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Appraising the Preclinical Evidence of the Role of the Renin-Angiotensin-Aldosterone System in Antenatal Programming of Maternal and Offspring Cardiovascular Health Across the Life Course: Moving the Field Forward: A Scientific Statement From the American Heart Association

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Abstract

There is increasing interest in the long-term cardiovascular health of women with complicated pregnancies and their affected offspring. Emerging antenatal risk factors such as preeclampsia appear to increase the risk of hypertension and cardiovascular disease across the life course in both the offspring and women after pregnancy. However, the antenatal programming mechanisms responsible are complex and incompletely understood, with roots in alterations in the development, structure, and function of the kidney, heart, vasculature, and brain. The renin-angiotensin-aldosterone system is a major regulator of maternal-fetal health through the placental interface, as well as kidney and cardiovascular tissue development and function. Renin-angiotensin-aldosterone system dysregulation plays a critical role in the development of pregnancy complications such as preeclampsia and programming of long-term adverse cardiovascular health in both the mother and the offspring. An improved understanding of antenatal renin-angiotensin-aldosterone system programming is crucial to identify at-risk individuals and to facilitate development of novel therapies to prevent and treat disease across the life course. Given the inherent complexities of the renin-angiotensin-aldosterone system, it is imperative that preclinical and translational research studies adhere to best practices to accurately and rigorously measure components of the renin-angiotensin-aldosterone system. This comprehensive synthesis of preclinical and translational scientific evidence of the mechanistic role of the renin-angiotensin-aldosterone system in antenatal programming of hypertension and cardiovascular disease will help (1) to ensure that future research uses best research practices, (2) to identify pressing needs, and (3) to guide future investigations to maximize potential outcomes. This will facilitate more rapid and efficient translation to clinical care and improve health outcomes.

Keywords

AHA Scientific Statements; aldosterone; angiotensin-converting enzyme 2; hypertension; pre-eclampsia; pregnancy; renin-angiotensin system

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The antenatal period, spanning conception to birth, is critical for maternal and fetal health. Exposure to adverse health conditions and environmental stressors during this time period can have long-term consequences on the mother and her offspring. Briefly stated, antenatal programming happens when exposures occur from conception through birth that alter structural, physiological, and metabolic fetal development and maternal health to improve short-term survival but at the expense of programmed adverse cardiovascular health in the long term (ie, developmental plasticity).[1] For example, maternal hypertension, the most common medical comorbidity in pregnancy, is a major health concern and is associated with increased risks of short-term mortality and morbidity, as well as programmed chronic disease later in life in both the mother and the fetus.[2] Offspring of women with pre-eclampsia have lower birth weight and higher blood pressure throughout childhood and young adulthood compared with unexposed offspring.[3] Numerous preclinical models have confirmed this association,[4] yet the exact mechanisms remain incompletely understood. Several of the major components of the renin-angiotensin-aldosterone system (RAAS) regulate several key physiological processes in both mother and fetus during pregnancy and the development and function of the kidney and cardiovascular system. Most notably, these include the angiotensin-converting enzyme (ACE)/angiotensin II (Ang II)/Ang II type

1 receptor (AT_1R) and the ACE2/angiotensin-(1–7) (Ang-[1–7])/Mas receptor pathways. Dysregulation of the circulating and tissue-specific RAAS contributes to the pathogenesis of numerous antenatal conditions, including hypertensive disorders in pregnancy.[5] RAAS dysregulation is one potential mechanism for the long-term programming of hypertension in offspring exposed to preeclampsia and other adverse antenatal factors.[4,6]

Greater risk of long-term hypertension and cardiovascular disease is also observed in women after preeclampsia, highlighting that the burden of programmed cardiovascular disease is not limited to the offspring.[7] It is important to note that, despite decades of research, recommendations for preeclampsia treatment have not changed,[8] and the prevalence of hypertension in pregnancy continues to increase.[9] Treatment strategies vary considerably around the world, with significant disparities in the screening and follow-up for the development of hypertension and cardiovascular disease in affected women during the postpartum period and beyond, including in women from disenfranchised populations in the United States.[10] In addition, emerging evidence indicates that pregnancy may place women at greater risk for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, which in turn may increase the risk of several pregnancy complications, including preeclampsia and low birth weight, possibly related to altered ACE2 expression, the binding site for SARS-CoV-2.[11,12] Thus, the short- and long-term increased risk of hypertension and cardiovascular disease in the mother and offspring attributable to pregnancy complications remains a critical health concern.

Despite decades of high-quality studies that have provided insights into the mechanisms responsible for the programming of cardiovascular disease associated with complicated pregnancies, there remains a crucial need in the field to further characterize dysregulatory events affecting the RAAS during this critical period of life. Primary reasons for persistent knowledge gaps include heterogeneity in the methods used in many preclinical models and the complex nature of the RAAS that makes accurate and reliable quantification challenging. Thus, the goal of this scientific statement is to summarize the current state of knowledge related to preclinical evidence of antenatal programming mechanisms of long-term maternal and offspring cardiovascular health as it relates to the role of several of the major RAAS pathways using well-characterized preclinical models of developmental programming. This scientific statement identifies gaps in knowledge that require further research. Moreover, this scientific statement emphasizes the importance of better understanding programming mechanisms for both investigators and clinicians to develop targeted interventions to prevent or mitigate the increased risk of hypertension and cardiovascular disease. This is the first step in an approach to reduce future cardiovascular risk in women with complicated pregnancies and their children.

A comprehensive literature search was conducted from approximately September 15, 2021, to November 15, 2021, that encompassed preclinical and clinical studies and reviews that were published in PubMed, Scopus, and other relevant databases using standardized methods. Key search words included but were not limited to pregnancy, preeclampsia, RAAS, high blood pressure, hypertension, cardiovascular, renal, brain, placental insufficiency, hypoxia, glucocorticoids, maternal undernutrition, offspring, and chronic health. The selection of writing group members was based on a wide range of

expertise, including clinical and preclinical researchers representing different backgrounds, geographic regions, sexes, races, and ethnicities.

