

REVIEW

Pre-eclampsia and offspring cardiovascular health: mechanistic insights from experimental studies

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ABSTRACT

Pre-eclampsia is increasingly recognized as more than an isolated disease of pregnancy. Women who have had a pregnancy complicated by pre-eclampsia have a 4-fold increased risk of later cardiovascular disease. Intriguingly, the offspring of affected pregnancies also have an increased risk of higher blood pressure and almost double the risk of stroke in later life. Experimental approaches to identify the key features of pre-eclampsia responsible for this programming of offspring cardiovascular health, or the key biological pathways modified in the offspring, have the potential to highlight novel targets for early primary prevention strategies. As pre-eclampsia occurs in 2–5% of all pregnancies, the findings are relevant to the current healthcare of up to 3 million people in the U.K. and 15 million people in the U.S.A. In the present paper, we review the current literature that concerns potential mechanisms for adverse cardiovascular programming in offspring exposed to pre-eclampsia, considering two major areas of investigation: first, experimental models that mimic features of the *in utero* environment characteristic of pre-eclampsia, and secondly, how, in humans, offspring cardiovascular phenotype is altered after exposure to pre-eclampsia. We compare and contrast the findings from these two bodies of work to develop insights into the likely key pathways of relevance. The present review and analysis highlights the pivotal role of long-term changes in vascular function and identifies areas of growing interest, specifically, response to hypoxia, immune modification, epigenetics and the anti-angiogenic *in utero* milieu.

CLINICAL IMPORTANCE

Traditionally pre-eclampsia was viewed as a self-limiting maternal condition that resolved completely with delivery of the placenta [1,2]. However, it is

now recognized that a woman who has a pregnancy complicated by pre-eclampsia [3,4] has a 4-fold increased risk of hypertension, and a 2-fold increased risk of ischaemic heart disease and stroke [3] in the 10–15 years after the pregnancy. Intriguingly, the offspring of that

Key words: blood pressure, cardiovascular disease, *in utero*, offspring, pre-eclampsia, pregnancy, vascular function.

Abbreviations: BP, blood pressure; BMI, body mass index; CYP, cytochrome P450; eNOS, endothelial NO synthase; HUVEC, human umbilical vein endothelial cell; IMT, intima-media thickness; I/R, ischaemia/reperfusion; IUGR, intra-uterine growth restriction; L-NAME, *N*^G-nitro-L-arginine methyl ester; LV, left ventricular; LVH, LV hypertrophy; MMP, matrix metalloproteinase; PlGF, placental growth factor; PKC ϵ , protein kinase C ϵ ; ROS, reactive oxygen species; RUPP, reduced uterine artery perfusion; SBP, systolic BP; sEng, soluble endoglin; sFlt-1, soluble fms-like tyrosine kinase-1; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinases; VEGF, vascular endothelial growth factor.

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pregnancy will also have increased BP (blood pressure) during childhood and nearly double the risk of stroke in later life [5–16]. As pre-eclampsia affects 2–5% of pregnancies, 1.2–3 million people in the U.K. and 6–15 million in the U.S.A. [1,2] are offspring of pregnancies complicated by pre-eclampsia. Experimental approaches to identify the key biological variation that infer the increased risks in this large cohort therefore have the potential to pivotally alter our understanding of the early development of cardiovascular disease and hypertension in the population. The fact that exposed children and adults can be easily identified based on precise clinical criteria, namely a diagnosis of pre-eclampsia in their mother, also raises the possibility for novel, very early, targeted, primary prevention strategies for cardiovascular risk reduction in a high risk group within the community [17].

