Indeed, in a study of placental tissues from IUGR and preeclamptic pregnancies, mitochondrial protein expression and function (increased respiratory chain complex activity) were found to be increased in different areas of IUGR placentas (245) while mitochondrial DNA content was increased in cord blood from monochorionic twins with IUGR (65), providing a theoretical capacity for increased mitochondrial production of ROS in the placenta. Interestingly, in a study by Chiaratti et al. (74), placental mitochondrial DNA content was found to be in-
versely correlated to mouse fetal weight in a model of maternal low protein. Skeletal mitochondrial function was also shown to be increased in the mouse offspring of a maternal low-protein-diet pregnancy (186), an effect that was thought to protect offspring against high-fat diet-induced obesity but may also result in increased ROS production. In another study, however, in a baboon model of maternal protein restriction, the expression of mitochondrial-related genes was affected to a greater extent in female than male fetuses with subsequently reduced mRNA expression of mitochondrial respiratory chain transcripts in both sexes (301). Furthermore, mitochondrial DNA content was reduced in SGA infants, and SOD activity was increased (98) in a possible compensatory manner. In placental trophoblast cultures, increased maternal adiposity was associated with reduced mitochondrial respiratory function despite a 6- to 14-fold higher production of ROS (254). This area, therefore, requires further investigation and may present a potential therapeutic avenue.

In one of the few studies to investigate specific antioxidant levels, Atanasova et al. (17) demonstrated increased antioxidant (glutathione peroxidase, MnSOD, and glutathione reductase) mRNA expression in the offspring of dexamethasone-exposed monkeys. In this study, all three of the antioxidants measured were affected if the insult occurred in late gestation. On the other hand, if the insult occurred early in gestation, only glutathione reductase mRNA was altered (17). In addition, urinary levels of F2-isoprostane, a marker of oxidative stress, were unaltered despite the increase in antioxidant levels, potentially suggesting a successful compensatory mechanism in this model. Further studies in dexamethasone-treated sheep demonstrated that production of ROS was altered in both a tissue- (decreased carotid, no effect on mesenteric and increased coronary artery superoxide production) and time-dependent manner (329, 330), while male sex and female hormonal status (ovariectomized vs. intact) increased ROS production in the aortas of maternal nicotine-exposed offspring (443). These data illustrate the complexity and difficulty in elucidating the pathophysiology of developmental programming.

The effect of antioxidants on vascular function has also been studied to determine the mechanistic pathways and possible intervention strategies (these will be further discussed in sect. III). Treatment with 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (Tempol), an SOD mimetic, has been shown to ameliorate the pulmonary endothelial dysfunction, pulmonary hypertension, and ventricular hypertrophy observed in offspring of nutrient-restricted mice (321). Growth restriction in offspring of nutrient restricted dams was also partially prevented by a dietary micronutrient supplementation that included vitamins C and E, vitamins with known antioxidant actions (124). While this diet also included selenium and folate, the production of aortic superoxide was prevented by the diet, suggesting an effect of the antioxidant components. Increased cardiac and aortic oxidative stress, along with femoral artery vascular dysfunction, observed in the offspring of hypoxic rat dams was also normalized by treatment with vitamin C (135). Furthermore, vitamin C treatment was able to ameliorate the hypertensive and femoral dysfunction phenotypes observed in offspring of sheep exposed to hypoxia, potentially via a mechanism involving increased SOD activity (400). In the same model, however, the xanthine oxidase inhibitor allopurinol was not able to ameliorate hypertension but did partially reduce femoral vascular resistance (188). A note of caution was the observation of detrimental effects in control animals following the use of antioxidants, including reduced femoral artery vasodilation in control rats following treatment with vitamin C (135) and significantly decreased basal blood pressure and increased basal heart rate in fetal sheep following treatment with allopurinol (188). These detrimental outcomes are discussed in more detail in section IIIA2.

Application of exogenous SOD was shown to improve vasodilation to ACh and bradykinin in mesenteric arteries of offspring from rats fed a nutrient-restricted diet, while the endogenous expression and activity of this enzyme were reduced (123). This was further investigated in mice where a sex-specific effect was observed; male offspring of protein-restricted rats had a greater disruption of antioxidant balance than females leading to increased NADPH-induced superoxide production and reversal of vascular dysfunction with SOD and catalase treatment (332). Inhibition of 11β-HSD in the mother led to the opposite effect, whereby female offspring demonstrated increased NADPH-induced superoxide production and reversal of vascular dysfunction by SOD and catalase treatment (333). Exogenous SOD was also shown to improve vasodilation in the aorta of rat offspring of nicotine-exposed dams (442). Oxidative stress in the aorta, as shown by NADPH oxidase-4 expression, was increased in offspring of the rat maternal low-protein-diet model (399). In this study, the programming effect could be prevented by dietary supplementation with coenzyme Q, a mitochondrial electron carrier and potent antioxidant; however, in later life, the balance of the endogenous antioxidants MnSOD and catalase was altered, and this was not recovered by the treatment regime (399).

Systemic treatment with antioxidants, such as the lipid peroxidation inhibitor lazaroïd, has also been shown to be instrumental in reducing mean arterial pressure in hypertensive rat offspring of the maternal low-protein-diet model (57). In addition, the SOD mimetic Tempol attenuated the enhanced stress-induced blood pressure responses seen in offspring of 11β-HSD inhibitor-treated mice (333). It appears, therefore, that the antioxidant balance is disrupted in many models of in utero programming and may play a pivotal role in the development of hypertension via reduced vascular function.
D. Glucocorticoids

Glucocorticoids are steroid hormones that influence almost every organ system via modification of physiological functions including blood pressure, the immune system, fluid and electrolyte homeostasis, and metabolism. Because of their pervasive role in developmental physiology, much attention has focused on the role of glucocorticoids on in utero programming of adult disease. Indeed, as is reviewed in the following section, the action of glucocorticoids likely mediates a variety of underlying mechanisms that contribute to programmed hypertension.

Cortisol (corticosterone in rodents) is secreted by the adrenal cortex under the regulation of the HPA axis, in a classical endocrine negative-feedback loop (FIGURE 5). These steroid hormones exert their effects by binding to intracellular glucocorticoid receptors, which are members of the ligand-activated nuclear receptor family of transcription factors (447), although newer evidence suggests that rapid nongenomic effects may be also mediated via cell membrane receptors (376). During fetal life, glucocorticoids are essential for organ growth development. Of critical importance is the role of glucocorticoids in lung development, which has led to their widespread clinical use for acceleration of lung maturation and surfactant production in fetuses at risk of premature delivery (424). On the other hand, negative effects of glucocorticoids on fetal development and long-term health are also well reported. Multiple studies have also shown that glucocorticoids administered during pregnancy can cause LBW in humans (38, 125, 269). Studies of glucocorticoid administration to pregnant animals have corroborated this negative effect on birth weight (178, 268; reviewed in Ref. 104).

1. Fetal exposure to glucocorticoids

It is apparent that long-term programming effects of exogenous corticosteroids may be observed in the absence of alterations in birth weight, as seen in rats (294) and sheep (99) in which increased blood pressure and altered renal development after a short-term exposure to glucocorticoids was demonstrated. Fetal programming by corticosteroids may also occur in complicated pregnancies such as in pre-eclampsia and IUGR; indeed, indirect evidence suggests that these pregnancies are considered “stressful” to the developing offspring (140, 141). The fetal programming effects of exposure to endogenous versus synthetic (exogenous) glucocorticoids, however, may vary significantly. For example, endogenous and synthetic glucocorticoids differentially activate receptor subtypes, thus causing tissue-specific effects. Furthermore, there may be differences in local concentrations in tissues, which may be governed by differences in transport (e.g., across the blood-brain barrier) and metabolism of endogenous and synthetic glucocorticoids (318, 320).

Although glucocorticoids are highly lipophilic molecules and should readily cross biological barriers, such as the placenta, fetal glucocorticoid levels are normally much lower than the levels in the maternal circulation. This is thought to be mediated by placental 11β-HSD2, which catalyzes the conversion of active glucocorticoids (cortisol in humans and corticosterone in rats) into their inactive 11-keto metabolites (cortisone and 11-dehydrocorticosterone, respectively) (365). The presence of 11β-HSD subtypes in many tissues suggests that it also modulates the ability of corticosteroids to access their receptors at a cellular level. Two distinct isoforms of the enzyme 11β-HSD have been identified. 11β-HSD1 is NADPH-dependent and acts mainly in the liver, although it is also expressed in the placenta and 11β-HSD2 is NAD-dependent and present in tissues such as the kidney and placenta (109, 392). Many
researchers have theorized that dysfunction of placental 11β-HSD may play a role in glucocorticoid excess in pregnancies complicated by IUGR (28). Indeed, in human placental tissue Wachter et al. (423) found reduced 11β-HSD protein levels and activity in placental tissue from pregnancies with IUGR. The authors speculate that IUGR fetuses may be exposed to increased levels of maternal corticosteroid because of reduced expression and activity of 11β-HSD. Thus a developing fetus has the potential to become exposed to excessive corticosteroids through a variety of reasons, including excess maternal corticosteroids in “stressed pregnancies” (which may be due in part to dysfunctional placental 11β-HSD) or through exogenous administration (as in cases of preterm birth, maternal adrenal insufficiency, etc.). The following section will expand on evidence in support of the role of glucocorticoids in fetal programming of hypertension.

2. Role of glucocorticoids in programmed hypertension

Evidence suggests that fetal origins of adult hypertension may be caused, at least in part, by excessive activity of glucocorticoids (21). For example, in umbilical cord blood from IUGR neonates, corticotropin releasing hormone (CRH) was elevated when compared with normally grown fetuses (140). Middle-aged men and women who were born with LBW were shown to have significantly elevated levels of fasting plasma cortisol, which was strongly correlated with current blood pressure, even when corrected for current BMI (304). Evidence also supports the concept that programmed hypertension may be secondary to overactivation of the HPA axis in offspring as a result of a suboptimal intrauterine environment. In population-based studies, Reynolds et al. (325) demonstrated that patients born SGA exhibited enhanced responses to an ACTH (also known as corticotropin) stimulation test, causing increased urinary excretion of cortisol and the mineralocorticoid aldosterone (also produced by the adrenal gland), both metabolites showing a positive correlation to increased blood pressure. These studies provide evidence linking HPA axis overactivity and increased corticosteroids in the programming of hypertension in adults born with LBW.

Clinical trials investigating the neonatal benefit of prenatal administration of corticosteroids for preterm infants has provided interesting clinical data on the possible programming effects of corticosteroids. The MACS (multiple courses of antenatal corticosteroids for preterm birth) trial found that multiple courses of corticosteroids did not improve neonatal outcome; however, birth weight was significantly reduced in the experimental group (269, 270). Furthermore, children born at term and exposed to multiple doses of corticosteroids may have an increased risk of neurodevelopmental delay at 5 years of age (16a). Blood pressure was not reported in this study. Other long-term follow-up studies of offspring whose mother received betamethasone during pregnancy, however, did not show an effect on blood pressure with either a single course (92) or multiple courses of antenatal corticosteroids (251), although there was evidence of insulin resistance in adults exposed to antenatal corticosteroids (92). Therefore, these data, and the potential for long-term programming effects in adulthood, should be considered when utilizing antenatal corticosteroids in pregnancies at risk for premature delivery.

To corroborate a direct causal relationship between corticosteroid excess and programmed hypertension, a variety of animal models have been investigated. Administration of betamethasone to pregnant baboons in utero increased blood pressure in the offspring (203). In sheep, maternal exposure to dexamethasone led to programming effects on offspring blood pressure (99, 261), which has also been shown in rat and primate models of maternal corticosteroid exposure (94, 436). Furthermore, Benedictksson et al. (28) found that in untreated rats, placental 11β-HSD activity correlated positively with term fetal weight and negatively with placental weight. Furthermore, offspring of pregnant rats treated during pregnancy with dexamethasone (which is not metabolized by 11β-HSD) had lower birth weight and higher blood pressure in adulthood than offspring from controls. Therefore, these data support the human data discussed above that exposure to excess glucocorticoids may link LBW with programmed hypertension.