MATERNAL-PLACENTAL-FETAL INTERFACE AND THE RAAS

Maternal Cardiovascular Physiology During Pregnancy

Maternal cardiovascular and renal adaptations to pregnancy are essential to accommodate the physiological stress imparted by the growing fetus and placenta. Marked systemic vasodilation with decreased systemic vascular resistance and subsequent lower blood pressure characterizes early pregnancy starting at 4 to 6 weeks of gestation. This likely stimulates the maternal circulating RAAS by the end of the first trimester to retain sodium and fluid to increase plasma volume progressively throughout gestation, up to 40% to 50% higher than the prepregnancy baseline.[13] Cardiac output, renal blood flow, and glomerular filtration rate increase to ≈50% over baseline; these changes are apparent by the second trimester and persist until term.

The RAAS in Normal Pregnancy Physiology

The RAAS, a crucial regulator of blood pressure and fluid-electrolyte balance, particularly in pregnant women and the fetus, is a key contributor to cardiovascular and kidney development (Figure 1A). Assessment of RAAS components in the maternal circulation during pregnancy suggests that overall activation that contributes to the aforementioned physiological cardiovascular changes. In normotensive, healthy pregnant women, blood pressure remains lower while plasma renin activity (PRA) and aldosterone remain elevated until late in pregnancy when blood pressure increases.[14] Increased angiotensinogen production and PRA lead to increased angiotensin I concentrations, favoring augmented Ang II production that occurs despite reduced serum ACE activity, in part as a result of activation of additional RAAS pathways.[15] Ang II–mediated increased aldosterone concentrations directly stimulate renal sodium and fluid retention to increase blood volume.

Circulating and local tissue Ang II exerts key physiological functions in many crucial steps of placentation, including trophoblast invasion and migration, as well as spiral artery remodeling.[16] RAAS components show a dynamic distribution throughout pregnancy. The AT_1R is expressed in trophoblasts in early pregnancy but also in villous endothelial cells at term.[17] Prorenin, (pro)renin receptor, AT_1R, and Ang II type 2 receptor proteins are also expressed throughout gestation in trophoblasts at the maternal-fetal interface and in invasive trophoblasts, whereas ACE is concentrated predominantly in the fetal circulation, particularly in endothelial cells.[18] The incremental ACE protein expression in fetal endothelial cells throughout pregnancy favors enhanced Ang II production in placental vessels from the fetal side, where angiogenesis, an essential process for maintaining fetal perfusion, continuously occurs.[18] However, the expected increased Ang II production in the fetus and mother must be finely modulated to prevent excessive vasoconstriction and cardiovascular remodeling that could occur if Ang II concentrations increase above the expected physiological range.[19]

Pregnancy also stimulates the ACE2/Ang-(1–7) pathway to balance increased ACE/Ang II pathway activity and to contribute to maternal hemodynamic adaptations and placentation, trophoblast invasion, decidualization, and vascular remodeling.[15,20] ACE2 breaks down Ang II, and Ang-(1–7), acting on its Mas receptor, antagonizes Ang II signaling through AT_1R modulation.[6] Estrogens regulate the progressive RAAS activation observed throughout gestation in part by directly stimulating angiotensinogen production and increasing ACE2 expression and activity in local tissue.[21] In rats, renal ACE2 and Ang-(1–7) are progressively upregulated throughout pregnancy.[22] ACE2 and Ang-(1–7) are also expressed in trophoblasts, villous vessel endothelial cells, primary villi vascular smooth muscle cells, and the syncytium and decidua.[23] ACE2/Ang-(1–7) expression and activity in the placenta are dynamic, with greater concentrations in the decidua in early pregnancy that progressively change toward the placental villous endothelial cells and trophoblasts in late gestation.[23] The presence of the ACE2/Ang-(1–7) pathway in invasive trophoblasts surrounding the spiral arteries, as well as in endothelial cells and vascular smooth muscle cells, suggests that the ACE2/Ang-(1–7) pathway helps regulate uterine artery tone and reduce maternal systemic vascular resistance.[15] Therefore, ACE2-mediated conversion of Ang II into Ang-(1–7) and intracellular signaling between AT_1R and Mas receptor are likely key factors regulating Ang II physiological effects during pregnancy.

Additional RAAS pathways contribute to the Ang II–Ang-(1–7) balance during pregnancy but are less well characterized; thus, their role in antenatal programming presents another important knowledge gap. The (pro) renin receptor is crucial for many developmental and physiological processes during pregnancy through several signaling pathways, including Wnt/b-catenin and mitogen-activated protein kinase.[24] Neprilysin, another metallopeptidase that converts angiotensin I to Ang-(1–7), has unclear effects during pregnancy. Compared with pregnant women with healthy weight, pregnant women with overweight or obesity (body mass index ≥25 or ≥30 kg/m^2, respectively) have lower endothelial cell neprilysin expression in the fetus and placenta, and fetal weight is associated inversely with circulating neprilysin levels in cord blood.[25] Uterine mast cell and natural killer cell secretion of chymase, a serine protease that generates Ang II independently of ACE, may contribute to decidual vessel remodeling and subsequent fetal growth.[26]

The RAAS in Pregnancy Pathologies

Placental insufficiency is a hallmark of many adverse pregnancy events that program later disease in the offspring. Short- or long-term interruptions in the sufficient delivery of blood, oxygen, or nutrients to the fetus can alter fetal growth and organ development, leading to abnormal tissue structure and function, especially in the kidneys. Adequate fetal perfusion requires sufficient maternal cardiovascular and placental health; hence, interruptions in the maternal, placental, or fetal RAAS can potentially adversely affect fetal and maternal cardiovascular health in the short and long term (Figure 1B).