EPIDEMIOLOGICAL CLUES TO THE MECHANISM

The epidemiological data used to uncover the relationships between maternal pre-eclampsia and offspring cardiovascular health provide clues to the broad nature of the biological variation that underlies the risk [15]. Meta-analysis of available BP data indicates that children exposed to pre-eclampsia *in utero* have approximately 2–3 mmHg higher SBP (systolic BP) during childhood and young adult life [18]. This difference, if maintained into adulthood, would be associated with a 12% increased risk from stroke [19,20]. In reality, long-term follow-up studies demonstrate a doubling of risk [6]. The key biological pathways relevant to offspring cardiovascular health are therefore likely to have additional effects, on other features of stroke risk, beyond clinic BP measures alone.

Pre-eclampsia is a condition with a heritability of approximately 55% [21], which is contributed to by both maternal and fetal genes. It is plausible that some of the alterations in risk seen in the offspring of pre-eclamptic pregnancies relate to shared genetic factors between the mother and child. The modification to these pathways also appears to be induced specifically by *in utero* exposure to pre-eclampsia. Siblings of offspring born to pregnancies complicated by pre-eclampsia, who themselves were born to uncomplicated pregnancies, do not demonstrate the vascular abnormalities seen in the offspring [11]. This observation has clear parallels with the ‘developmental programming’ hypothesis [22], which holds that an insult or deprivation during a critical period of growth and development can lead to long-term alterations in the risk of chronic disease [23]. This hypothesis was developed in response to epidemiological data that associated a low birthweight, a pregnancy-

related outcome with multi-factorial determinants, to a variety of cardiovascular and non-cardiovascular outcomes, including mortality from coronary artery disease [24,25], BP [24], the metabolic syndrome [26] and non-insulin-dependent diabetes (Type 2 diabetes) [27].

Offspring of pre-eclamptic pregnancies, particularly those with early-onset pre-eclampsia, can also have a low birthweight due to IUGR (intra-uterine growth restriction) or pre-term birth [23]. Prematurity and IUGR have themselves been associated with alterations in cardiovascular risk factors [28,29]. Furthermore, early- and late-onset pre-eclampsia are increasingly being recognized as different subclasses of disease, which may have different implications for cardiovascular risk in the offspring [30]. This complicated combination of *in utero* insults means that in many currently available human studies it is challenging to separate the effects of exposure to pre-eclampsia from other *in utero* insults. However, if a baby is born at term without IUGR, but their mother had pre-eclampsia, they still have an increased risk of stroke [6] and vascular abnormalities [31] that persist after adjustment for birthweight. It therefore seems plausible that there is a unique insult within the *in utero* environment during pre-eclampsia which is incremental to, or acts in synergy with, any risks inferred by IUGR. This hypothesis is supported further by the observation that pre-term birth largely protects offspring from the adverse associations between birthweight and later cardiovascular disease [24,32]. Pre-eclampsia is one of the leading causes of pre-term birth, and the largest differences in later BP are seen in those offspring of pre-eclamptic pregnancies born pre-term [12]; findings that focus attention on key insults that may operate early in pregnancy.

On the basis of these observations, we have now gone on to review the current experimental literature to gain insight into potential mechanisms for *in utero* cardiovascular programming in relation to pre-eclampsia. We have focused on experiments that use models designed to replicate features of pre-eclampsia, in particular the early detrimental exposures that characterize the fetal experience in this condition. We have compared the findings with those from human studies of offspring, or tissues, from pre-eclamptic pregnancies. The cumulative body of work supports associations between pre-eclampsia exposure and offspring cardiovascular health, as well as a potential pivotal role for long-term programmed changes in vascular biology (Table 1 and Figure 1).

THE *IN UTERO* INSULT

Disturbed placentation in early pregnancy is considered the key pathological event that leads to subsequent

Table 1 Evidence of cardiovascular programming following pre-eclampsia

An overview of some of the evidence for cardiovascular programming in offspring of animal models and humans born to pre-eclamptic mothers. Positive findings are underlined in roman and negative findings are indicated in italic.