3. Glucocorticoid mechanisms

The mechanistic basis of fetal corticosteroid exposure programming adult hypertension remains uncertain, although the RAS has been implicated as its role in programmed hypertension is well established (196). Dexamethasone treatment of pregnant rats late in gestation causes IUGR in both male and female offspring. At 6 mo of age, female offspring were hypertensive, with increased angiotensinogen and plasma renin activity (284). Conversely, male offspring displayed altered glucose homeostasis, increased ACTH and corticosterone, but had no change in the RAS and no hypertension (284, 285). In another study, however, maternal dexamethasone treatment in rats induced hypertension in male offspring that was associated with a decreased nephron number (294). Although these studies in rats demonstrate conflicting results, overall they demonstrate that prenatal glucocorticoids may program adulthood cardiovascular and metabolic physiology via sex-specific mechanisms. Activation of the RAS may underlie programmed hypertension associated with prenatal corticosteroid exposure, particularly in females (284). Langely-Evans (211) sought to determine whether the hypertensive phenotype in offspring from dams fed a low-protein diet was mediated by fetal exposure to glucocorticoids.
corticoids of maternal origin. Dams were injected with metyrapone (an inhibitor of corticosteroid synthesis) alone or with a replacement dose of corticosterone. Blood pressures of rats exposed to maternal low-protein diets in utero were significantly elevated at 7 wk of age, and this was prevented in animals that were treated with metyrapone (211). Replacement of corticosterone during pregnancy showed different effects in males versus females: blood pressure remained normal in males while corticosteroid administration reversed the protective effects of metyrapone in female offspring (211). These intriguing data illustrate that maternal low-protein diet programs hypertension in offspring via corticosteroid-dependent mechanisms that are modulated by additional sex-specific mechanisms.

Corroborating the theory that fetal programming by maternal protein restriction is mediated by glucocorticoids, McMullen et al. (253) studied the mechanisms leading to increased blood pressure in a model of glucocorticoid excess. At 4 wk of age, IUGR rat offspring from a maternal low-protein diet exhibited increased systolic blood pressure compared with controls. Male offspring exhibited glucocorticoid-dependent hypertension (preventable with metyrapone) with no modulation of renal AT$_1$ or AT$_2$ receptor expression. In contrast, female offspring exhibited glucocorticoid-independent hypertension associated with reduced expression of renal AT$_2$ receptors (253). These results are in contrast to those previously published by Langely-Evans which showed hypertension to be glucocorticoid dependent in both males and females at 7 wk of age (211). Together, these demonstrate the complexity and sex specificity of fetal programming of hypertension (253).

Prenatal dietary protein deprivation is also known to alter renal development and nephron number (which has been previously discussed in sect. IIA1). Habib et al. (155) sought to investigate whether this observation was also glucocorticoid dependent. Similar to the previous studies, maternal metyrapone administration ameliorated the increase in blood pressure in the low-protein male offspring, which was associated with an increase in the number of glomeruli compared with the vehicle-treated low-protein group. These data provide evidence that decreased nephron number and hypertension in the maternal low protein rat model are mediated in part by glucocorticoid production (155).

Controversy exists, however, regarding the direct role of glucocorticoids in programming of hypertension. Dexamethasone treatment in pregnant dams may cause decreased food intake, and the associated poor maternal weight gain and malnutrition may be responsible for programming of hypertension, thus confounding the results in these animal models. In an attempt to address this issue, Woods et al. (436) determined the extent to which nutritional factors contribute to the programming of offspring hypertension by maternal glucocorticoids. Pregnant rats were treated with dexamethasone on days 1–10 (early dex) or days 15–20 (late dex) of pregnancy. Additional groups of pregnant animals were pair-fed to the early and late dexamethasone-treated groups to control for the effect of dietary intake. The dams treated with dexamethasone reduced their food intake and did not gain a normal amount of weight while they were receiving treatment. At 21 wk of age, offspring from the early dex-treated dams had normal blood pressures, whereas late dex offspring were hypertensive. However, late dex-pair fed offspring were equally hypertensive (436). These data demonstrate that not only are the programming effects of dexamethasone temporal in nature, the long-term effects of maternal glucocorticoid administration may, at least in part, be secondary to a reduction in maternal food intake.

The sheep model of glucocorticoid treatment during pregnancy has been well characterized. Ovine fetuses exposed to high concentrations of synthetic (dexamethasone) or naturally occurring glucocorticoids (cortisol) in utero during early gestation develop high blood pressure in adulthood (99). To investigate potential mechanisms involved, the role of the renal RAS was studied in corticosteroid treated ewes. ANG II, AT$_1$, and AT$_2$ receptor expression was increased in fetal kidneys following dexamethasone or cortisol treatment (262). Dexamethasone treatment also caused decreased fetal nephron number, although global renal hemodynamics and excretory function were not affected (260). These results indicate that treatment with dexamethasone early in sheep gestation causes significant alterations in the RAS in the fetal kidney, possibly resulting in long-term functional consequences. Furthermore, in this sheep model, a nephron deficit after exposure to glucocorticoids in utero is acquired before birth, and thus may be a potential cause, rather than a consequence, of increased blood pressure in adulthood.

Other evidence exists that the RAS may be involved in glucocorticoid-induced programmed hypertension. Gwathmey et al. (154) studied angiotensin receptor sensitivity and the production of angiotensin-mediated oxidative stress in betamethasone-treated sheep. Interestingly, AT$_1$ receptor sensitivity to losartan was twofold higher in betamethasone-treated offspring, while AT$_2$ binding was reduced. Furthermore, functional studies revealed that ANG II-stimulated ROS production (measured via dichlorofluorescin) was increased, while NO production was decreased in kidneys from offspring of betamethasone-treated sheep compared with controls (154). Therefore, imbalance in the ratio of AT$_1$ to AT$_2$ receptors may contribute to functional changes in angiotensin-induced blood pressure control and may stimulate oxidant-sensitive signaling pathways, thus leading to adult hypertension (154).
Additional mechanisms have also been investigated that may mediate glucocorticoid effects in programmed hypertension. For example, Tang et al. (398) found that betamethasone exposure caused sex-specific effects on renal function and salt handling (a precursor to high blood pressure, Ref. 355). In prenatal betamethasone-exposed males, glomerular filtration rate and the ability to excrete an acute salt load was reduced, while no effect on glomerular filtration rate or salt handling was noted in female offspring (398). Furthermore, vascular sensitivity to ET-1 was increased specifically in female offspring from betamethasone-treated sheep (220). Antenatal betamethasone treatment also decreased ANG-[1–7] levels in cerebrospinal fluid of adult sheep, which may contribute to altered RAS control of blood pressure in this model (247). Thus, in sheep models of antenatal betamethasone treatment, the mechanisms behind renal dysfunction and programmed hypertension are diverse and sex-specific.

In summary, prenatal exposure to synthetic glucocorticoids (such as dexamethasone or betamethasone) or to endogenous maternal glucocorticoids (via inhibition of the placental “barrier enzyme” 11β-HSD2) reduces birth weight in rats and sheep and contributes to hypertension in the adult offspring. Furthermore, the mechanisms leading to long-term alterations in renal structure and function appear to be sex-specific. Nevertheless, abundant evidence exists that glucocorticoids are an integral mediator of programmed hypertension. In particular, they interact with and regulate key components of the RAS. Given the widespread clinical use of corticosteroid administration for babies at risk of prematurity, combined with the advances in neonatal care which allows for many of these children to thrive well into adulthood, the long-term implications of these findings may be vast.

E. Epigenetics

Epigenetic modifications have been touted as important mediators of in utero “programming” effects, including adult-onset hypertension. Epigenetics is a phenomenon that involves heritable changes in gene expression that do not result from alterations in the nucleotide sequence, but by changes in the conformation of the DNA complex (158). Epigenetic adaptations include a variety of genomic alterations: DNA or histone methylation, histone acetylation (35), as well as newer players such as noncoding RNAs, which can directly affect cytosine methylation and histone modification, in addition to controlling translation (85). Epigenetic modifications are able to regulate gene activity by altering the structure or accessibility of a genomic region, thus providing a mechanism by which an altered phenotype may be transmitted to a subsequent generation without DNA mutations (224). For example, in some cases, epigenetic modifications inhibit gene expression by preventing a transcription factor from binding to a DNA sequence, whereas in other situations, chromatin structure may be altered in a way that promotes the expression of a particular gene (Figure 6). Once established, epigenetic modifications are stable through cell cycle replication, which likely promotes and sustains the abnormal gene function. It is known that there are “critical windows” during development at which point the fetus may be more susceptible to programming effects (181, 287); therefore, studying epigenetic changes at various stages during development is important. Indeed, DNA methylation can be influenced by exposure to suboptimal conditions during these sensitive times in development, potentially linking the prenatal and early postnatal environment with permanent changes in the genome and life-long physiological consequences (323). The following section will review evidence that epigenetic changes are involved in developmental origins of hypertension.

1. DNA methylation patterns

A variety of human data have emerged demonstrating that a poor intrauterine environment alters DNA methylation status in the offspring born from these pregnancies. For example, using a genome-wide cysteine methylation assay, Einstein et al. (110) demonstrated that DNA methylation was altered in cord blood from IUGR versus control neonates. This observation was also found in placentas from growth-restricted neonates compared with controls (20). In addition, in a large Norwegian cohort of mothers and babies, differential methylation patterns were correlated with LBW (113). Maternal psychological stress, which has been linked to adverse offspring health and developmental programming (136), is also associated with increased DNA methylation in cord blood from neonates (420). Malnutrition is thought to be a key factor in developmentally programmed diseases. Interestingly, in a randomized control trial in an African population with known nutritional deficiencies, micronutrient supplementation during pregnancy may benefit women by altering the DNA methylation profile compared with the control group (84).

The concept that maternal obesity is as detrimental to fetal development as malnutrition or hypoxia has also led researchers to study the role of epigenetics in the programming effects of maternal obesity (163). Indeed, cysteine methylation of the peroxisome proliferator-activated receptor γ coactivator 1-α (PPARγC1α) promoter was positively correlated with maternal BMI in umbilical cord blood from neonates. Interestingly, these epigenetic changes were independent of birth weight, and a similar correlation was evident in both large for gestational age, appropriately grown and IUGR offspring born to obese mothers (132). Lending strength to the hypothesis that maternal obesity adversely affects the developing fetus are data which show that abnormal methylation patterns in children born to obese women are not found in siblings who were born after maternal weight loss secondary to gastric bypass surgery (153).
Insulin-like growth factor II (IGF-II) is a key factor in human growth and development, and its regulation is known to involve maternally imprinted methylation. Healthy adults, who were prenatally exposed to famine during the Dutch Famine, had decreased DNA methylation of the IGF-II gene compared with their unexposed, same-sex siblings. This correlation was particularly strong if exposure was peri-conceptual or early in gestation (164). In a variety of different human populations, therefore, evidence suggests that epigenetic changes may result from a poor intrauterine environment and persist into adult life. The link to disease causation of specific phenotypes such as hypertension warrants further investigation.

The genes involved in the HPA axis are important in regulating circulating levels of cortisol, and aberrant expression patterns have been associated with hypertension and vascular dysfunction (448). Furthermore, genes in the HPA axis have been implicated in the mechanisms behind programmed hypertension (104). The role of epigenetics in modifying the HPA axis in humans and its contribution to programmed hypertension has been investigated. The Motherwell birth cohort has previously been shown to be a population with dietary imbalance during pregnancy, associated with high blood pressure in offspring (377). The authors performed a followup study which demonstrated altered DNA methylation of 11β-HSD, glucocorticoid receptor expression, and IGF-II in offspring from the mothers who had a poor diet compared with controls. Methylation status was positively correlated with increased adiposity and diastolic blood pressure in adults (103). These studies provide evidence of an epigenetic link between early-life maternal diet, fetal growth, and the development of hypertension in adult humans.

Additional mechanistic evidence exists in humans linking epigenetics to programmed hypertension. In children born SGA, DNA methylation of the ACE was significantly reduced, and an inverse relationship between the degree of ACE methylation and blood pressure was demonstrated (317). Another pathway postulated to be involved in programmed hypertension is NOS expression (see sect. II B4). Indeed, in human umbilical vein endothelial cells from pregnancies complicated by IUGR, DNA methylation of the eNOS promoter was increased, while methylation of the arginase-2 promoter was decreased (205). Together, these results suggest that epigenetic changes of the RAS and/or NOS pathways may be clinically relevant as they contribute to the development of high blood pressure in IUGR offspring.

Epigenetics mechanisms have also been investigated in animal models of programmed hypertension. For example, methylation patterns were altered in placental tissue from
IUGR rats from a maternal low protein diet model (319). In a maternal undernutrition rat model of programmed pulmonary hypertension, DNA methylation in the lung was altered in IUGR offspring compared with controls, which correlated with increased pulmonary vascular resistance (321). Furthermore, in a global nutrient-restricted baboon model, widespread alterations in DNA methylation were observed in restricted offspring (276, 411). Therefore, evidence from a variety of animal models using different maternal/pregnancy perturbations suggest that epigenetic alterations play a role in developmental programming of hypertension.