Human intrauterine growth restriction, a proxy for placental insufficiency, is associated with higher cord blood Ang II concentration but no difference in fetal-placental AT_1R concentration compared with term pregnancies with delivery by elective cesarean section.[27] Uterine vessel ligation or clamping during mid or late pregnancy as a model of placental

insufficiency and maternal preeclampsia commonly induces reduced glomerular number and hypertension in the offspring. Uteroplacental insufficiency in the rat model of reduced uterine perfusion pressure at gestational day 14 is associated with attenuated intrarenal RAAS activity in neonatal rats.[28]

The apparent bidirectional relationship between preeclampsia and placental insufficiency remains a paradox in the field that can hinder accurate inferences about RAAS measurements observed in preclinical models and clinical studies. Placental insufficiency in preclinical models and in women may be a precursor to maternal hypertensive disorders. Preeclampsia, in turn, exacerbates placental insufficiency and may program adverse cardiovascular health in affected women and their offspring independently of placental insufficiency. Although the cause of preeclampsia is still unclear, intriguing preclinical and clinical evidence suggests that several potentially interrelated pathways are involved. Early-pregnancy placental ischemia is associated with release of soluble fms-like tyrosine kinase 1, a circulating soluble isoform of the vascular endothelial growth factor receptor that has antiangiogenic properties. It binds free vascular endothelial growth factor and placental growth factor, leading to an imbalance of antiangiogenic and proangiogenic factors, resulting in maternal endothelial dysfunction, hypertension, proteinuria, and glomerular endotheliosis.[29]

Increased levels of circulating AT_1R autoantibodies in women with preeclampsia, first described in 1999,[30] have been studied extensively both in preclinical models of placental hypoperfusion or preeclampsia and in clinical studies. These antibodies are directed to a specific epitope on the second extracellular loop of the AT_1R and bind to human trophoblasts. Multiple studies suggest that the antibodies may play an important role in the pathophysiology of preeclampsia by inducing vasoconstriction, hypertension, and increased coagulation.[31] Antibodies derived from women with preeclampsia induce placental soluble fms-like tyrosine kinase 1 production through AT_1R activation in pregnant mice, human placental villous explants, and human trophoblast cells.[32] Human studies have shown that these antibodies can be detected early in pregnancy in women who later develop preeclampsia and that, once preeclampsia develops, >95% of women have the antibodies, which also correlate with disease severity.[33] However, significant limitations remain in measuring these autoantibodies accurately, and their clinical significance remains unclear.[34]

RAAS contributions to preeclampsia development and progression remain elusive as well, with conflicting findings and much debate resulting mainly from a lack of consensus of methodological rigor.[35] It remains unknown whether RAAS dysregulation is a prerequisite for preeclampsia development or a consequence. Activation of tissue RAAS and, by extension, the circulating RAAS is necessary to meet and regulate the growing demands of the fetus to ensure a healthy pregnancy. Although there is consensus that placental alterations are a principal causal mechanism in preeclampsia, it remains unclear what role the RAAS has and whether the observed changes in the circulating RAAS in women with preeclampsia (eg, lower PRA) contribute to the dysregulation of maternal kidney and vascular function or are altered in response to changes induced by angiogenic factor dysregulation. Leading theories suggest that abnormal or shallow placentation with defective spiral arteriolar remodeling, reduced placental blood flow, and increased placental oxidative

stress contributes to preeclampsia pathogenesis.[35] This abnormal placental development may contribute to underexpression or overexpression of placental RAAS components and excess shedding of placental particles such as miRNA that target RAAS mRNA.[36]

Subsequent release of RAAS components into the maternal circulation from the fetal-placental unit (eg, increased Ang II, decreased Ang-[1–7]) may alter the systemic and tissue-specific maternal RAAS, including the intrarenal RAAS, subsequently reduce uteroplacental blood flow, and further drive placental damage.[35,37] Women who develop preeclampsia demonstrate decreased glomerular filtration rate, PRA, and aldosterone before and at the time of diagnosis, consistent with a volume-expanded circulation.[38] Placental and plasma soluble (pro)renin receptor levels are higher in women with preeclampsia compared with women with normal pregnancies.[39] During preeclampsia, neprilysin levels are higher in the placenta and in circulating extracellular vesicles derived from syncytio-trophoblasts.[40] Maternal vascular endothelial chymase expression is increased in preeclampsia.[41] In addition, these RAAS components may lead to secondary changes in neurohormonal regulation of cardiovascular and kidney function that program hypertension and cardiovascular disease.[42] However, the precise timing of these RAAS changes and their importance to later programmed disease remain unknown.

OVERVIEW OF MAJOR PRECLINICAL PROGRAMMING MODELS

Numerous preclinical models using different programming events in various animal species have demonstrated consistently that programmed RAAS alterations in the antenatal period can lead to hypertension development in women and their offspring (Figure 2). Among the most widely studied are models of placental insufficiency and maternal protein or global nutrition restriction or excess in rodents and sheep (Table 1). Within these models, specific antenatal events include maternal-fetal vascular supply disruptions, maternal stress, and exogenous exposures such as glucocorticoids. Although most models attempt to induce fetal growth restriction, it is well documented that programming mechanisms can occur independently of placental insufficiency or growth restriction. Because human nephrogenesis is complete by 36 weeks' gestation, most preclinical models target equivalent windows in animals in which nephrogenesis continues after birth (eg, the first 10–12 days of postnatal life in rodents) to investigate the effects of exposures during organogenesis on long-term kidney function and cardiovascular health.

However, the programming effects of antenatal exposures on the RAAS are heterogeneous, depending on the animal model (species, strain), exposure (timing, severity, duration), and tissue of interest. Choice of model, exposure, and timing often depends on whether the investigator is interested in a specific tissue (kidney, heart, brain, or vasculature) and sex- or age-specific effects on RAAS programming.[43] Variability in the timing of programmed events has led to heterogeneity among reported results and contributed to paradoxes in the field. Early, mid, late, or pan-gestation exposures can cause discrepant findings even in the same animal model. For example, maternal protein restriction–induced hypertension severity is greatest in mid to late gestation.[44] As presented in the following sections, the expression, concentration, and activity of ACE/ACE2, Ang II/Ang-(1–7), and AT_1R/Ang II

type 2 receptor/Mas receptor can be altered dramatically and permanently in different tissues to contribute to increased hypertension and cardiovascular risk.