Parameter	Animal model offspring				Human offspring						
					Perinatal			Later life			
	RUPP	Hypoxia	sFLT1	eNOS	Placenta	Cord blood	HUVECs	Neonate	Childhood	Adolescence	Young adult
BP											
↑BP	<u>[63–65]</u> [54–58,60–62,159,160,162,180]	<u>[72,100]</u>	<u>[78,181]</u>	<u>[82,182]</u>	–	–	–	<u>[183]</u>	<u>[114,184]</u> [10,15,177,178,185]	<u>[11]</u> [7,8,13,14]	<u>[12]</u>
Altered BP under stress	–	–	<u>[72]</u>	<u>[82]</u>	–	–	–	–	–	–	–
Vascular structural changes											
↑IMT/vascular wall thickness	<u>[63,64,86]</u>	<u>[87–89]</u>	–	–	–	–	–	<u>[92]</u>	–	–	<u>[12]</u>
↑Arterial stiffness	<u>[55,63]</u>	<u>[69]</u>	–	<u>[186]</u> <u>[113]</u>	–	–	–	–	<u>[15]</u>	–	<u>[12]</u>
Vascular functional changes											
Endothelial dysfunction	<u>[63,141]</u> <u>[54,110]</u>	<u>[69–71]</u>	–	<u>[112,113,182,186]</u>	–	<u>[116]</u>	<u>[187]</u>	<u>[31]</u>	<u>[15]</u>	<u>[11]</u>	<u>[12]</u>
Non-endothelial function	<u>[110,141]</u>	<u>[71]</u>	<u>[188]</u>	<u>[113,182]</u>	–	–	–	–	–	<u>[11]</u>	<u>[12]</u>
Cardiac structural and functional changes											
Altered cardiac structure	<u>[65]</u> <u>[57,86]</u>	<u>[72,95–98]</u>	<u>[181]</u>	–	–	–	–	–	<u>[105]</u>	–	–
LV dysfunction	–	<u>[95]</u>	–	–	–	–	–	<u>[189]</u>	<u>[105]</u>	–	–
↑IR injury	–	<u>[94,99–101,190]</u>	–	–	–	–	–	–	–	–	–
Renal structural and functional changes											
Altered structure and function	<u>[59,62,65]</u> <u>[57,58,61,64,65,86,159–161]</u>	–	<u>[191]</u>	–	–	–	–	–	–	–	–

Table 1 Continued

Parameter	Animal model offspring				Human offspring						
					Perinatal				Later life		
	RUPP	Hypoxia	sFLT1	eNOS	Placenta	Cord blood	HUVECs	Neonate	Childhood	Adolescence	Young adult
Metabolic changes											
↑BMI	[54,56–59,62,65,86,110,180,192,193] [194,195]	[71,72,88,94,95,100,131,196]	[77,181,188]	[82,113]	–	–	–	[92]	[178,184] [7,8,10,15]	[11,14] [16]	[12] [13]
↑Serum lipids	[56,63,195]	[196] [131]	[181]	–	–	[92,126–130]	–	–	[15] [114]	[14]	[12]
Altered glucose metabolism	[63] [56,194,195,197]	[196]	[181]	–	–	–	–	–	–	[14]	[12]
Altered sympatho-adrenal function											
Adrenal hormones	[62,161,195]	[72,155,157]	–	–	–	[198,199]	–	–	–	[154] [14]	–
Sympathetic changes	[200]	[72,158]	–	–	–	–	–	–	–	–	–
Genetic/epigenetic changes											
Gene expression	[57,61,65,160,161] [62,63,159]	[96,97,100,131,190,196]	–	[191]	[67,103,164,201–206]	–	–	–	–	–	–
Epigenetics	–	[101]	–	–	[135–137]	–	–	–	–	–	–
Other											
↓Antioxidants	–	–	–	–	[207]	[127,147]	–	–	–	–	–
↑Oxidative stress	[56]	–	–	–	[148]	[127,147,148]	–	–	–	–	–
↑Anti-angiogenic factors	–	–	–	–	–	[120,121]	–	–	[114]	–	–
↑PIGF/VEGF	–	–	–	–	–	[120]	–	–	[114]	–	–
Altered inflammation	[63]	[131]	[181]	–	–	–	[173–176]	[208]	[15,114]	–	–