2. Hypothalamic-pituitary-adrenal axis

As previously discussed in section II.D, glucocorticoids are important mediators of programmed hypertension, which have been shown in some studies to be altered by epigenetic modifications. For example, Baserga et al. (24) studied renal expression of components of this pathway in IUGR offspring. With the use of a uterine artery ligation model, IUGR offspring demonstrated altered methylation of the 11β-HSD2 promoter in kidneys at postnatal day 21 via sex-specific mechanisms. For example, in exon 2, DNA methylation was significantly decreased in IUGR males but increased in IUGR females compared with controls (24). These data provide evidence that renal HPA axis epigenetic modifications occur in an animal model of IUGR and are sex-specific.

Lillycrop et al. (225) found that in IUGR offspring from protein-restricted dams methylation of the glucocorticoid receptor was significantly decreased in the liver, which was associated with a 200-fold increase in receptor expression. Interestingly, folic acid supplementation in protein-restricted dams prevented hypomethylation of GRs and increased their expression in livers from IUGR offspring (225), suggesting that reduced availability of folic acid may be a contributing factor in epigenetic changes caused by dietary restriction. A direct link between diet, liver methylation, glucocorticoid receptor expression, and the mechanisms contributing to hypertension remains uncertain.

3. RAS

The RAS is considered to be an important pathway in the development of programmed hypertension (see sect. II, A2 and B3). Epigenetic changes have also been studied in genes associated with the RAS. In the maternal low-protein-diet model, the angiotensin receptor type 1b (AT1b) promoter was hypomethylated and mRNA expression was increased in the adrenal gland of IUGR offspring at 12 wk of age (40). Interestingly, a follow-up study by this group demonstrated that when pregnant rats were treated with metyrapone (an 11β-hydroxylase inhibitor which blocks the formation of corticosterone), the methylation status of the AT1b promoter region was indistinguishable from controls. Furthermore, systolic blood pressure in IUGR offspring was normalized and became similar to controls (39). These data highlight the susceptibility of the RAS to epigenetic changes and suggest a glucocorticoid-mediated role in RAS gene promoter methylation status.

While many studies support the concept that renal expression of RAS is altered in animal models of fetal programming and is likely to contribute to the programmed hypertensive phenotype, few studies have examined renal epigenetic alterations of RAS following maternal perturbations. The role of epigenetic changes in the kidney was studied in the uterine artery ligated rat model of IUGR and programmed hypertension. In this model, Pham et al. (303) found that increased apoptosis and decreased nephron number were associated with decreased methylation of p53, an important co-regulator of apoptosis and cellular senescence. Similarly, altered histone methylation was associated with global suppression of transcription in kidneys from a maternal low-protein diet in microswine (96). Although the authors speculate that these effects may contribute to fetal programming, further studies are needed to clarify whether altered renal function is influenced by epigenetic changes, thus providing a direct link from DNA methylation to developmentally programmed hypertension.

4. Transgenerational studies

Transgenerational studies have provided abundant evidence, all be it indirect, that epigenetics may contribute to programmed hypertension. Studies have demonstrated that phenotypic alterations (including hypertension and vascular dysfunction) may be transmitted to subsequent generations in the absence of continued pregnancy interventions, thus demonstrating a “transgenerational effect.” This phenomenon is attributed by many to be secondary to epigenetic modifications (347). For example, Ponzo et al. (309) found that global nutrient restriction in rats caused a deleterious phenotype observed in the F1 offspring (hypertension, impaired aortic vasodilator response to ACh, and alterations in NO production). The same phenotype was also demonstrated in males from both the second (F2) and third (F3) generations. These data are significant because they represent how an insult during pregnancy can result in a phenotype that is transmitted to the second and third generations without ongoing maternal perturbations (309). This study, however, was unable to address whether maternal or paternal influences contribute to the transgenerational effect of the fetal programming since control and IUGR offspring were mated to each other. In contrast to the malnourished rat model, in offspring from the maternal low-protein diet, hypertension was demonstrated in the F2 generation (including IUGR animals mated both to controls and IUGR); however, a hypertensive phenotype was not observed in any F3 animals (160).
Gallo et al. (129) investigated whether cardiovascular and renal changes were transmitted transgenerationally via the maternal line in a rat model of utero-placental insufficiency. Growth-restricted female offspring were mated with control males to produce F2 offspring. Interestingly, F2 growth-restricted males, but not females, developed elevated systolic blood pressure at 6 mo of age. Both male and female F2 restricted offspring demonstrated reduced nephron number at birth, but nephron number was similar to controls by postnatal day 35. Furthermore, alterations in the renal expression of RAS could not explain the observed differences in blood pressure or nephron number (129). Thus, in a rat model of utero-placental insufficiency, there appears to be transgenerational programming of hypertension via the maternal line, through mechanisms which are sex-specific. Although this study illustrates how being born growth restricted can have far-reaching effects extending into the subsequent generations, it also highlights the complexity behind the mechanisms leading to programmed hypertension.

A controversy that is often debated in the literature is whether the programming effects of a poor in utero environment occur at the level of the developing gametes (which ultimately affect the phenotype of the F2 offspring) or whether the poor in utero environment affects the ability of that offspring to adapt to its own pregnancy, thereby causing an F2 programming effect. Tran et al. (408) aimed to investigate this issue by performing embryo transfers from both control (cont) and nutrient-restricted (rest) pregnant rats, thereby creating four groups (cont-in-cont, rest-in-cont, cont-in-rest, rest-in-rest) (408). The authors hypothesized that rest-in-rest offspring would have similar characteristics (IUGR, altered metabolic profile and hypertension) to the cont-in-rest. However, rest-in-rest offspring underwent accelerated growth during the peripubertal phase then had stunted growth between 2 and 3 mo of age compared with cont-rest; demonstrating a confounding experimental influence of embryo transfer on the data. Furthermore, renal function, blood pressure, and insulin sensitivity were different between respective embryo transfer and non-embryo-transferred groups (408). Thus these elaborate studies were unable to determine the contribution of adverse maternal pregnancy environment versus intrinsic germ line effects. The data are important, however, in that they demonstrate long-term effects of in vitro embryo manipulation and, as such, the authors question whether these experiments have any utility in delineating between the maternal pregnancy environment and germ line effects in fetal programming. Additional studies in this area that focus on developmental programming in the F3 generation are required to aid in answering these unresolved questions.

5. Role of miRNA in programmed hypertension

Micro RNAs (miRNAs) are a class of small endogenous noncoding RNAs and are key epigenetic regulators of gene expression (85). By targeting complex biological pathways, miRNAs contribute to diverse physiological and pathophysiological processes (255), and while increasing evidence is becoming available regarding miRNAs and fetal programming (particularly of metabolic disease, Ref. 64), few studies have investigated the role of miRNA in programmed hypertension.

The RAS pathway is involved at many cellular levels and in many organ systems in the developmental programming of hypertension (see sect. II, A2 and B3). Altered miRNA may be involved in regulating the RAS pathway in animal models of programmed hypertension. For example, in fetal brains from a mouse model of maternal low-protein diet, mir27a and mir27b levels were significantly increased, which are regulators of ACE translation; while mir330, which regulates the AT_2 receptor, was significantly decreased (143). Furthermore, in a mouse model of caloric restriction, female offspring developed high blood pressure by 24 wk of age, whereas males did not. This was associated with a female-specific decrease in ACE 2 protein levels with no associated changes in ACE 2 mRNA expression but an increase in miRNA 429, which has a binding site on the ACE 2 gene and can impair translation. These data suggest that ACE 2 regulation by miRNA is an important mechanism contributing to the sex-specific programming of hypertension in this mouse model (147). Other miRNAs have been found to be altered in renal tissue from offspring of maternal low-protein rats, including mir200a, mir141, and mir429, which may contribute to glomerular dysfunction, fibrosis, and ultimately programmed hypertension (369). Thus understanding the role of miRNAs in fetal programming of adult disease such as hypertension is a rapidly evolving field, which may open up new avenues for pharmacological or dietary manipulation of miRNA effects in offspring.

In summary, evidence exists that the human genome is susceptible to epigenetic alterations that may contribute to lasting changes within an individual and, therefore, play a potentially important role in programmed hypertension. Altered DNA methylation patterns have been demonstrated globally, as well as in specific organs, in animal and human offspring from complicated pregnancies. Although studies demonstrate epigenetic differences in key blood pressure regulatory genes (such as those of the RAS, NOS, and HPA pathways), further studies are warranted to confirm a mechanistic link between epigenetic alterations and downstream physiological effects, such as vascular dysfunction or hypertension. The epigenetic modifications involved in prenatal programming of hypertension may also be specific to the model used, the timing of the insult, the tissue, and the gene of interest studied. In the future, therefore, large-scale human prospective cohort studies may be required to elucidate whether a true cause-and-effect relationship exists.
between epigenetic modifications and the hypertensive phenotype of fetal programming.

F. Sex Differences

As has been alluded to throughout the description of various mechanistic studies, developmental programming often has a differential effect depending on the sex. A few investigators have studied the specific effects of hormonal status, or manipulation of the hormonal status, on the outcomes in models of developmental programming. In a model of maternal nutrient restriction in sheep, female adult offspring (6 yr of age) were demonstrated to have reduced steroidogenic enzyme expression and subsequently reduced circulating and luteal progesterone levels (228). In the female offspring of protein-restricted rats, plasma testosterone levels were increased along with increased blood pressure (359). In this study, treatment of the offspring with the androgen receptor antagonist flutamide (10 mg·kg⁻¹·day⁻¹ sc, for 10 days) ameliorated the hypertensive phenotype and reversed the enhanced mesenteric artery responses to ANG II, suggesting that the alterations in RAS might be androgen-dependent. A similar situation occurred in male IUGR adult (13–16 wk of age) offspring of the maternal RUPP model, whereby serum testosterone and blood pressure were increased, and these were both ameliorated by gonadectomy at 10 wk of age (290). Indeed, maternal treatment of otherwise healthy pregnant rats with testosterone propionate (0.5 mg·kg⁻¹·day⁻¹ sc, GD 15–19) was, itself, able to cause growth restriction of female offspring with increased blood pressure and mesenteric artery dysfunction (360), supporting a role for altered hormonal status in the development of cardiovascular diseases. Even in the postnatal period, exposure of healthy swine offspring to estradiol valerate (50 µg/kg im, PD 0–13) caused altered vascular expression of estrogen receptors and factors which might impact vascular remodeling, VEGFA, and MMP-9, which lasted into adulthood (70). Thus there appears to be both decreased progesterone in females and increased testosterone in both males and females depending on the insult. In addition, some of the observations in females of effects unmasked by ovarietomy suggest a protective effect of estrogen.

Not all sex differences, however, are dependent on hormonal influences; chromosomal differences may also have a role to play. In the field of hypertension, researchers have identified a gene that contributes significantly to blood pressure regulation in the spontaneously hypertensive and stroke-prone spontaneously hypertensive rats (SHR and SP-SHR, respectively) and which was localized to the X chromosome (169), although autosomal genes were also involved and the evidence for sex-linked inheritance of hypertension is inconsistent (79, 202). This is an area that is currently unexplored in the developmental programming field and which, given the frequent discovery of sexually dimorphic responses to compromised in utero environ-

ments, might be a worthwhile avenue of research, particularly in regard to tailored (personalized) medicine approaches.

G. New Research Avenues

There is an impressive volume of research studies that have considerably expanded our knowledge of the effects of developmental programming on hypertension in adult life; however, there still remain areas in which little is known or results are controversial. An area that seems to be of critical importance is in the timing of both the insult and the following progression of developmental changes. While some research has been performed to investigate critical windows in which an insult might occur, a greater understanding of the time course of developmental origins of disease would provide insight into the relative impact of various prenatal and postnatal factors on the cardiovascular outcomes. For example, what is the effect of IUGR versus preterm birth? During which periods of development are offspring at most risk of undergoing adverse adaptations? Furthermore, understanding the pathological time course would provide new avenues for the identification of biomarkers to accurately predict offspring affected by developmental programming and allow for early implementation of monitoring or treatment strategies (further discussed in sect. III). In addition, therapeutic strategies might be better chosen dependent on the pathological progression, for example, initial transitory changes occurring early in life might be followed by more permanent compensatory alterations. An area that might follow this pattern is vascular dysfunction in which early epigenetic changes may increase vascular oxidative stress, among other effects, and cause permanent alterations in vascular function pathways which reflect an “early aging” phenotype.