EVIDENCE FOR RAAS-MEDIATED ANTENATAL PROGRAMMING

Maternal Health

Women who had preeclampsia have an increased risk of developing hypertension and cardiovascular disease at an earlier age.[45] Many of the maternal cardiovascular and RAAS pathophysiological alterations that occur in the antenatal period may contribute to maternal programmed hypertension. However, the precise mechanisms involved remain incompletely understood in preclinical models and clinical studies.[46] In the reduced uterine perfusion pressure model in the rat, affected females demonstrate salt-sensitive blood pressure 3 weeks postpartum independently of PRA or plasma aldosterone.[47] In this model, affected females have worse glomerular filtration rate and left ventricular ejection fraction despite no differences in mean arterial pressure or kidney or heart structure.[48] AT_1R autoantibody–induced preeclampsia is associated with greater left ventricular mass index, left ventricular remodeling, and cardiac susceptibility to ischemia in rats 16 weeks postpartum.[49] In addition, AT^1R autoantibody blockade during pregnancy in the reduced uterine perfusion pressure model improves maternal blood pressure during pregnancy and maternal blood pressure, cardiac hypertrophy, and cardiac mitochondrial function 10 weeks postpartum.[50] However, much more investigation is needed to better delineate the role of the RAAS in mediating maternal antenatal hypertension programming.

Offspring Health

The RAAS plays a major role in fetal kidney development. RAAS manipulation during gestation can have profound effects on the developing fetus. This is perhaps best illustrated by the fact that the use of RAAS inhibitors to manage maternal hypertension during pregnancy is strongly contraindicated because of a high risk of fetal birth defects, including renal agenesis, tubular dysgenesis, kidney failure, and fetal death. In addition to higher blood pressure, human adolescents born preterm have higher circulating RAAS activity toward the ACE/Ang II pathway and away from the ACE2/Ang-(1–7) pathway, associations that are magnified in female individuals and those with obesity.[51] Human male adolescents born prematurely with very low birth weight who were exposed to preeclampsia have higher circulating aldosterone levels compared with those who were unexposed.[52] However, a major paradox in the field is whether preterm birth and lower birth weight in and of themselves are necessary and sufficient to cause programming or the antecedent exposures (eg, preeclampsia) actually program future disease.[1,53]

Various genetically modified animal models could be used to study the effects of antenatal RAAS manipulations on offspring outcomes. However, most studies that use genetic manipulations have targeted the offspring’s genome rather than that of the parents. As a result, dissociating the effects of such manipulations, specifically during the antenatal period, to program adult cardiovascular function, versus the ongoing effects of genetic manipulations throughout the life course through epigenetic mechanisms, is difficult. Thus,

most evidence comes from preclinical surgical, dietary, or pharmacological interventions commonly used to induce antenatal RAAS programming.

Antenatal dexamethasone programs increased blood pressure and increased renal renin and ACE expression in male rat offspring, and in utero delivery of antioxidants, dimethyl fumarate, or melatonin can prevent these effects.[54,55] Antenatal dexamethasone exposure in sheep upregulates fetal pulmonary and circulating RAAS components and impairs cardiovascular function through RAAS-dependent mechanisms.[56] In sheep, antenatal betamethasone induces sex-specific alterations in renal proximal tubule cell responses to Ang II and Ang-(1–7), cerebral RAAS expression and content, and RAAS-dependent impaired baroreflex and heart rate variability.[57–59] Intracerebroventricular Ang-(1–7) administration can attenuate the deleterious effects of antenatal betamethasone on blood pressure and autonomic dysfunction in sheep.[60] Antenatal betamethasone causes developmental stage-specific effects on renin expression in female sheep and increases blood pressure and renal sympathetic nerve activity, although administration of the AT_1R blocker losartan immediately after birth does not attenuate this effect, which supports additional RAAS-independent effects.[61]

A maternal low-protein diet during pregnancy leads to several RAAS programming effects. In mice, these include sexually dimorphic blood pressure control and increased RAAS expression in the lungs, pancreas, and brain.[62,63] In rats, maternal protein deprivation increases blood pressure in offspring through several RAAS-dependent mechanisms.[64] Manipulating the maternal gut microbiota can attenuate maternal high-fat diet–induced increased blood pressure and RAAS alterations in male offspring.[65]

Various other antenatal experimental manipulations can induce deleterious phenotypes mediated through the RAAS. For example, antenatal nicotine exposure sensitizes male rat offspring to the hypertensive effects of Ang II, whereas antioxidants attenuate this effect.[66] Maternal exogenous Ang II infusion during pregnancy sensitizes male rat offspring to kidney damage induced by a high-sodium diet.[67] Antenatal hypoxia in rats causes sex-specific programming of blood pressure responses to Ang II.[68] The reduced uterine perfusion pressure model of placental insufficiency and intrauterine growth restriction that mimics many of the maternal characteristics of preeclampsia also programs Ang II hypersensitivity and hypertension.[69] Uteroplacental insufficiency in the rat model of reduced uterine perfusion pressure at gestational day 14 is associated with Ang II–dependent hypertension, with increased renal ACE activity and renin and angiotensinogen mRNA expression but no changes in Ang II or AT^1R in adult offspring.[28] In summary, a wide array of antenatal manipulations are used in rodent and sheep models to investigate RAAS programming and later offspring cardiovascular health. Collectively, these studies highlight the diverse array of mechanisms that alter the RAAS. However, they also prompt many questions about the utility of measuring and manipulating the RAAS in the antenatal period to develop novel diagnostic and therapeutic tools.

Second Hits: Additive and Multiplicative Programming Effects

Programmed RAAS modifications in the antenatal period can also sensitize both the mother and the offspring to subsequent programming stimuli that directly cause hypertension

and other cardiovascular disease risk factors, often referred to as second hits.[51] Indeed, it is conceivable that many antenatal programming factors require additional adverse exposures over the life course to unmask hypertension programming.[53] These can include environmental exposures such as unmet social needs and adverse childhood experiences in humans and the development of other conditions such as obesity.[51,70–73] During pregnancy, maternal Ang II infusion in the rat sensitizes offspring to later high-fat diet–induced hypertension.[74] Conversely, a maternal high-fat diet during pregnancy sensitizes offspring rats to the pressor effects of Ang II.[75] Adult sheep offspring exposed to antenatal betamethasone demonstrate sex- and obesity-dependent modulation of insulin sensitivity and related RAAS alterations, including increased ACE and ACE2 expression and an imbalance between the Ang II/AT_1R and Ang-(1–7)/Mas receptor signaling pathways.[76] Similarly, antenatal betamethasone modifies renal responses to exogenous RAAS peptides in a sex-dependent manner.[77] When exposed to maternal dietary high-sodium load during gestation, rat offspring exhibit an array of cardiometabolic alterations that parallel the effect of exogenous Ang II.[78] After birth, antenatal dexamethasone exposure in maternal rats is associated with weight gain and glucose intolerance 12 months after weaning.[79] Thus, sensitization of both mother and offspring to subsequent stimuli represents a clinically significant yet underappreciated consequence of antenatal RAAS manipulation.