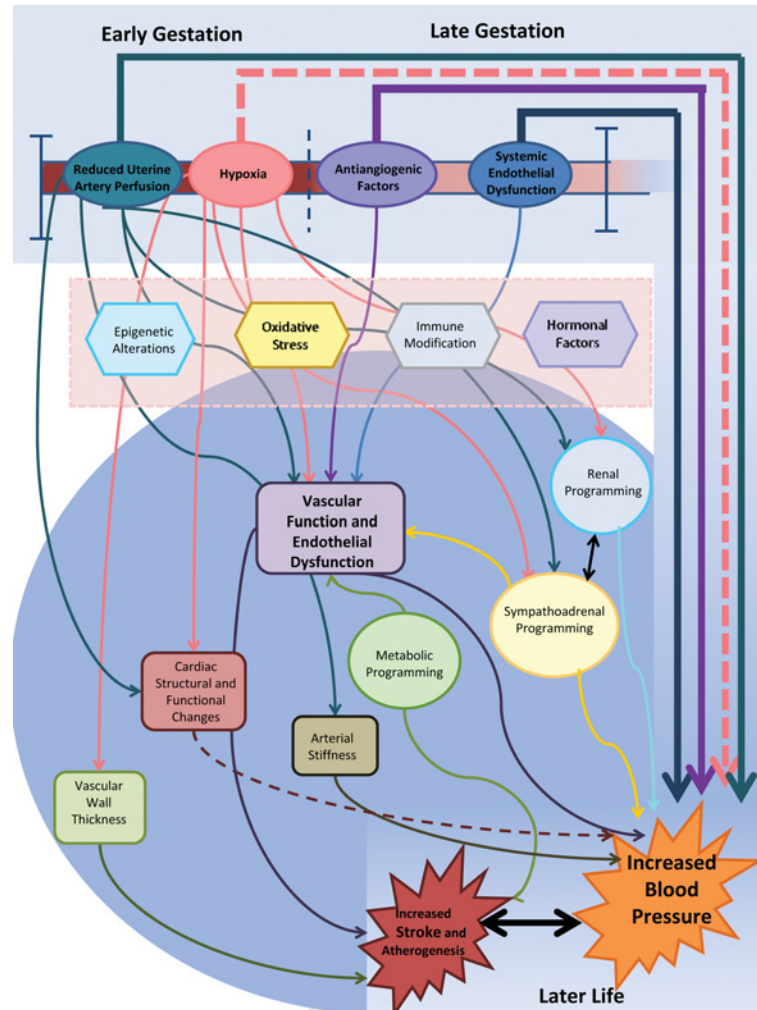


Figure 1 Potential mechanism of cardiovascular programming in the offspring following a pre-eclamptic pregnancy

Summary of the potential and demonstrated mechanisms by which *in utero* exposure to pre-eclampsia may lead to altered cardiovascular physiology and risk in the offspring in later life.

development of pre-eclampsia [2,33]. Normal placental development is characterized by invasion of maternal spiral arteries by fetal cytotrophoblasts and their transformation into high-calibre capacitance vessels, which provides low-resistance placental perfusion to sustain fetal growth [34]. In pre-eclampsia, the spiral arteries inadequately remodel so that uterine flow is reduced by 50% and there is chronic placental ischaemia or, at best, intermittent flow that induces an ischaemia/reperfusion phenomenon [35]. ROS (reactive oxygen species) and cytokines released from the ischaemic placenta trigger a systemic oxidative [36] and inflammatory [35,37] state. The placenta also overexpresses anti-angiogenic factors that inhibit the normal function of pregnancy-related pro-angiogenic factors, including VEGF (vascular endothelial growth factor) and PlGF (placental growth factor) [38]. The combination of these factors is believed to underlie the systemic endothelial dysfunction that is consist-

ently found during symptomatic pre-eclampsia in the mother.