In addition, some detail has yet to be discovered regarding the specific pathways affected, an aspect which could drive the development of novel therapeutics. In terms of vascular function, the endothelin, prostaglandin, and endothelium-derived hyperpolarization pathways are lacking in sufficient evidence to promote or exclude them as potential targets for the development of interventions. Furthermore, tissue management of oxidative stress via endogenous SOD isoforms requires further study to determine how this central factor is critical in the development of a pathological state.

Historically, animal studies have concentrated on the male sex to reduce the variability in findings. This oversight of the female sex, however, has been changing in more recent years, and there is a considerable array of data in the developmental origins of disease field which support differences in the responses of each sex to a compromised environment during development. This area will be critical in determining which therapeutic options would be advantageous in
each individual. Much remains to be determined, however, in specifying how each of the sexes responds. Whether the sexually dimorphic responses are due to underlying genetics or, as the majority of the literature appear to support, hormonal influences will determine the opportunities available for therapeutic options.

III. THERAPEUTIC INTERVENTIONS

The financial burden of cardiovascular disease on the health care system is enormous and, therefore, with the increasing knowledge of potential mechanisms leading to programming effects on cardiovascular health, this has naturally led to the investigation of therapeutic interventions. The window of opportunity for therapeutic intervention extends from the in utero environment (hence maternal treatment) into the postnatal life, with the treatment of the offspring at any point in their life. Previous reviews have proposed a life course trajectory for the development of hypertension and suggest that intervention at early stages of life may be instrumental in altering this trajectory in a beneficial manner (159). Interventions that have been tested range from pharmacological therapies to lifestyle approaches, and these have been used either to further elucidate the pathways involved or as possible translational approaches. Some of the findings are presented herein; however, this should not be considered an exhaustive list.

A. Pharmacotherapy

1. RAS intervention

Given the extensive involvement of the RAS in both renal and vascular systems, and its demonstrable effects on the development of hypertension, this mechanism has engendered considerable attention for therapeutic intervention. Indeed, ACE inhibitors are already widely prescribed in the treatment of hypertension and congestive heart failure, and therefore, there is also extensive safety and efficacy data already available.

During investigations of the developmental origins of cardiovascular diseases, ACE inhibitors have been used to treat offspring of maternal low-protein rats. In this model, adult IUGR offspring had increased blood pressure (mean and systolic) from 7 to 12 wk of age. Blood pressure was normalized following either chronic [0.9 mg·kg⁻¹·day⁻¹ for 1 wk at age 10–12 wk (208)] or acute [150 μg/kg iv or icv for 20 min at age 9–12 wk (307)] treatment with the ACE inhibitor enalaprilat. Enalaprilat [10 mg·kg⁻¹·day⁻¹ gavage for 2 wk at age 2–5 wk (150)] was also shown to be effective in reducing the increased mean arterial pressure observed in adult IUGR offspring born from the rat RUPP model. None of these treatment regimens had an effect in control animals. In these studies, the effect of enalaprilat on underlying mechanisms was not further investigated. Acute administration of an AT₁ receptor antagonist (losartan, 30 μg/kg icv or 20 mg·kg⁻¹·day⁻¹ gavage) has also been shown to be effective in reducing mean arterial pressure in maternal low-protein offspring (307, 358) while another AT receptor antagonist, candesartan cilexetil (50 μg·kg⁻¹·day⁻¹ for 8 wk at age 9–17 wk) reduced systolic blood pressure in adult IUGR offspring of maternal undernutrition mice and protected them from cardiac enlargement and coronary perivascular fibrosis (193).

The ability of these compounds to treat the hypertensive phenotype observed in these programming models is not surprising given the extensive historical use of the same compounds as antihypertensive therapy following various etiologies. There is currently a lack of evidence concerning whether targeting of the RAS is particularly effective in developmental origins of hypertension; however, more mechanistic studies might well support this approach.

2. Antioxidants

As previously described, the development of systemic oxidative stress is thought to be a central culprit in the progression of cardiovascular diseases in offspring born from compromised pregnancies. Several antioxidants have been tested in models of developmental programming to assess their efficacy; however, the results are controversial.

In a study by Cambonie et al. (57), mean arterial pressure in adult offspring of a maternal low-protein rat model was increased at 10–12 wk of age. Maternal treatment with a lipid peroxidation inhibitor (lazaroid, 10 mg·kg⁻¹·day⁻¹ gavage, GD 0-term) was able to prevent the increased blood pressure observed in the offspring in addition to ameliorating an increased constrictive vascular phenotype. Interestingly, while there was an early (GD 21) index of decreased antioxidant status (decreased total liver glutathione levels), this did not persist into postnatal life, nor were the total redox ratios or levels of 4-hydroxynonenal (HNE) and 1,4-dihydroxynonenone (DHN) protein adducts (markers of lipid peroxidation) altered, suggesting a subtle, if any, altered level of oxidative stress in the offspring. By adulthood, renal levels of 8-isoprostaglandin-F₂α (produced via lipid peroxidation of arachidonic acid catalyzed by oxygen free radicals) were increased in low-protein offspring, but these were not significantly reduced by maternal treatment with lazroid. The beneficial effects of maternal lipid peroxidation inhibition, therefore, appear to have only partially been mediated by an improved antioxidant status (57).

Tempol, a SOD mimetic, has been used in several studies of developmental programming of hypertension. In the eNOS knockout mouse, a model of IUGR, tempol (1 mM in drinking water) was able to increase the birth weight and crown-rump length of offspring when administered to the dam from GD 12.5–18.5. Offspring birth weight was also in-
creased in control, C57Bl/6J mice; an effect which might have detrimental outcomes (388). In a mouse model of maternal undernutrition, pulmonary vascular dysfunction, pulmonary hypertension, and right ventricular hypertrophy were observed in adult (14 wk old) male offspring. Administration of tempol (10 μM in drinking water, GD 7-term) to the pregnant dams ameliorated these detrimental phenotypes (321). C57Bl/6J mice treated with the 11β-HSD inhibitor carbenoxolone (12.5 mg·kg⁻¹·day⁻¹ sc, GD 12–19) to increase fetal exposure to maternal glucocorticoids, led to offspring with increased sensitivity to conditioned fear/stress manifested as heightened blood pressure and heart rate responses. When dams were concurrently treated with tempol (1 mM in drinking water, GD 0-term), these adverse responses were attenuated. Interestingly, neither the endothelial dysfunction nor the increased vascular oxidative stress observed in this model was corrected by antioxidant therapy (333). In a study of maternal protein restriction during gestation in rats, hypertension was observed in adult male offspring (9–12 wk of age) that was associated with heightened carotid vascular responses to ANG II and aortic superoxide production. Acute administration of tempol (1 mM) during ex vivo vascular studies normalized the vasoconstrictive phenotype, suggesting that it might be due to heightened oxidative stress; however, the effect of tempol on vascular superoxide production per se was not assessed (450).

Coenzyme Q (an endogenous antioxidant) has been shown to be reduced in IUGR prewean and adult offspring from rats fed a low-protein diet, and this was associated with increased aortic oxidative stress and accelerated DNA damage. These effects were reversed when offspring were given a dietary supplementation of coenzyme Q in the post-weaning period (399), demonstrating that the window of opportunity for treatment extends into post-weaned life.

An area that was briefly discussed in section II C was the use of vitamin supplementation to improve antioxidant capacity, namely, vitamin C or E. In the studies previously mentioned, vitamin C (ascorbate, 8.9 ± 0.4 mg·kg⁻¹·day⁻¹ iv) was provided to fetal sheep in utero 1 h before and during a 30-min maternal exposure to a hypoxic environment, and this was able to prevent the hypertensive and vascular dysfunction phenotype observed in this model. Since SOD activity was increased and the plasma nitrate/nitrite ratio was reduced during this treatment period, improved antioxidant status was thought to be a potential mechanism of action (400). The same group demonstrated that maternal hypoxia also caused increased vascular oxidative stress and endothelial dysfunction in rats, effects that were reversed by maternal vitamin C treatment (5 mg/ml in drinking water, GD 6–20). This study demonstrated an important contraindication, however, in that maternal vitamin C supplementation in normoxic animals actually induced endothelial dysfunction in this group (135). In the same model, vitamin C treatment was also shown to reduce baseline mean arterial pressure in both the hypoxia-exposed offspring and their normoxic controls at 4 mo of age (189), suggesting that the redox balance is a finely tuned mechanism that can have detrimental effects when offset in either direction. Combined antioxidant (vitamins C, 150 mg·kg⁻¹·day⁻¹ and E, 250 mg·kg⁻¹·day⁻¹), selenium (0.3 mg·kg⁻¹·day⁻¹), and folate (4 mg·kg⁻¹·day⁻¹) supplementation by gavage from GD 1-term in maternally undernourished rats was shown to reverse indexes of vascular endothelial dysfunction, reduce NO production, and increase superoxide production, but was unable to ameliorate the detrimental renal effects of undernutrition: proteinuria, reduced glomerular filtration rate, and glomerular number (124).

The knowledge gained from animal studies has encouraged clinical trials to be conducted to determine the potential benefits of antioxidant therapy in compromised pregnancies in women; however, these have not been as promising as might have been expected. Systematic reviews of randomized controlled trials, using various antioxidant treatments, have failed to demonstrate improved pregnancy outcomes, such as preterm birth, preeclampsia, fetal growth restriction, or fetal/neonatal mortality (81, 345, 351, 395, 402). More worryingly, several randomized controlled trials and systematic reviews have demonstrated increased complications such as risk of preeclampsia (427), LBW offspring (311), maternal hypertensive disorders (23, 81, 340), and prescription of maternal antihypertensive therapy and hospital admissions for hypertension (345). While the evidence to date has not shown improved pregnancy outcomes with the antioxidant therapies tested, ongoing research is important to assess newer, as yet untested, antioxidant options. In addition, the majority of animal studies have focused on maternal treatment to prevent pathological outcomes while postnatal administration of antioxidants, for example, coenzyme Q (399) or resveratrol (344), in offspring may provide a better approach to the prevention of cardiovascular diseases; this aspect also warrants further investigation.

3. Resveratrol

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) has become a widely researched compound in recent years due to its pleiotropic actions as an antioxidant, in improving insulin sensitization and vascular function, and in prevention of atherosclerosis, cardiac dysfunction, and hypertension (reviewed in Refs. 222, 455), all of which are important in the progression of cardiovascular disease. Interest in resveratrol has extended into the programming field and in rats exposed to hypoxia in utero; the cardiac (344) and metabolic (102) dysfunction observed in growth restricted offspring exposed to a high-fat diet in adult life (3–12 wk of age) were prevented by concurrent treatment with resveratrol (4 g/kg diet). This field is a likely area of future research.
into the beneficial effects of resveratrol treatment in the prevention of programmed hypertension.

4. Statins

Statins are another class of drugs that are already widely used in the prevention of cardiovascular diseases. One group has investigated their use in a mouse model of high-fat diet-induced programming. In this model, pravastatin (5 mg·kg⁻¹·day⁻¹ in drinking water, GD 0–weaning) was able to partially correct the hypertensive phenotype observed in the dams and to fully correct the hypertensive phenotype observed in the offspring. Pravastatin treatment also had beneficial effects on the offspring’s metabolic phenotype by normalizing serum cholesterol, plasma glucose, and insulin and preventing liver lipid accumulation (111, 112).

B. Dietary Interventions

1. Nutrition

It has been extensively demonstrated through animal studies and human conditions that maternal diet can have a profound impact on fetal development in utero. Following his publications on the theory of the developmental origins of health and disease, Dr. Barker has championed many studies of maternal nutrition throughout the world, including studies in India (randomized controlled trial, daily micronutrient supplement of green leafy vegetables, fruit and milk; no increase in birth weight unless provided ≥3 mo preconception, Ref. 312), Saudi Arabia (sex differences in the in utero growth trajectory in response to maternal nutrition, Ref. 11), and Tunisia (maternal adherence to Ramanadan reduces fetal growth, Ref. 12), while other studies are ongoing in the United States, United Kingdom, Finland, France, Netherlands, China, and Thailand. As with antioxidant studies, however, long-term follow-up studies in the infants of these pregnancies are still lacking and further investigation into the relative importance of various micronutrients, for example, iron, zinc, selenium, folate, glycine, and calcium, to name but a few, requires elucidation (316, 451).