ADDITIONAL RAAS PROGRAMMING MECHANISMS

Our understanding of the mechanisms underlying RAAS programming in offspring has advanced significantly in recent years as a result of the development of innovative techniques and different approaches to identify genetic and epigenetic changes in target genes. For example, models of maternal nutrient deprivation or excess demonstrate major epigenetic alterations to several RAAS gene promoters. These alterations occur in various tissues that directly affect adaptive kidney and cardiovascular structure and function across the life course, with adverse effects that are likely perpetuated over multiple generations.

Epigenetic mechanisms, including DNA methylation, posttranslational histone modification, and noncoding RNA, are biological processes that modify gene expression without altering the DNA sequence. Activation of epigenetic mechanisms attributable to changes in maternal nutrition during pregnancy suggests that metabolic alterations trigger adaptive cardiovascular and renal processes mediated through the RAAS during placentation and fetal development. As an example, changes in methylation of the promoter for the AT_1R gene, *AGTR1*, are widely studied and concisely reproduced in various models. Maternal low-protein diet results in AT_{1b}R subtype promoter undermethylation and early adrenal overexpression in affected offspring,[80] which is further shown to be associated with maternal glucocorticoid levels in early pregnancy. DNA methylation is found at AT^{1b}R CpG sites in the fetal heart of offspring exposed to maternal high-salt diet.[81] DNA methylation and histone modification of the *AGTR1* promoter are observed in both the aorta and mesenteric arteries of offspring exposed to maternal high-sucrose diet during gestation.[82] These findings suggest that AT_1R is one of the primary RAAS components that undergo epigenetic modification as a potential programming pathway. However, an important limitation for translating these findings to humans is that, although rodents have 2 subtypes of genes transcribing AT_1R, humans only have 1 AT^1R gene, *AGTR1*.[83] Studies using selective

knockout have identified $AT^{1a}R$ as the closest homolog to human $AT^{1}R$.[84] Therefore, further studies are needed to confirm whether in humans *AGTR1* is a major RAAS gene that antenatal events target epigenetically and whether these changes are transmitted across generations in a clinically meaningful manner. Although this is encouraging and in line with several clinical and epidemiological studies, further investigations are warranted to better elucidate which mechanisms are truly inherited through epigenetic processes and which are actually attributable to shared environmental exposures between parents and offspring. Indeed, this line of investigation is especially interesting as a mechanism for how social determinants of health such as unmet social needs and adverse childhood experiences may contribute to RAAS programming and the long-term effects of health disparities in perinatal care.

There is emerging evidence that exosomes and microRNA may in part mediate RAAS dysregulation–induced signaling.[5,36] Last, alterations to the maternal microbiota and related metabolites during pregnancy are associated with RAAS dysregulation and subsequent programmed hypertension and hence have been studied as therapeutic targets during pregnancy.[85]

METHODOLOGICAL LIMITATIONS AND BEST RESEARCH PRACTICES

Manipulating the RAAS during specific phases of gestation is difficult for many biological and technical reasons. Complex pharmacokinetic changes occur during pregnancy, including expansion of specific fluid compartments and subsequent modifications in volume distribution, compound-specific placental transport, and potentially lethal outcomes for the offspring resulting from, for example, failed kidney development. Dietary manipulations can be difficult because the experimenter must demonstrate that the mother has an overall nutritional balance of key nutrients and experiences the intended consequence (eg, sodium depletion) within a carefully defined and targeted time line. Thus, in the study of the effects of antenatal manipulations on offspring biology, use of cross-fostering may be warranted. Genetic manipulations to specifically target the antenatal period can be complicated because of the potential expression of targeted genes in maternal tissues before pregnancy and during lactation versus in the offspring. Creative application of conditional manipulations (eg, Cre-Lox, tetracycline sensitive) can overcome these limitations, but careful validation of such models is required. Last, although commonly used to great effect, rodents exhibit importantly different placentation and fetal developmental biology compared with humans. Thus, results from rodent studies must be interpreted appropriately. Sheep provide an exceptional model given that their placentation biology is similar to that of humans, but sheep models involve substantially higher (and potentially prohibitive) costs and facility considerations that are much less commonly available. Ultimately, experimental RAAS manipulations during the antenatal period are possible but require consideration of many additional variables to achieve sufficient scientific rigor.

Accurate and reliable quantification of all relevant RAAS components requires well-validated methods to achieve appropriate rigor and reproducibility and to enable appropriate data comparisons across studies (Table 2). Limitations for angiotensin peptide measurement include naturally low concentrations (femtomole per milliliter), ongoing metabolism

(degradation and generation), interfering substances, and poor specificity and cross-reactivity attributable to sequence homogeneity. Samples for plasma analysis should be collected in EDTA tubes containing the appropriate protease inhibitor cocktail (especially for renin) that is validated for the intended assay, whereas urine samples often require acidification or inhibitors. Samples should immediately be frozen or undergo extraction for purification. Various drugs can interfere with RAAS measurement, including common sedatives and paralytics used in preclinical studies. To date, there are no validated, reliable, commercially available ELISAs to quantify angiotensin peptides.[86] Currently accepted best practices recommend assays such as mass spectrometry, high-performance liquid chromatography, and liquid chromatography–mass spectrometry; assays such as radioimmunoassays can have value if they have been validated with these aforementioned gold-standard techniques.[34] Quantification of RAAS enzymatic activity and content also requires rigorous collection and processing methods. One must differentiate between quantifying full-length and soluble enzyme forms. Serum is generally preferred over plasma for enzymatic analysis given the risk of assay interference. These methods and assays are thus costly and labor intensive and require measurement in established laboratories with extensive experience measuring RAAS components.