Offspring of pre-eclamptic pregnancies therefore develop from the first trimester onwards within an environment of placental insufficiency and restricted oxygen supply. By the start of the second trimester there is, in addition, exposure to elevated circulating anti-angiogenic factors, which predates the later emergence of the clinical pre-eclamptic syndrome and the diffuse maternal inflammatory, oxidative and dysfunctional endothelial state. Experimental studies have an important role to help us understand how, and whether, these specific insults have an impact on the developing cardiovascular system of the offspring. Their findings have the potential to unravel the developmental programming that underlies the observed long-term cardiovascular disease risk in the offspring.

However, the ideal animal model of pre-eclampsia does not exist, in part because the spontaneous development

of pre-eclampsia during pregnancy is a condition largely unique to humans [39,40]. To mimic the human syndrome a model would need to be characterized by the development of pregnancy-specific hypertension, proteinuria and associated alterations in vascular function and biomarkers. To replicate the condition perfectly, these changes must be secondary to impaired trophoblastic invasion [40]. Although a variety of models based on different aspects of the pathophysiology of pre-eclampsia exist [39,40], no model has been able to successfully mimic all of the pathophysiological features of pre-eclampsia or to accurately replicate the first trimester origin of the human condition. For example, the currently available animal models are limited in their capacity to produce the metabolic alterations which are evident in human pre-eclamptic pregnancies [41,42]. Another limitation is that the models have been primarily developed to investigate pre-eclampsia in the mother not the offspring. However, fortuitously, the adequacy of animal models developed to study pre-eclampsia has, in part, been defined by their ability to induce IUGR. As a result, these 'pre-eclampsia' models have been adopted to study how IUGR has an impact on the offspring [43]. The findings from these offspring studies, in particular those that maintain experimental set ups that would be expected to induce pre-eclampsia-like syndromes in the dams, thereby provide insights into the role of pre-eclampsia-related insults. We have used data available from animal models to dissect out whether there is evidence that key features of the pre-eclamptic *in utero* environment induce long-term changes characteristic of the cardiovascular risk phenotype seen in offspring of pre-eclamptic pregnancies, namely elevated resting BP.

Uterine perfusion

Mechanical reduction in maternal uterine artery blood flow [RUPP (reduced uterine artery perfusion)], by unilateral or bilateral uterine artery ligation, in animal models induces a pregnancy-specific increase in maternal BP [44]. The model is well established and has been applied in a range of species from initial work in dogs to rats [40], rabbits [45], sheep [46], guinea-pigs [47] and non-human primates [48]. In dogs, other cardinal features of pre-eclampsia are evident such as renal glomerular endotheliosis and, in some instances, proteinuria [49]. In rats, RUPP pressure between days 14 and 19 of pregnancy induces a full pre-eclampsia pregnancy phenotype, including markedly elevated BP and changes in renal artery flow and glomerular filtration rate [50]. Furthermore, other distinct biological changes occur in the mother, specifically dysfunction of endothelium-dependent vascular relaxation [51,52] and increased circulating levels of sFlt-1 (soluble fms-like tyrosine kinase-1) with a resultant reduction in VEGF and PlGF [53].

Variations of the RUPP model have been used to study the influence of reduced uterine perfusion on offspring BP and, interestingly, BP does not differ from controls in a large number of the studies [54–62]. Variation in the species investigated or techniques for BP measurement have been postulated to account for this disparity from the observations in humans. However, when all of the studies are considered together (Figure 2), the time when uterine perfusion is reduced appears to be critical. If at 14 days into gestation, changes in resting BP are consistently evident in the offspring throughout life. When uterine perfusion is only introduced late in gestation, despite inducing growth restriction, the studies show either no increase in BP or modest changes in later life that may require stimuli to elicit variability [63–65]. These findings argue for early exposure to uterine perfusion abnormalities to be critical to the BP programming in the offspring. However, early ligation has been associated with the full range of pre-eclampsia-like features in the dams, including deranged anti-angiogenic factors and vascular dysfunction. Therefore an evaluation of the specific relevance of the individual insults is not possible from this model.