2. Folate

Folate can act as a methyl donor and, in that manner, influence DNA methylation. Given the epigenetic alterations that have been shown to occur following a compromised in utero environment, maternal folate supplementation may prevent the occurrence of these detrimental changes. Indeed, a study has investigated this possibility in rat models. In male offspring of protein-restricted dams, increased systolic blood pressure in 4- to 5-mo-old animals was prevented by folate supplementation during pregnancy (5 mg/kg diet, GD 0-term); endothelial dysfunction, however, was not improved (405). One primary mechanism by which low folate levels may contribute to fetal programming is via epigenetic alterations. Specifically, telomere length has been implicated in the fetal programming of hypertension (157). Entiniger et al. (114) conducted a prospective, longitudinal study on maternal serum folate levels in the first trimester of pregnancy and newborn telomere length from cord blood. Their results show that newborns of mothers in the lowest quartile of total folate levels had ~10% shorter telomeres than those newborns from mothers in the highest folate quartile (114). Interestingly, folic acid is being considered as a potential therapeutic option in reversing the epigenetic changes responsible for programmed hypertension. Indeed, in the maternal low-protein rat model, epigenetically altered DNA methylation patterns in regions that are known to be associated with cardiovascular disease were largely reversed by supplementing the protein restricted diet with folic acid (9).

3. Calcium

Multiple randomized controlled trials have investigated the effects of calcium supplementation during pregnancy. Systematic reviews of the literature have demonstrated a reduced maternal risk of compromised pregnancy: maternal hypertension, preeclampsia, and preterm birth (29, 171), and included several trails in which a follow-up of childhood cardiovascular health following maternal calcium supplementation had been performed. In one study systolic blood pressure in 7-yr-old offspring was significantly reduced, particularly in overweight offspring, following maternal calcium supplementation (2 g/day, 20 wk gestation-term) (27) while in another a trend for reduced blood pressure in offspring at 4–7 yr of age did not reach significance (170). In a more recent review of the literature, however, the effect of maternal calcium supplementation on preterm birth or infant LBW was not found to be significant (49), leaving the benefits of maternal calcium supplementation debatable.

C. Lifestyle Modifications

1. Exercise

Exercise has long been promoted as a safe, nonpharmacological approach to preventing cardiovascular diseases in the general population, and there is a large body of evidence to support its use in this regard. Less is known, however, regarding its benefit and safety in the developmental programming of hypertension. In pregnant women, exercise has been demonstrated to reduce factors such as maternal antioxidant levels at labor, blood pressure and heart rate (356, 449), and risk of preeclampsia (121, 386), while the risks of gestational diabetes (19, 278) or hypertensive disorders of pregnancy in Hispanic women (66) were not reduced. Most studies, however, have not assessed fetal or offspring outcomes, particularly as relates to their cardio-
vascular health. In a small study of healthy pregnancies in 51 women, increased physical activity during pregnancy was associated with decreased birth weight (302). This phenomenon was confirmed in a recent meta-analysis that demonstrated a reduction in LGA infants without an increased risk of SGA infants (429). In a follow-up of 20 offspring from the 51 healthy pregnancies, increased maternal activity was also inversely related to systolic blood pressure in the children at 8–10 years of age (306), suggesting a potential beneficial effect of maternal activity on offspring cardiovascular health that requires further investigation.

Exercise training in the offspring of rodent models of maternal nutrient restriction (131) or bilateral uterine artery ligation (207) has been shown to lead to beneficial metabolic effects on glucose and insulin handling. A recent study in growth-restricted offspring of hypoxia-exposed rats demonstrated a differential effect of aerobic exercise from 10 to 16 wk of age on the improvement of vascular function in males and females; these data suggested that exercise training in female offspring may not be beneficial in this population (322). It is possible that exercise may represent a secondary stressor that may not be well tolerated in developmentally programmed offspring.

2. Gastrointestinal bypass surgery

Obesity is known to be a contributor in creating an adverse in utero environment in which developmental programming of offspring can occur. Maternal weight loss prior to pregnancy, therefore, can improve outcomes for both the mother and the fetus. One group has compared the long-term outcomes in sibling offspring (2–24 yr of age) born either before or after their mothers underwent bariatric gastrointestinal bypass surgery. Offspring born after surgery had improved cardiovascular and metabolic outcomes including improved fasting insulin levels, lower blood pressure, and reduced homeostatic model assessment (HOMA) index. These improvements were associated with differential gene methylation of glucoregulatory, inflammatory, and vascular disease pathways (152). A retrospective study of women who had undergone Roux-en-Y gastric bypass surgery, however, demonstrated that while the surgery had beneficial maternal effects of reducing pregnancy-related hypertension, it also increased the likelihood of infants being born SGA (4), an outcome that could reflect a detrimental in utero environment.

D. New Therapeutic Avenues

1. Epigenetics as a therapeutic target

While epigenetic alterations influence gene expression, it is important to emphasize that epigenetic changes are reversible (as opposed to genetic alterations that are not reversible). Epigenetic-based therapeutics offer an exciting opportunity to reverse disease-associated epigenetic abnormalities. Indeed, many studies in cancer, autoimmune, and cardiovascular diseases have described the effects of dietary and pharmacological agents for targeting epigenetic changes and treatment of pathology, including (but not limited to) statins, resveratrol, cocoa, ASA, and folic acid (reviewed in Ref. 361). Indeed, this is intensely studied in the field; however, given the unique challenges of therapeutic intervention in fetal programming and limitations in treatments in pregnant women, few studies have focused on targeting epigenetic pathways in fetal programming of hypertension with the exception of folic acid (see sect. IIIB2).

2. Timing of treatment

The studies into the effects of RAS intervention and antioxidant supplementation highlight different approaches as to when to target therapeutic interventions. While RAS intervention has primarily been tested in adult offspring, the effect of inhibiting the RAS in utero has not been tested. Known contraindications of ACE or AT receptor inhibition during pregnancy, however, rule this out as a potential therapeutic avenue (10, 378, 396). Both the detrimental effects of maternal RAS intervention and the increased maternal and fetal risks associated with maternal antioxidant supplementation highlight the sensitivity of the pregnancy state to interference, making it a delicate period in which to attempt pharmacotherapies. It is interesting to note that maternal lifestyle modifications such as exercise may also not be universally beneficial when applied during gestation. While a more generalized approach in altering the dietary profile during pregnancy might be thought to provide a safer approach, the studies performed to date have not been able to exact meaningful results: neither nutritional nor calcium supplementation dramatically increased infant birth weight, and folate supplementation was unable to improve vascular dysfunction. There remain many unexplored nutritional avenues, however, and it might be hoped that this direction remains a viable potential therapeutic intervention. In addition, the use of vasodilators, such as sildenafil (Viagra), are currently under investigation to determine their effects on improving both maternal and fetal pregnancy outcomes (130), and the effect of this treatment on the offspring health later in life requires further investigation.

A technique that has been used to demonstrate the extension of the period of developmental plasticity into the postnatal environment is the use of cross-fostering. In the offspring of heterozygous eNOS knockout mice, vascular dysfunction that was observed at 10–11 wk of age could be prevented by cross-fostering the offspring from birth until weaning onto wild-type mothers (77). This demonstrates that not only the in utero environment, but also maternal nutrition in postnatal life, has an impact on the development of the offspring’s cardiovascular system. This extends the potential therapeutic window and might be useful in
Nutritional deficiencies are also thought to play a significant role in the developmental origins of hypertension. For this reason, some investigators have studied the association of maternal dietary intake with blood pressure in children. Indeed, first-trimester maternal daily dietary intake (folate, homocysteine, and vitamin B₁₂) was assessed in a population-based prospective cohort study of 2,863 mothers, and blood pressure was measured in their children at 6 yr of age. However, no correlations between maternal dietary factors and childhood blood pressure were found to be statistically significant (412). On the other hand, another study found that higher total maternal n-3 PUFA levels were associated with lower childhood systolic blood pressure (419). These studies illustrate the potential for maternal serum markers, whether placental, hormonal, or dietary, to be predictive of adult later-life hypertension in their children, an area which warrants further investigation.

Overall, these studies illustrate that the identification of biomarkers (nutritional or biochemical) would be a useful tool for the prediction of cardiovascular outcomes in children born from compromised pregnancies at an early stage. The successful implementation of biomarkers in other fields, such as heart failure (385a), indicates that this area provides a promising avenue for future research, leading to the tailoring of therapeutics in specific populations.

There is much work to be done to determine both the best timing of intervention and the best therapeutic approach to preventing the development of cardiovascular diseases in this susceptible population; however, there are some promising indications and a wealth of data regarding potential mechanisms to be targeted. Previous reviews have discussed in more detail the concept that a “life course approach” is required for the prevention of non-communicable disease states such as those programmed by an adverse environment during developmental stages (137, 159). This concept proposes that intervention can be more effective when implemented in early stages when plasticity is highest and the risk of disease still relatively low. This strategy, however, relies heavily on the successful identification of populations at risk. In our own laboratory, we conducted a study to determine whether a noninvasive technique such as ultrasonography could be used to determine vascular stiffness in a model of developmentally programmed offspring with a view to establishing a reliable method of early evaluation of cardiovascular risk in young children and neonates. This research identified an increased aortic stiffness as early as day one of life in rat offspring exposed to hypoxia in utero (unpublished data), in line with previously published data (sect. II81), using a method that could be easily applicable to neonatal humans. Together with the recognition of reliable biomarkers, approaches such as this might pave the way towards the identification of at risk populations.

3. Prediction of developmental programming

There is an attractive lure to the concept of early identification of those who will ultimately be affected by early origins of chronic disease, such as hypertension. Indeed, this field has been extensively investigated in preeclampsia, which may share many pathophysiological mechanisms with developmental programming, where there has been a search for serum biomarkers, which may predict the development of this syndrome. Many candidate markers have emerged as promising for the prediction of preeclampsia, including inhibin A, sFlt-1, PlGF, PAPP-A, and placental-specific protein 13 (8, 177, 288), although minimal research has focused specifically on their correlation with fetal programming of hypertension. One recent study found an interesting correlation between lower second trimester maternal PlGF levels and narrower retinal arterioles in children at age 12, which was independent of maternal or childhood demographics including birth weight (134). However, their data did not show any association between sFlt-1 levels and childhood vascular caliber. Nevertheless, these data are among the first to show that maternal serum biomarkers may indicate persistent effects on vascular structures in their offspring.
Along with the new avenues of research into mechanistic pathways (sect. IIG) and the investigation of biomarkers or methods to identify individuals at risk (sect. IIID3), potential therapeutics are at the forefront of developmental origins of hypertension research; however, a successful therapy has yet to be found. Mechanistic research has identified central players such as epigenetic changes, renal dysfunction, oxidative stress, and a dysfunctional RAS; however, as was discussed in the previous section on the timing of treatment, the translation of this knowledge to a useful therapeutic avenue has been fraught with difficulty. There are areas that continue to show promise and, with refinement, current strategies might be differently targeted to overcome unwanted side effects. For example, while maternal antioxidant treatment has been shown to cause detrimental outcomes in the offspring, targeted placental or mitochondrial antioxidant therapy has yet to be tried. Indeed, the placenta may provide an ideal target for other strategies, such as the prevention of fetal exposure to maternal stress hormones, since it is a natural barrier between the maternal and fetal systems.

IV. SUMMARY AND CONCLUSIONS

As demonstrated by the extensive literature base represented in this review, there is now a well-established link between the pre-, peri-, and postnatal environments in which a fetus develops and cardiovascular health in later life. A particular emphasis of this review was on the mechanisms that might be responsible for the subsequent development of hypertension in the offspring of compromised pregnancies (FIGURE 1). In the United States, approximately one in three American adults have high blood pressure, and this leads to an estimated $46 billion/year in health costs (2011 statistics; http://www.cdc.gov/bloodpressure/faqs.htm). The numbers are similar in Canada where the proportion of adults diagnosed with hypertension was seen to more than double from 1995 to 2005 (409), a trend which indicates the continually worsening state of affairs. While hypertension is typically defined as having a blood pressure greater than 140/90 mmHg, studies have shown that the risk of developing cardiovascular disease increases throughout the blood pressure range in a graded relationship, such that those with only slightly increased blood pressure (prehypertensive) may already face an increased risk of developing cardiovascular disease at an earlier age than their “normal” counterparts (190). This vastly expands the potential “at risk” population and may encompass a large proportion of developmental programming effects where the initial phenotype may be presymptomatic but the increased risk is, nevertheless, present due to underlying alterations. The rising costs of chronic cardiovascular diseases such as hypertension provide a critical need for the development of potential therapeutic strategies to offset the development of these conditions, a secondary focus of this review.