RAAS component tissue expression and quantification can be estimated with established techniques with appropriate rigor such as immunohistochemistry, Western blotting, and tissue enzymatic assays. The AT_1R is expressed broadly throughout the body, whereas the Ang II type 2 receptor may have relatively lower expression in various cardiovascular and renal tissue. Mas receptor expression generally mirrors that of the Ang II receptors. When investigating tissue and whole-cell expression, one must consider that many RAAS components are also expressed in mitochondria and nuclei.[87,88] Receptor measurement has been particularly challenging, in part because commercially available antibodies have poor specificity, leading to concerns about data obtained from immunolabeling techniques such as Western blot and immunohistochemistry.[89] It is highly recommended that investigators use proper negative and positive controls. Alternatively, reverse transcription–polymerase chain reaction can assess gene expression of human *AGTR1* or both murine $AT_{1a}R$ and $AT_{1b}R$.[84] However, it is important to note that, depending on the method used, the data may provide differential regulatory information, including differential expression of mRNA versus protein, transcriptional regulation, turnover, and activity. Therefore, combining several methods is usually preferred to provide more robust insights into receptor regulation and signaling.

Last, it is critical to perform a comprehensive analysis of the major RAAS pathway components simultaneously to infer pathophysiological changes appropriately because the analysis of isolated markers may result in misinterpretation of the functional status of the system. In addition, the circulatory RAAS and several epigenetic markers are not tissue specific even in preclinical studies; thus, changes in RAAS components in the blood cannot be ascribed to changes in any particular tissue. Although normative values of RAAS components do not exist in animals or humans, verified content across numerous sample sources and species is established.[90] This same RAAS methodological rigor applies to translational and clinical studies. Thus, because the interpretation of RAAS status can be even more complex in humans, it is extremely important that samples are collected,

processed, and analyzed with the use of best practices.[12,34] Clinical studies should obtain tissue samples whenever feasible and ethical to better determine tissue-specific RAAS alterations, especially from participants at baseline at disease diagnosis or before treatment initiation. This approach allows a better understanding of the incidence, rather than the prevalence, of RAAS alterations and can mitigate sources of confounding bias and time-dependent bias.[91]

CONCLUSIONS AND FUTURE DIRECTIONS

Despite persistently increased perinatal mortality risk and long-term cardiovascular risk that antenatal events confer to women and their offspring, standard practices for screening pregnant women remain underused.[92] In addition, Black women have a higher prevalence of preeclampsia and worse pregnancy outcomes, with disparities in access to adequate prenatal care being a critical factor.[93] This gap in health care has become even more relevant during the coronavirus disease 2019 (COVID-19) pandemic. However, the role of RAAS programming in these health disparities is not known. Little is known about how environmental exposures and social determinants of health may additionally program the RAAS to increase the risk of subsequent hypertension and cardiovascular disease. Investigation is urgently needed into how unmet social needs and adverse childhood experiences may contribute to RAAS programming of disease through epigenetic pathways and, in particular, how novel primordial prevention and therapeutic strategies can be developed to target these emerging mechanisms.[53]

Our understanding of the mechanisms responsible for antenatal programming of hypertension remains limited despite decades of high-quality research in this area. Although RAAS dysregulation is established as an important driver of antenatal programming, crucial gaps in the field remain, in part because methodological limitations and a lack of consensus have significantly hindered translation to clinical practice. RAAS programming provides an opportunity to establish novel diagnostic and prognostic biomarkers and to develop new approaches to improve short- and long-term maternal and child cardiovascular health, including mitigating health disparities pertaining to personalized medicine and nutritional interventions.

There is emerging interest in the role that the RAAS could theoretically play in SARS-CoV-2 infection and COVID-19–related adverse maternal and offspring outcomes, given that ACE2 is the binding site for SARS-CoV-2 and is expressed in the placenta, as well as the lungs, heart, vasculature, brain, and kidneys. Virus-induced ACE2 downregulation could lead to local ACE/Ang II pathway upregulation at the expense of ACE2/Ang-(1–7) downregulation, leading to more Ang II–mediated vasoconstriction and inflammation in the placenta and cardiovascular tissues generally.[12] In particular, proinflammatory and prothrombotic signaling may result in systemic endothelial and microvascular dysfunction, increasing the risk for embolization and immune system dysregulation. However, there remains a lack of robust clinical data to support preclinical evidence of these mechanisms. SARS-CoV-2–related RAAS dysregulation during pregnancy could theoretically contribute to adverse perinatal outcomes, including preeclampsia, preterm birth, and growth restriction, and program adverse cardiovascular changes that may increase the risk of developing

hypertension and cardiovascular disease, but more preclinical and clinical data are necessary to support these hypotheses.[94] Indeed, ongoing preclinical and clinical studies will provide important data supporting or refuting these interesting theories.

Based on these mechanisms, promising therapeutics include novel AT_1R-selective agonists (TRV027), exogenous Ang-(1–7) and its analogs (TXA127), and soluble ACE2 (APN01). Theoretically, soluble ACE2 or ACE2/Ang-(1–7)–derived compounds would be of interest in restoring the balance between the ACE/Ang II and ACE2/Ang-(1–7) pathways in the placenta, given that Ang II is necessary for placental function and fetal development and that traditional RAAS-blocking medications are contraindicated in pregnancy and the first month of life in infants. Future translational studies and clinical trials should consider the unique opportunity that the maternal-fetal dyad offers in preventing or mitigating antenatal programming of cardiovascular disease.

Preclinical studies have provided crucial insight into adverse perinatal and long-term cardiovascular outcomes in women and their offspring, but much more work is needed to translate those findings to clinical practice more efficiently. RAAS programming has enormous potential to inform the development of diagnostic and prognostic biomarkers and novel therapeutics to improve perinatal outcomes in the short- and long-term cardiovascular health in women and their offspring. Consensus on RAAS methods is imperative to foster more rapid translation into clinical research and practice.