Hypoxia

Placental insufficiency is likely to lead to offspring of pre-eclamptic pregnancies developing in an environment of significant hypoxia from early in pregnancy. In all pregnancies, early placental development takes place under low oxygen conditions as this predates the formation of an effective maternal–fetal circulation [66,67]. Even in later stages of normal pregnancy, fetuses whose mothers have normal placental perfusion and breathe 21% inspired O₂ are still exposed to intervillous oxygen levels equivalent to only 8–11% O₂. The abnormal placentation in pre-eclampsia is therefore likely to result in significantly lower oxygen delivery to the fetus for the majority of gestation. Interestingly, even in pregnant mice with normal placental development, exposure to an inappropriate level of hypoxia from early in pregnancy (9.5% O₂ from day 7.5 to day 17 of gestation) induces a range of pre-eclampsia-like symptoms in the mother, including hypertension, proteinuria, IUGR, renal pathology and elevations in maternal sEng (soluble endoglin) [68]. The underlying reason for this is not clear, although it could be because the degree of hypoxia induced at the level of the placenta is sufficient to trigger the 'pre-eclampsia cascade' of events, similar to that present in a mother with abnormal placental development. Consistent with this hypothesis, less severe hypoxia (11% O₂) or hypoxia later in gestation (9.5% O₂ from day 10 or 15 to day 17 of gestation) does not induce these changes [68].

When the data from models of pregnancy hypoxia are collated (Figure 3), despite a range of changes in other cardiovascular measures, there is a striking lack of