This review details many pathways (FIGURE 7) that might potentially be targeted by therapeutic interventions; however, as has been discovered during clinical trials, the path from mechanism to treatment is fraught with difficulty. Consideration must be given to multiple factors including, among others, translation of results from animals to humans, differential timing of organ development among species, animal modeling of human conditions, the timing of treatment, and potential sex-specific pathologies (reviewed in Refs. 179, 343, 357). While few women approach their clinicians for advice prior to pregnancy, public education is necessary to alert potential parents of the impact of their preconceptual health, pregnancy considerations, and the postnatal environment in which their child will develop. An area that was briefly touched on in some of the studies presented is the susceptibility of the offspring of compromised pregnancies to “second hits.” Children born of complicated pregnancies, therefore, with either low, normal, or high birth weight or who experience rapid childhood weight gain (or postnatal nutritional “mismatch”), should alert clinicians to increase their surveillance for the development of hypertension and should prompt education regarding primary prevention to help minimize additional risk factors (smoking, alcohol use, weight gain, etc.). Since birth weight and prematurity are affected substantially by maternal nutrition and health during pregnancy, optimization of maternal health and early childhood nutrition could attenuate this programming cycle and reduce the global burden of hypertension and cardiovascular disease in the future.

While the developmental origins of health and disease theory has made its mark on the scientific communities, and has also reached mainstream media as an important determinant of childhood and adult health and risk of cardiovascular disease, clinical practice of obstetrics and gynecology, neonatology, and pediatrics has yet to become significantly influenced by evidence that periconceptual, in utero, and early postnatal life stages have a major impact on long-term adult health. Part of the reluctance may be secondary to a paucity of convincing mechanistic evidence that demonstrates “cause and effect” (how does a poor prenatal environment cause hypertension in adulthood?) or perhaps due to a lack of interventions shown to be effective at preventing or treating these negative programming effects. While a huge body of literature has addressed this in a variety of animal models, translating these findings into a human population is not straightforward. Indeed, clinicians hesitate to change clinical practice (particularly addressing early interventions for later-life conditions) without substantial and convincing human data. This fact exposes a huge need for developmental programming research in human populations, including studies that are specifically designed to test hypotheses related to mechanistic pathways. Only in this manner will there be the potential to develop therapeutic interventions and thus hopefully engage all clinicians to
alter their management of offspring from complicated pregnancies.

A more widespread acceptance of the developmental origins theory would likely benefit from an update in clinical practice guidelines. Indeed, the long-term maternal health implications of preeclampsia are now well established and have been incorporated into women's health care via American Heart Association guidelines (53, 267). Clinical practice guidelines by multiple international regulatory societies (SOGC, ACOG, RCOG, etc.) currently exist regarding optimal maternal weight gain, dietary requirements and exercise, management of hypertension and diabetes, and fetal monitoring in high-risk pregnancies. The collective goal of these guidelines is to create the best possible in utero environment for the developing fetus and for early detection of complications. In the neonatal period, nutritional support for preterm and/or growth-restricted babies is also critical and may benefit the long-term prognosis of these children (215, 216). Furthermore, advances are being made in the global recognition of the importance of fetal origins of adult cardiovascular disease. The prevention and management of cardiovascular disease is a strategic priority for the World Health Organization, which now recognizes low birth weight as an important risk factor to be considered by health caregivers (1). Public health campaigns are one effective method to educate society about pregnancy complications and long-term health risks. Increased publicity may empower patients to become their own best advocates in monitoring for the development of cardiovascular disease, as well as encourage the maintenance of a healthy lifestyle. Thus it is critical to educate all health care providers about the need for increased surveillance in children born from complicated pregnancies, who may be at significant risk of cardiovascular disease.

Despite the enormity of this health problem, there are several promising avenues of research into the mechanisms involved in the programming of hypertension and potential treatment strategies. For instance, the use of early postnatal intervention with antioxidants or resveratrol to improve health in adult offspring might prove to be beneficial in the

FIGURE 7. Schematic overview of pathways affected by in utero programming. As has been illustrated through the course of this review, many pathways that are affected by in utero programming of hypertension are also closely interrelated. There is evidence that epigenetic alterations may contribute to increased oxidative stress, altered RAS function, altered HPA function, heightened sympathetic responsiveness, and endothelial dysfunction (particularly as relates to the NO pathways). These changes ultimately combine to result in dysfunction of the two major blood pressure regulatory systems, namely, the kidney and systemic vasculature. ACE, angiotensin converting enzyme; ANG II, angiotensin II; ANG(1–7), angiotensin 1–7; AT₁, angiotensin 1 receptor; AT₂, angiotensin 2 receptor; ET₁, endothelin-1; GFR, glomerular filtration rate; HPA, hypothalamic-pituitary axis; NO, nitric oxide; RAS, renin-angiotensin system.
reversal and/or prevention of disease. Further research into the lifestyle of pregnant women may also elucidate nutritional or exercise-related modifications with far-reaching benefits to the health of their children. As more is discovered and more trials are completed, more successful management of hypertension can be expected to follow; however, as long as pregnancy complications such as fetal growth restriction, preeclampsia, preterm birth, etc., continue to occur, their offspring will be at increased risk for future health concerns such as hypertension.

V. APPENDIX: ANIMAL MODELS OF PROGRAMMING

The following contains a detailed description of the animal models used to investigate the developmental origins of cardiovascular health and disease. References are included in the accompanying tables.

A. Dietary Manipulations

Seminal observations of the consequences of intrauterine programming were made following events such as war and famine (e.g., the Dutch Famine) in which entire populations were subjected to a sudden decrease in available nutrition. Following these events, both retrospective and prospective studies have shown strong associations of cardiovascular disease with maternal undernutrition. Animal models have since been developed to reproduce these situations to further investigate the mechanisms involved and have been extensively represented in the literature (TABLE 2).

1. Maternal undernutrition

Animal models of undernutrition fall largely into two groups: global food restriction or specific restriction of certain aspects of the diet. Global nutrient restriction is a dietary modification that has been employed to create growth-restricted offspring and comprises of a proportional reduction in all components of the diet. In one approach, the experimental group is fed a proportion of the dietary intake of control animals fed ad libitum, determined either during a preexperimental time period or during the previous day. Alternatively, nutrient restriction is calculated as a proportion of recommended dietary nutrient requirements for the species/strain of animals being used. The majority of studies have employed a 50% restriction in sheep or rats (Sprague-Dawley or Wistar) with additional studies using higher (60%) or lower (15–40%) levels of restriction in a variety of species and timing protocols through the gestational term.

Dietary proteins are important in the supply of essential amino acids, energy, and synthesis of tissues, such as muscle, during growth and development. An important component of malnutrition, such as that experienced during the Dutch Famine, is a lack of these dietary proteins. A substantial volume of work has been performed using animal models in which protein levels are specifically reduced while the total diet remains isocaloric to investigate the specific role of protein restriction on fetal growth and development. The source of protein in rodent diets is generally milk-derived casein, and this is reduced either as a percentage by weight, ranging from 5 to 9% of the total diet, or as a percentage by metabolizable energy. In either case, the protein content of the diet is reduced by 50–75% compared with a control diet. The caloric value of the diet is maintained by substitution of protein with corn/maize starch and sucrose while all other nutritional components remain the same. A diet composed of 9% protein (compared with an 18% control) provided to Wistar rats has been one of the most extensively used models for the investigation of developmental effects.

Malnutrition may also involve a lack of micronutrients without a lack of total energy or protein intake. These micronutrients include essential vitamins and minerals that are important in various components of cardiovascular health such as antioxidant status (selenium, vitamin A), cell division and growth (folate), anemia (iron, folate), and enzyme synthesis (zinc). An alteration in specific elements of the diet, for example, levels of zinc, iron, or selenite (which can be reduced to form selenium), has been used to investigate the effects of maternal nutrition on fetal development. Zinc restriction was provided to rats at a level of 8 ppm compared with a control diet containing 30 ppm. Iron deficiency in rats has been induced by providing a diet containing 7.5 mg/kg Fe compared with a control diet of 50 mg/kg. Selenite supplementation was provided at a level of 0.14 mg/kg, in sheep studies.

2. Maternal overnutrition

A dietary imbalance in favor of overnutrition has also been found to be responsible for developmental programming in offspring, and this is particularly relevant in today’s society. Aspects of the so-called “Western diet” common in developed countries and characterized by high fat, high sugar, refined grains, and (in humans) processed meats are replicated in models of maternal overnutrition in animals to mimic current dietary habits. As with other pathological conditions, observational studies in women presenting as patients have been employed to gain insights into the mechanisms behind programming phenotypes, including follow-up studies in women who have undergone bariatric gastrointestinal surgery prior to their pregnancy. In addition, the following animal models of maternal overnutrition have been developed (TABLE 3).

3. Diet-induced obesity

Obesity is induced prior to gestation through a variety of dietary modifications in a variety of species such as CD-1...
Table 2. **Summary table of models of developmental origins of cardiovascular disease that have employed maternal undernutrition in various species to cause a compromised pregnancy**

<table>
<thead>
<tr>
<th>Model</th>
<th>Type of Insult</th>
<th>Species</th>
<th>Timing of Insult</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient restriction</td>
<td>60% restricted</td>
<td>Sprague-Dawley rats</td>
<td>GD 15–21 (term ~21 days)</td>
<td>431</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Western Whiteface sheep</td>
<td>GD 50-130 (term ~145 days)</td>
<td>379</td>
</tr>
<tr>
<td>50% restricted</td>
<td>Sprague-Dawley rats</td>
<td>GD 10-term (term ~21 days)</td>
<td>199, 200</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GD 11-term (term ~21 days)</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Throughout gestation</td>
<td>59, 165, 415, 446</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wistar rats</td>
<td>Throughout gestation</td>
<td>123, 124, 308</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sheep [strain n/r]</td>
<td>GD 28–78 (term ~145 days)</td>
<td>228</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Merino sheep</td>
<td>GD 118–140 (term ~145 days)</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Welsh Mountain sheep</td>
<td>GD 1–31 (term ~145 days)</td>
<td>78, 310</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Targhee sheep</td>
<td>GD 64–135 (term ~145 days)</td>
<td>275</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C57Bl/6J mice</td>
<td>GD 7-term (term ~19 days)</td>
<td>321</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C57Bl/6J mice</td>
<td>GD 10.5–17.5 (term ~19 days)</td>
<td>193</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Guinea pigs</td>
<td>GD 1–35 (early) or GD 36–70 (late) (term ~59–72 days)</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Protein restriction</td>
<td>Pair fed to hypoxic dams</td>
<td>Wistar rats</td>
<td>GD 15–20 (term ~21 days)</td>
<td>201</td>
</tr>
<tr>
<td></td>
<td>Microswine</td>
<td>GD 85–PD 14 (term ~115 days)</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>70% restricted</td>
<td>C57Bl/6J mice</td>
<td>Throughout gestation</td>
<td>391</td>
<td></td>
</tr>
<tr>
<td>70% restricted</td>
<td>Sprague-Dawley rats</td>
<td>Throughout gestation</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>65% restricted</td>
<td>Wistar rats</td>
<td>GD 3-term</td>
<td>358, 359</td>
<td></td>
</tr>
<tr>
<td>60% restricted</td>
<td>Wistar rats</td>
<td>GD 10-term</td>
<td>417</td>
<td></td>
</tr>
<tr>
<td>59% restricted</td>
<td>Wistar rats</td>
<td>GD 13-term</td>
<td>155</td>
<td></td>
</tr>
<tr>
<td>56% restricted</td>
<td>Wistar rats</td>
<td>Throughout lactation</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>55% restricted</td>
<td>Balb/c mice</td>
<td>Throughout gestation and lactation</td>
<td>186</td>
<td></td>
</tr>
<tr>
<td>50% restricted</td>
<td>C57Bl/6J mice</td>
<td>Throughout gestation</td>
<td>331, 332</td>
<td></td>
</tr>
<tr>
<td>50% restricted</td>
<td>Wistar rats</td>
<td>Through out gestation and lactation</td>
<td>242</td>
<td></td>
</tr>
<tr>
<td>50% restricted</td>
<td>56% restricted</td>
<td>2 wk before mating, throughout gestation and lactation</td>
<td>212</td>
<td></td>
</tr>
<tr>
<td>55% restricted</td>
<td>Balb/c mice</td>
<td>Throughout gestation and lactation</td>
<td>39, 40, 46, 57, 206, 211, 225, 252, 253, 271, 282, 307, 308, 328, 348, 405, 406, 450</td>
<td></td>
</tr>
<tr>
<td>50% restricted</td>
<td>C57Bl/6J mice</td>
<td>Throughout gestation</td>
<td>331, 332</td>
<td></td>
</tr>
<tr>
<td>50% restricted</td>
<td>Wistar rats</td>
<td>Through out gestation and lactation</td>
<td>242</td>
<td></td>
</tr>
<tr>
<td>50% restricted</td>
<td>2 wk before mating, throughout gestation</td>
<td>212</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% restricted</td>
<td>2 wk before mating, throughout gestation</td>
<td>212</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Continued
and C57Bl/6J mice, Sprague-Dawley rats, sheep, and Japanese macaques. Obesity is generally induced by an increase in fat intake (sourced from animal lard and ranging from 16–60% total fat content compared with a control diet of 3–6%), but may also include high sugar levels (33% compared with control levels of 7%) or a nonspecific increase in calories (overfeeding). To ensure overfeeding, the diet is either administered via an intragastric cannula or is provided as a highly palatable formulation.