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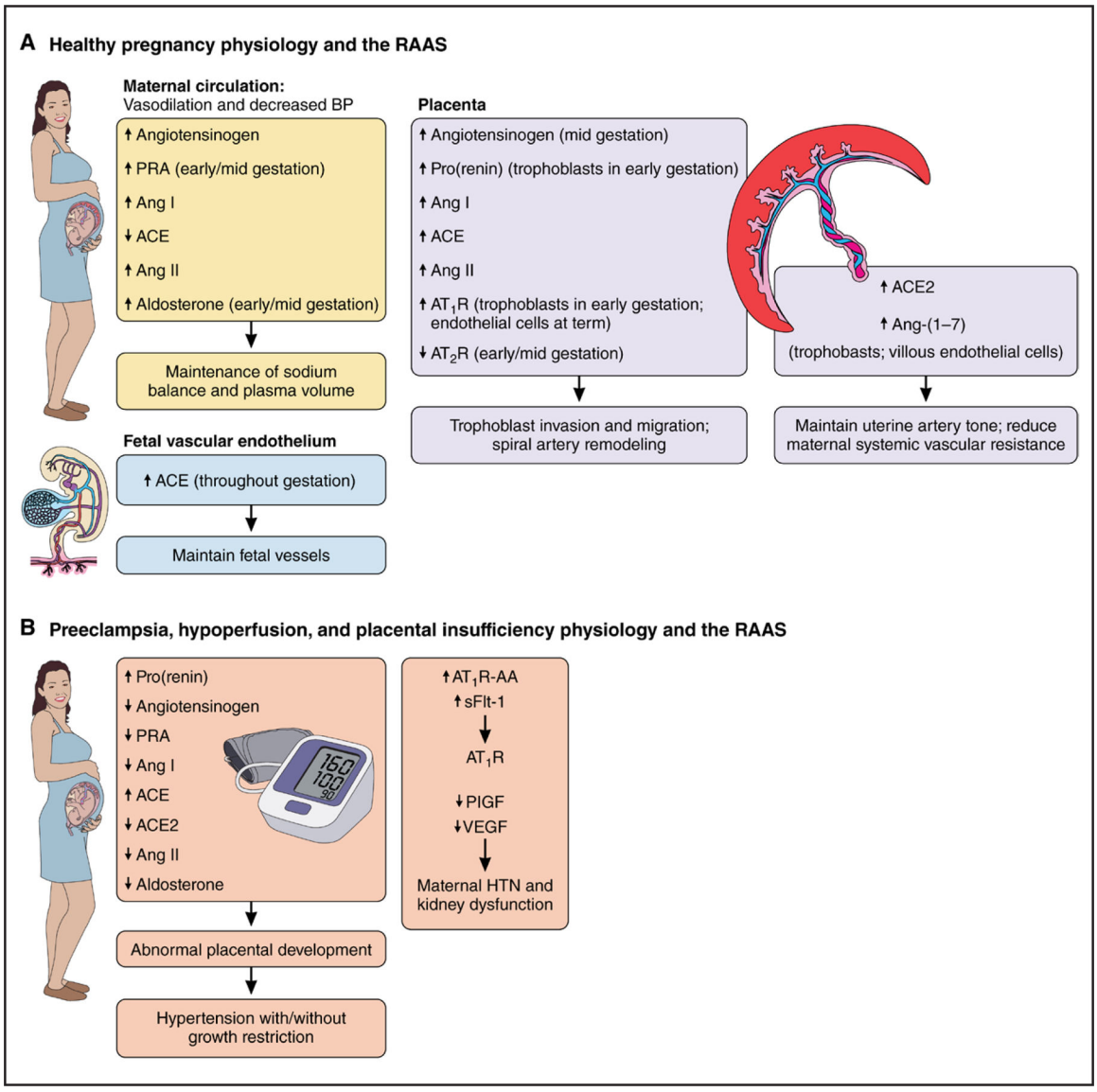

Figure 1. Physiological roles of the RAAS in healthy and pathological pregnancies.

A, Expression and actions of the major RAAS components during healthy pregnancy. **B**, Expression and actions of the major RAAS components during pathological pregnancies, including preeclampsia and placental insufficiency. Ang indicates angiotensin; ACE, angiotensin-converting enzyme; AT_1R, angiotensin type 1 receptor; AT_2R, angiotensin type 2 receptor; AT_1R-AA, angiotensin II type 1 receptor autoantibody; BP, blood pressure; HTN, hypertension; PlGF, placental growth factor; PRA, plasma renin activity; RAAS, renin-angiotensin-aldosterone system; sFlt-1, soluble fms-like tyrosine kinase 1; and VEGF, vascular endothelial growth factor.