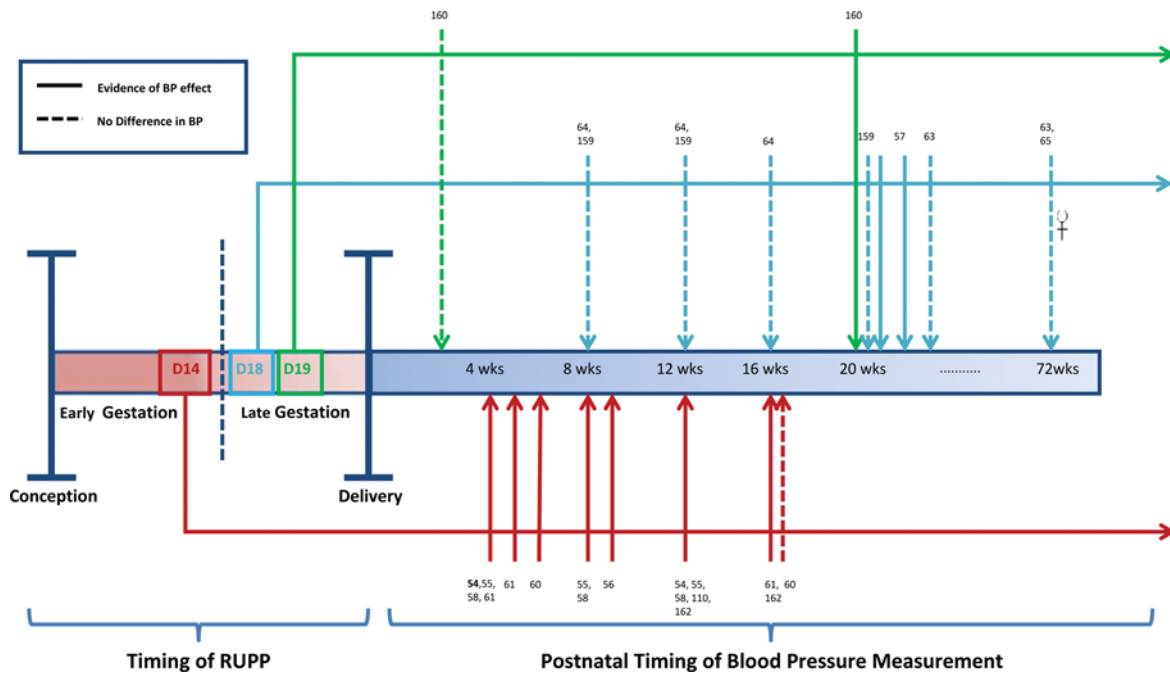


Figure 2 Timing of the reduction in uterine artery perfusion and offspring BP

Summary of the results of studies (reference number given) considering the effect of RUPP at different points of gestation on offspring BP. Continuous lines indicate that studies have demonstrated an increase in BP in RUPP animals compared with controls at this time point, whereas broken lines indicate no difference in BP compared with controls. The colour of the lines indicates the timing of the *in utero* insult; the red line showing the result of studies in which uterine artery perfusion was reduced at day (D) 14, the blue line at day 18 and the green line at day 19 of gestation. This suggests that the timing of the insult may be critical in determining the effect on offspring BP. Studies of mid-gestation (14 days of gestation) reduction in uterine artery flow have demonstrated relatively consistent increases in BP from early life (4 weeks of age). Interventions later in gestation (days 18 and 19 of gestation) have not indicated consistent increases in early life, although some studies have indicated later development of hypertension which may be linked to dietary factors.

any consistent reports of elevated BP in the offspring. This may be because the majority of studies are based on the use of oxygen concentrations higher than 9.5% and/or exposed animals later in pregnancy [69–71]. Even in models in which oxygen availability is reduced early in development (days 5–20 of pregnancy), the level of maternal hypoxia (10% O₂) has been relatively higher than that normally associated with induction of a pre-eclampsia-like syndrome in the mother. BP variation in the offspring has been demonstrated in response to stress [72], but this BP phenotype is distinct from that observed in human offspring of pre-eclamptic pregnancies, who have increased resting BP in early life [18]. These findings may suggest that hypoxia alone is insufficient to induce the long-term effects of pre-eclampsia seen in the offspring, or that the levels of hypoxia currently used are not representative of the levels offspring are exposed to during development.

Anti-angiogenic factors

Multiple studies have now demonstrated a key step in the progression of pre-eclampsia from placental underperfusion and hypoxia to a clinical syndrome is an increase in levels of circulating anti-angiogenic factors.

These include sFlt-1, the alternatively spliced truncated form of VEGF receptor 1 [73], and sEng [74–76]. sFlt-1 inhibits the normal function of pregnancy-related angiogenic factors, including VEGF and PlGF [38], and a Sprague–Dawley model of pre-eclampsia based on adenovirus vector administration of sFlt-1 early in the second trimester (days 8–9 of pregnancy), when the levels are seen to increase in human pregnancy, has been developed [74]. This model overexpresses sFlt-1 and the transfected rats develop hypertension, heavy proteinuria and glomerular endotheliosis. These signs also occur in non-pregnant rats treated with sFlt-1 [74], indicative of the broader relevance of deranged anti-angiogenic factors to vascular homeostasis. The same intervention in mice induces a slightly different phenotype with maternal hypertension, fetal growth restriction and impairment of maternal platelet count [77]. Lu et al. [78] have investigated the effect of administration of an adenovirus carrying sFlt-1 to pregnant mice on offspring BP over the first 6 months of life. They found a sustained increase in BP in male offspring evident from the first day of life, which persisted during the period of measurement. As with other models of pre-eclampsia, a range of clinical features of the condition are induced [79] and