In addition, some models use dietary modifications only during the pregnancy period, a subset in which maternal obesity is not necessarily achieved. As with models of obesity, either high-fat (20%, animal lard source) or high-sugar (33% compared with control levels of 7%) diets are used.

### Table 2. Summary table of models of developmental origins of cardiovascular disease which have employed maternal overnutrition in various species to cause a compromised pregnancy

<table>
<thead>
<tr>
<th>Model</th>
<th>Type of Insult</th>
<th>Species</th>
<th>Timing of Insult</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity</td>
<td>33% high-sugar, 16% high-fat diet (control 7% sugar, 3% fat)</td>
<td>C57Bl/6J mice</td>
<td>6 wk before mating, throughout gestation and lactation</td>
<td>352, 353</td>
</tr>
<tr>
<td>Overfeeding</td>
<td>40% excess calories</td>
<td>Sprague-Dawley rats</td>
<td>3 wk prior, throughout gestation</td>
<td>41</td>
</tr>
<tr>
<td>Overfeeding</td>
<td>50% excess calories</td>
<td>Rambouillet x Columbia sheep</td>
<td>60 days before mating, throughout gestation</td>
<td>453</td>
</tr>
<tr>
<td>High-fat diet</td>
<td>20% high-fat diet</td>
<td>Sprague-Dawley rats</td>
<td>10 days before mating, throughout gestation</td>
<td>198</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Japanese macaques</td>
<td>Throughout gestation and lactation</td>
<td>14, 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C57Bl/6J mice</td>
<td>Adult life, throughout gestation and lactation</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C57Bl/6J mice</td>
<td>Adult life, throughout gestation and lactation</td>
<td>111, 112</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD-1 mice</td>
<td>14 wk before mating, throughout gestation and lactation</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sprague-Dawley rats</td>
<td>8 wk before mating, throughout gestation and lactation</td>
<td>151</td>
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<tr>
<td>High-sucrose diet</td>
<td>20% sucrose solution</td>
<td>Sprague-Dawley rats</td>
<td>Throughout gestation</td>
<td>223</td>
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<tr>
<td></td>
<td></td>
<td>C57Bl/6J mice</td>
<td>6 wk before mating, throughout gestation and lactation</td>
<td>354</td>
</tr>
<tr>
<td>High-cholesterol diet</td>
<td>0.25% cholesterol</td>
<td>New Zealand White rabbits</td>
<td>2 wk before mating, throughout gestation and 1 wk postpartum</td>
<td>296</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ApoE−/− and C57Bl/6J mice</td>
<td>Postnatal</td>
<td>7</td>
</tr>
<tr>
<td>High-salt diet</td>
<td>8% salt (control 0.15%)</td>
<td>Sprague-Dawley rats</td>
<td>Throughout gestation and lactation</td>
<td>305</td>
</tr>
</tbody>
</table>
crose (20–26% sucrose solution) diets have been applied. In addition, a specific increase in cholesterol (0.25%) has been employed to investigate this particular aspect of the diet. An alternative dietary excess that has been employed is a high-salt (8%) diet in Sprague-Dawley rats.

### B. Diabetes and Dyslipidemia

As observed in dietary intervention models, a maternal metabolic imbalance can be detrimental to fetal development in utero. This has been observed in women entering pregnancy with either preexisting diabetes or in women who subsequently develop gestational diabetes. The following animal models have been used to investigate the effects of a metabolic imbalance during pregnancy (TABLE 4).

#### 1. Genotypic models

The agouti mouse has a spontaneous mutation that causes a heightened agouti yellow coat pigmentation and a tendency towards adult-onset obesity, diabetes, and tumor genesis in heterozygous individuals (101). While mice homozygous for this spontaneous mutation are embryonic lethal, heterozygote mice have been used to investigate the effects of maternal metabolic status on developmental programming. The background strain for this model is either KK/HJ or C57Bl/6J mice, which can be employed as relevant controls.

The low-density lipoprotein (LDL) receptor homozygous knockout mouse displays high serum cholesterol and triglyceride levels at 3–4 mo of age which become very high when exposed to a high-fat diet. As such, this mouse strain is used as a model of spontaneous maternal hypercholesterolemia. The apolipoprotein E (ApoE) knockout mouse displays an increase in total plasma cholesterol with moderate increases in triglyceride levels and has, therefore, also been used as another model of spontaneous maternal hypercholesterolemia. The control strain for both of these models is the C57Bl/6J mouse.

#### 2. Hypoinsulinemia/hyperglycemia

Streptozotocin is a naturally occurring chemical that causes the destruction of pancreatic β cells which produce insulin. As a result of treatment with even a single dose of streptozotocin, animals develop a significant level of diabetes or hyperglycemia due to insufficient levels of insulin production, similar to type 1 diabetes. Since animals do not spontaneously develop insulin resistance (type 2 diabetes) or gestational diabetes, treatment with streptozotocin (35–50 mg/kg) has been employed to initiate hyperglycemia during gestation in Sprague-Dawley rats. The development of diabetes varies between animals and can be severe in some cases; therefore, the diabetes is either controlled to a set level using insulin and glucose injections or animals are chosen for inclusion in the study based on their monitored blood glucose levels.

#### 3. Hyperinsulinemia/hypoglycemia

An alternative approach to inducing metabolic dysfunction is to provide continual insulin treatment to initiate hyperinsulinemia and concurrent hypoglycemia. This method has been used in Wistar rats and has been shown to cause growth restriction and hypertension in the offspring.

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**Table 4. Summary table of models of developmental origins of cardiovascular disease which have employed maternal exposure to altered metabolic factors in various species to cause a compromised pregnancy**

<table>
<thead>
<tr>
<th>Model</th>
<th>Type of Insult</th>
<th>Species</th>
<th>Timing of Insult</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous obesity</td>
<td>Genetic modification</td>
<td>Agouti mice</td>
<td>Adult-onset, throughout gestation</td>
<td>56</td>
</tr>
<tr>
<td>Spontaneous hypercholesterolemia</td>
<td>Genetic modification</td>
<td>ApoE−/− mice</td>
<td>Throughout life, throughout gestation</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LDLR−/− mice</td>
<td>Adult-onset, throughout gestation</td>
<td>209</td>
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<tr>
<td>Gestational diabetes</td>
<td>Streptozotocin, 35 mg/kg, in 0.4 mM citrate buffer vehicle, diabetic status monitored</td>
<td>Sprague-Dawley rats</td>
<td>GD 0 (term −21 days)</td>
<td>413</td>
</tr>
<tr>
<td></td>
<td>Streptozotocin, 50 mg/kg, in 10 mM citrate buffer vehicle, glucose/insulin-controlled diabetes</td>
<td>Sprague-Dawley rats</td>
<td>GD 13 (term −21 days)</td>
<td>192, 366</td>
</tr>
<tr>
<td>Hyperinsulinemia</td>
<td>Insulin, 2–4 U/day, slow release implant, subcutaneous</td>
<td>Wistar rats</td>
<td>1 wk before mating, throughout gestation</td>
<td>52</td>
</tr>
</tbody>
</table>
C. Hypoxia

While an imbalance in nutrient supply is established as a causative factor for poor fetal development, a lack of sufficient oxygen has also been observed to have independent effects. This has been observed in humans and also in the following animal models ( TABLE 5).

1. High altitude

Fetal growth restriction has been observed in women who live, or carry their pregnancy, at high altitude (387), and the risk of developing preeclampsia is higher in these populations than in those nearer to sea level (197). The mechanisms leading to these observations, however, are complex, involving evolutionary adaptations in both placental and maternal cardiovascular function (258, 272). The long-term “programming” effects of high altitude on offspring health are not fully understood and would be an interesting area of future study. The effects of high altitude have been reproduced in pregnant animal models housed in research facilities at high altitude (3,600-3,820 m elevation), locations which can be compared with counterparts at sea level (300–520 m elevation). While the majority of these animal

<table>
<thead>
<tr>
<th>Model</th>
<th>Type of Insult</th>
<th>Species</th>
<th>Timing of Insult</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>High altitude</td>
<td>3,600 m elevation (control 420-520 m)</td>
<td>Chicken eggs</td>
<td>Throughout gestation</td>
<td>168, 350</td>
</tr>
<tr>
<td></td>
<td>3,801 m elevation (control 300-335 m)</td>
<td>Sheep (strain n/r)</td>
<td>Throughout gestation</td>
<td>298</td>
</tr>
<tr>
<td></td>
<td>3,820 m elevation (control 346 m)</td>
<td>Sheep (strain n/r)</td>
<td>GD 30-term (147 days)</td>
<td>174, 375</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>10% O2 (control 21% O2)</td>
<td>Wistar rats</td>
<td>GD 15-20 (term 21 days)</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>10.5% O2 (control 21% 02)</td>
<td>FVB/NJ mice</td>
<td>GD 15.5-17.5 (term 19 days)</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>11.5% O2 (control 21% 02)</td>
<td>Sprague-Dawley rats</td>
<td>GD 15-21 (term 21 days)</td>
<td>441</td>
</tr>
<tr>
<td></td>
<td>12% O2 (control 21% 02)</td>
<td>Sprague-Dawley rats</td>
<td>GD 15-21 (term 21 days)</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>Wistar rats</td>
<td>GD 1-10 (term 21 days)</td>
<td>161</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13% O2 (control 21% 02)</td>
<td>Wistar rats</td>
<td>GD 10-20 (term 21 days)</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>15% O2 (control 21% 02)</td>
<td>Chicken eggs</td>
<td>GD 0-19 (term 21 days)</td>
<td>227, 257</td>
</tr>
<tr>
<td>Intermittent hypoxia</td>
<td>4.5% O2, 8 h/day, (control 21% 02)</td>
<td>Wistar rats</td>
<td>GD 14-term (21 days)</td>
<td>274</td>
</tr>
<tr>
<td></td>
<td>12% O2, 4 h morning + 4 h afternoon/day, (control 21% 02)</td>
<td>New Zealand White rabbits</td>
<td>GD 10-19 (term 30 days)</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>6% O2, 30 min, 3x</td>
<td>Welsh Mountain sheep</td>
<td>GD 130 (term 145 days)</td>
<td>188, 400</td>
</tr>
</tbody>
</table>
studies have involved sheep, incubation of chicken eggs at altitude has also been used to investigate the effect of the partial pressure of oxygen throughout gestation or following the implantation stages.

2. Maternal hypoxia

Hypoxia chambers have facilitated the investigation of low oxygen levels on pregnancy outcomes in rodents. The ease of adjustment of oxygen has allowed the study of various levels of oxygen, ranging from 10–13%, through different stages of pregnancy in Sprague-Dawley and Wistar rats, FVB/NJ mice, and chicken eggs. Furthermore, the effect of intermittent periods of hypoxia can be investigated and have been employed in Wistar rats and New Zealand White rabbits. A limitation of the hypoxic environment models is the concurrent reduction in food intake and ambulatory activity that occurs as a response to the reduced oxygen environment. While nutritional restriction in a normal oxygen environment is well established as a model of compromised in utero environment, in hypoxic conditions the nutrient intake is likely appropriate for the unique metabolic demands of this environment given the reduced activity levels. Larger equipment has been employed to investigate the effects of hypoxia in sheep. The benefit of this model is the concurrent instrumentation of the fetal lamb to allow monitoring of the acute cardiovascular effects of a compromised in utero environment on both maternal and fetal outcomes.

D. Placental Insufficiency

The previous sections demonstrate the critical role of both nutrient and oxygen transfer between the maternal and fetal circulations in the development of the fetus. Since the placenta is the organ that mediates this transport, an alternative approach to initiating a model of developmental programming has been to impact the ability of the placenta to perform this transfer. Such models are described in the following section (TABLE 6).

1. Reduced blood flow models

A primary method of decreasing the placental ability to mediate nutrient and oxygen transfer in rodents and rabbits is to reduce blood flow to the placenta, thereby mediating a global reduction in nutrient and oxygen supply. There are two methods of achieving a reduction in blood flow: these are the reduced utero-placental perfusion pressure (RUPP) and bilateral uterine artery ligation models. Both of these are surgical models that employ mechanical restriction of blood flow.