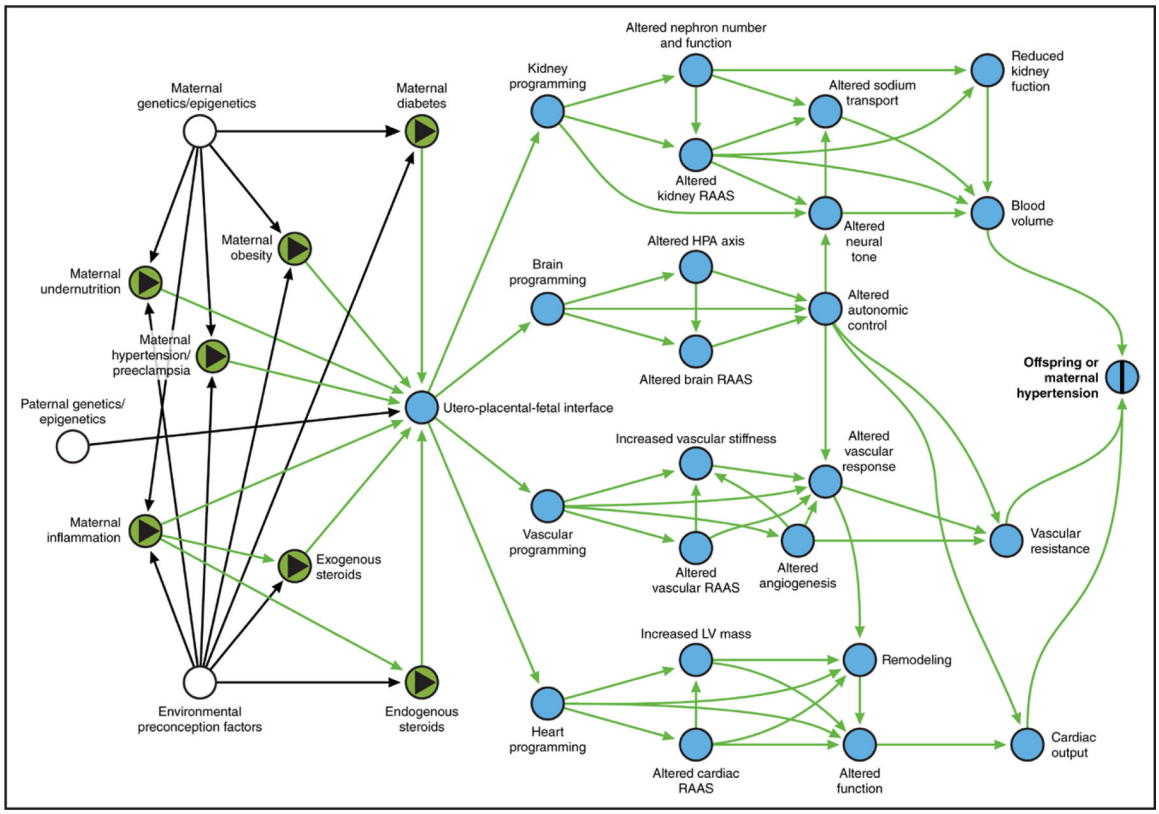

Figure 2. Conceptual causal model of representative antenatal exposures and their effects on various tissue programming mechanisms.

Directed acyclic graph with exposures noted as green ovals with black triangles and outcome as blue oval with black vertical bar. Intermediate mechanisms as mediators on the causal path are noted as blue ovals; causal paths are noted as green arrows. White ovals are unmeasured factors; black arrows are noncausal and nonbiasing paths. HPA indicates hypothalamic-pituitary axis; LV, left ventricular; and RAAS, renin-angiotensin-aldosterone system. Figure created with www.dagitty.net.

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This table represents the relationships of writing group members that may be perceived as actual or reasonably perceived conflicts of interest as reported on the Disclosure Questionnaire, which all members of the writing group are required to complete and submit. A relationship is considered to be "significant" if (a) the person receives $5000 or more during any 12-month period, or 5% or more of the person's gross income; or (b) the person owns 5% or more of the voting stock or share of the entity, or owns $5000 or more of the fair market value of the entity. A relationship is considered to be "modest" if it is less than "significant" under the preceding definition.

† Significant.

Reviewer Disclosures

Reviewer	Employment	Research grant	Other research support	Speakers' bureau/ honoraria	Expert witness	Ownership interest	Consultant/ advisory board	Other
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This table represents the relationships of reviewers that may be perceived as actual or reasonably perceived conflicts of interest as reported on the Disclosure Questionnaire, which all reviewers are required to complete and submit. A relationship is considered to be "significant" if (a) the person receives $5000 or more during any 12-month period, or 5% or more of the person's gross income; or (b) the person owns 5% or more of the voting stock or share of the entity, or owns $5000 or more of the fair market value of the entity. A relationship is considered to be "modest" if it is less than "significant" under the preceding definition.

†Significant.

Table 1.

Overview of Preclinical Models of Programmed Hypertension Mediated Through Antenatal RAAS Dysregulation by Species

Experimental model	Species	Antenatal programming exposure
Placental dysfunction	Rat	Reduced uterine perfusion pressure (35%–45%)
		Uterine artery/vessel ligation
		Maternal or fetal hypoxia (10.5%–12% oxygen or 25% reduction in fetal PaO_2)
	Sheep	Umbilico-placental embolization
		Removal of uterine/endometrial caruncles
		Maternal hypoxia (25% reduction in fetal PaO_2)
		Natural twinning
Nutrient manipulation	Rat	Protein restriction (6%–18%)
		Global undernutrition (40%–70%)
		High-salt (8%–30%) or low-salt (0.03%) diet
		High-fat (20%) diet
	Mouse	Protein restriction (6%–12%)
		Global undernutrition (70%)
	Sheep	Global undernutrition (85%)
Pharmacological intervention	Rat	Diabetes induced by streptozotocin or glucose infusion
		L-NAME–induced preeclampsia
		Antenatal glucocorticoids: increased maternal endogenous glucocorticoids or clinical administration
	Mouse	Antenatal glucocorticoids: increased maternal endogenous glucocorticoids
	Sheep	Antenatal glucocorticoids: increased maternal endogenous glucocorticoids or clinical administration
		Exogenous fetal or maternal cortisol infusion

L-NAME indicates L-N^G-nitro arginine methyl ester; and RAAS, renin-angiotensin-aldosterone system.

Table 2.

Summary of Methodological Limitations in RAAS Quantification: Considerations for Rigor and Reproducibility

Renin-angiotensin-aldosterone component	Weakness or limitation	Best practice consideration
Peptides	Improper collection practices Improperly validated ELISAs Naturally low concentrations (femtomole/milliliter) Ongoing metabolism Interfering substances Poor specificity and cross-reactivity Interference from sedatives and paralytics	Design experiments with appropriate quantification methods a priori Collect plasma in EDTA with appropriate protease inhibitor cocktail validated for intended assay Extract for purification Use mass spectrometry, high-performance liquid chromatography, or well-validated RIAs
Enzymes	Improper collection and processing Interfering substances	Use serum over plasma Use validated assays
Receptors	Poor specificity of antibodies for use in Western blots and immunohistochemistry	Use radiolabeled peptide binding Use proper positive and negative controls
mRNA or protein expression	Differential expression of mRNA vs protein, transcriptional regulation, turnover, and activity	Combine several methods
Interpretation	Difficult-to-interpret values without normative ranges in isolation	Ensure that values are biologically plausible Assess multiple components simultaneously Interpret in the context of both major pathways Consider tissue expression when available

RAAS indicates renin-angiotensin-aldosterone system; and RIA, radioimmunoassay.