Bilateral ligation is achieved using sutures and can be modified to cause either a complete or partial obstruction of the uterine arteries. Due to the vascular anatomy, compensatory blood flow via the ovarian arteries continues to provide partial blood supply to the uterus and, therefore, a range of birth weights from normal through varying levels of growth restriction to complete reabsorptions is observed in the offspring. Complete ligation is generally performed later in term [gestational day (GD) 18–19 in rats, GD 2.5 in rabbits] due to the severity of this insult and subsequent fetal loss.

The RUPP model employs partial restriction of both the uterine arteries and the abdominal aorta using silver clips of a set diameter to effect a 40–60% reduction of blood flow to the uterus and the fetal units with no compensatory flow. The RUPP model has been extensively used as a model of preeclampsia due to the observation of increased maternal blood pressure and proteinuria in some species.

2. Direct placental insults

Ovine models have a particular method of placentation whereby the fetuses are supplied by a polycotyledonary placenta that is formed of 70–100 cotyledons (fetal attachments). The maternal side of the placentae, the caruncles, is visible in the nonpregnant uterus, and this allows for a direct reduction in placental units prior to mating via carunclectomy. Placental function in sheep has also been reduced via embolization, which employs the injection of latex microspheres to provide a physical barrier to placental blood flow.

E. Fetal Exposure to Environmental and Toxic Factors

As discussed in the previous sections, placental dysfunction can reduce the supply of essential nutrients and oxygen to the fetus. An additional role of the placenta is to protect the fetus from maternal factors that could be harmful to its development. In some cases, however, this function of the placenta is reduced or the placenta itself can become a source of circulating factors. Assessment of both maternal and fetal blood in patients suffering from complicated pregnancies has identified an array of compounds that are elevated when compared with normal pregnancies and that can lead to detrimental outcomes in the offspring. Models of the developmental origins have mimicked these conditions in animals (TABLE 7).

1. Corticosteroids and stress

An increase in maternal stress or inflammation can cause the release of corticosteroids such as glucocorticoids. Animal models of the developmental origins of hypertension have investigated the effect of pharmacologically increased corticosteroid levels using treatment with either betamethasone, (0.17 mg·kg$^{-1}$·day$^{-1}$ for 2 days in sheep) or dexamethasone (0.02–5 mg·kg$^{-1}$·day$^{-1}$ for 2–8 days in C57Bl/6 mice, Wistar rats, sheep, and marmoset mon-
The placenta produces an inactivator, 11β-hydroxysteroid dehydrogenase (11βHSD) 2, which catalyzes the conversion of active glucocorticoids (cortisol in humans and corticosterone in rats) into their inactive 11-keto metabolites (cortisone and 11-dehydrocorticosterone respectively). Other investigators have used animal models in which placental 11β-HSD is either absent, 11β-HSD knockout mice, or inhibited using carbenoxolone treatment (12.5 mg·kg⁻¹·day⁻¹ for 7 days in C57Bl/6J mice). In these models, the function of the placenta to breakdown corticosteroids is lost.

As mentioned, corticosteroids are increased in the presence of maternal stress or inflammation. In this regard, increased ambient temperature, chronodisruption (a constant light cycle), and maternal dehydration have all been employed to induce stress and subsequent developmental programming effects in sheep and Sprague-Dawley rats.

### Toxin exposure

The observation of smoking mothers has demonstrated the detrimental effects of fetal exposure to substances such as nicotine and cannabis. Animal models have been used to further investigate these effects. Both C57Bl/6 mice and Sprague-Dawley rats have been provided with nicotine prior to mating as well as throughout gestation and lactation to mimic the human situation. Nicotine was administered via a mini osmotic pump (5.76 mg·kg⁻¹·day⁻¹) or in drinking water made palatable by the addition of saccharin (0.2 mg/ml).

Cocaine use is another substance that fetuses may be exposed to during in utero development. The effects of cocaine exposure have been investigated in Sprague-Dawley rats administered with cocaine (15 mg/kg twice a day intraperitoneally).

### Table 6. Summary table of models of developmental origins of cardiovascular disease which have employed reduced placental function in various species to cause a compromised pregnancy

<table>
<thead>
<tr>
<th>Model</th>
<th>Type of Insult</th>
<th>Species</th>
<th>Timing of Insult</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RUPP, reduced utero-placental perfusion pressure</td>
<td>Mechanical restriction of the uterine and abdominal aorta with silver clips</td>
<td>Sprague-Dawley rats</td>
<td>GD 14-term (term ~21 days)</td>
<td>5, 6, 150, 180, 289, 290, 292, 293</td>
</tr>
<tr>
<td>Bilateral uterine artery ligation</td>
<td>Complete suture ligation of the uterine arteries</td>
<td>Wistar rats</td>
<td>GD 17 (term ~21 days)</td>
<td>363</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brown Norway rats</td>
<td>GD 18 (term ~21 days)</td>
<td>299</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wistar rats</td>
<td>GD 18 (term ~21 days)</td>
<td>4, 129, 207, 408, 434</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wistar rats</td>
<td>GD 19 (term ~21 days)</td>
<td>281, 303</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sprague-Dawley rats</td>
<td>GD 19.5 (term ~21 days)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dunkin-Hartley guinea pigs</td>
<td>GD 28-30 (term ~67 days)</td>
<td>48</td>
</tr>
<tr>
<td>20–30% (mild) or 40–50% (severe) suture ligation of the uterine arteries</td>
<td>Rabbits (strain n/r)</td>
<td>GD 25 (term ~30 days)</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>Single umbilical artery ligation</td>
<td>Complete suture ligation of one umbilical artery in a twin pregnancy</td>
<td>Sheep (strain n/r)</td>
<td>GD 110 (term ~147 days)</td>
<td>32</td>
</tr>
<tr>
<td>Carunclectomy</td>
<td>Removal of the majority of caruncles</td>
<td>Merino sheep</td>
<td>11 wk before mating</td>
<td>93, 264, 327</td>
</tr>
<tr>
<td>Embolization</td>
<td>Injection of microspheres</td>
<td>Border Leicester x Merino sheep</td>
<td>GD 110-130 (term ~147 days)</td>
<td>454</td>
</tr>
<tr>
<td></td>
<td>Injection of 15- or 30-μm latex microspheres</td>
<td>Western sheep</td>
<td>GD 116–133 (term ~147 days)</td>
<td>401</td>
</tr>
<tr>
<td></td>
<td>Injection of 40- to 70-μm latex microspheres</td>
<td>Border Leicester x Merino sheep</td>
<td>GD 120–140 (term ~147 days)</td>
<td>256</td>
</tr>
</tbody>
</table>
Table 7. Summary table of models of developmental origins of cardiovascular disease which have employed maternal exposure to various environmental factors in various species to cause a compromised pregnancy

<table>
<thead>
<tr>
<th>Model</th>
<th>Type of Insult</th>
<th>Species</th>
<th>Timing of Insult</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroids</td>
<td>Betamethasone acetate, 0.17 mg/kg, intramuscular injection, 2 doses 24 h apart</td>
<td>Sheep [strain n/r]</td>
<td>GD 80–81 (term ~145 days)</td>
<td>34, 154, 191, 220, 246–248, 371–374, 398</td>
</tr>
<tr>
<td></td>
<td>Betamethasone acetate, 87.5 µg/kg, intramuscular injection, 4 doses 12 h apart</td>
<td>Baboons</td>
<td>GD 122–136 (term ~185 days)</td>
<td>203</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone sodium phosphate, 0.02 mg·kg⁻¹·day⁻¹, mini osmotic pump, 60 h</td>
<td>C57Bl/6J mice</td>
<td>GD 12.5–14.5 (term ~19 days)</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone, 0.1 mg·kg⁻¹·day⁻¹, subcutaneous</td>
<td>C57Bl/6J mice</td>
<td>GD 10–18 (term ~19 days)</td>
<td>331</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sprague-Dawley rats</td>
<td>GD 1–10 (term ~21 days)</td>
<td>436</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wistar rats</td>
<td>GD 15–21 (term ~21 days)</td>
<td>39, 284, 286</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone, 0.2 mg·kg⁻¹·day⁻¹, 2 days, intraperitoneal</td>
<td>Rats [strain n/r]</td>
<td>GD 11–20 (term ~21 days)</td>
<td>294</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone, 0.05, 0.12 or 0.2 mg·kg⁻¹·day⁻¹, oral in diet</td>
<td>Velvet monkeys</td>
<td>GD midgestation-term</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone, 0.28 mg/kg, intravenous infusion, 48 h</td>
<td>Sheep [strain n/r]</td>
<td>GD 27–28 (term ~145 days)</td>
<td>329, 330</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone, 0.48 mg/h, intravenous infusion, 48 h</td>
<td>Merino sheep</td>
<td>GD 27–28 (term ~145 days)</td>
<td>99, 260, 262</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone, 5 mg·kg⁻¹·day⁻¹, oral in NutriCal/water vehicle</td>
<td>Marmoset monkeys</td>
<td>GD 42–48 (early gestation) or GD 90–96 (late gestation) (term ~144 days)</td>
<td>17</td>
</tr>
<tr>
<td>Repeated corticosteroids</td>
<td>Betamethasone acetate, 0.5 mg/kg, intramuscular injection</td>
<td>Sheep [strain n/r]</td>
<td>GD 104, 111, 118, and 124 (term ~145 days)</td>
<td>178, 268</td>
</tr>
<tr>
<td>Cortisol</td>
<td>Cortisol, 5 mg/h, intravenous infusion, 48 h</td>
<td>Sheep [strain n/r]</td>
<td>GD 26–28 (term ~145 days)</td>
<td>260–262</td>
</tr>
<tr>
<td>11β-Hydroxysteroid dehydrogenase knockout</td>
<td>Genetic manipulation</td>
<td>11β-hydroxysteroid dehydrogenase 2−/− mice</td>
<td>Throughout life, throughout gestation</td>
<td>172, 438</td>
</tr>
<tr>
<td>11β-Hydroxysteroid dehydrogenase inhibition</td>
<td>Carbenoxolone, 12.5 mg·kg⁻¹·day⁻¹, subcutaneous</td>
<td>C57Bl/6J mice</td>
<td>GD 12–19 (term ~19 days)</td>
<td>332, 333</td>
</tr>
<tr>
<td>Elevated temperature</td>
<td>12:12 h at 40/35°C</td>
<td>Rambouillet x Columbia sheep</td>
<td>GD 35–115 (term ~147 days)</td>
<td>100, 342</td>
</tr>
<tr>
<td>Chronodisruption</td>
<td>Constant light [12:12 light-dark control]</td>
<td>Sprague-Dawley rats</td>
<td>GD 10–18 (term ~21 days)</td>
<td>128</td>
</tr>
</tbody>
</table>

Continued
3. Alcohol exposure

Alcohol consumption during pregnancy is also known to have adverse fetal outcomes. These have been investigated in sheep that were provided with 40% alcohol at 1 g/kg, vol/vol for 5 days/wk for 5 wk. Fetal growth restriction has been observed in a Sprague-Dawley rat model that was provided with a liquid diet including 6% vol/vol ethanol throughout pregnancy.

4. Soluble fms-like tyrosine kinase-1

A factor that has been shown to be elevated in maternal plasma in conditions of complicated pregnancies, particularly those affected by preeclampsia, is soluble fms-like tyrosine kinase-1 (sFlt-1). Elevation of sFlt-1 in animals has, therefore, been used to investigate the effect of this particular factor on the development of the fetus. To date, elevated sFlt-1 levels have been investigated in mid-gestation (GD 8) in CD-1 mice through adenovirus introduction.

Hypoxia inducible factor-1 (HIF-1α) is known to induce the production of sFlt-1, and the effect of overexpression of HIF-1α has been investigated in C57Bl/6J mice. Overexpression was achieved through adenovirus introduction.

5. RAS

An imbalance in the RAS is one of the primary candidates for involvement in both altered kidney function and vascular function. While the proposed RAS pathways have been extensively studied in many models of fetal development, a specific model has also been developed in which immunization of Wistar rats leads to the expression of antibodies to the angiotensin receptor 1 (AT1-Ab). The AT1-Ab can act as an AT1 receptor agonist and impair placental development,
thereby impairing fetal growth and development. AT1 autoantibodies have been shown to be increased in women with preeclampsia (349).

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DISCLOSURES

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DEVELOPMENTAL PROGRAMMING OF HYPERTENSION


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