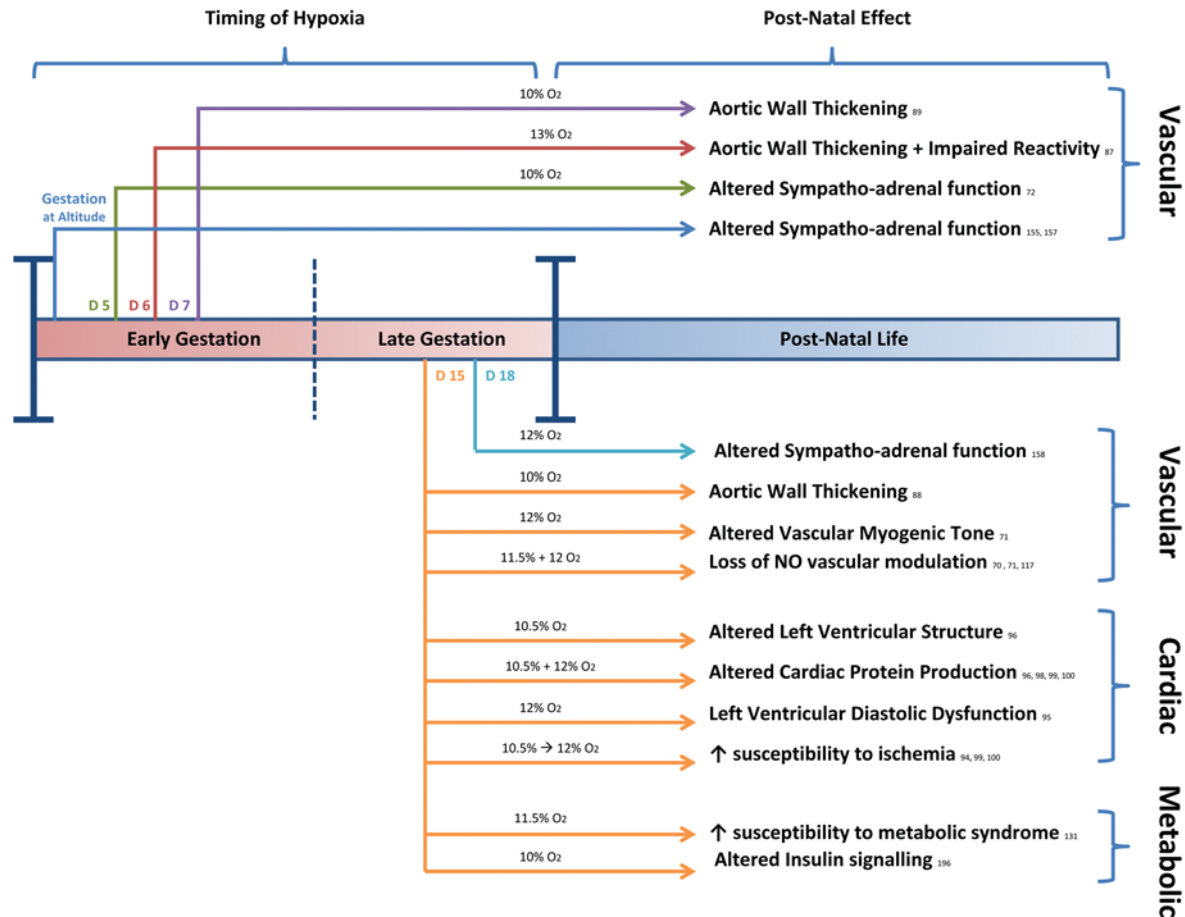


Figure 3 Cardiovascular changes associated with *in utero* exposure to hypoxia

The major findings from studies (reference number given) considering the effects of pre-natal chronic hypoxia at different points of gestation on offspring cardiovascular physiology are outlined. The colour of the lines indicates the timing of commencement of *in utero* hypoxia; The dark blue line indicates hypoxia for the entire period of gestation, the green line from day 5, the red line from day 6, the purple line from day 7, the orange line from day 15, and the pale blue line from day 18 of gestation. The percentage of oxygen used in each study is indicated above the lines. Studies have been divided into those where hypoxia was commenced in early pregnancy (before day 10 in rat models or from conception in other animals) and those where hypoxia was commenced later in pregnancy. Studies later in pregnancy have demonstrated vascular, cardiac and metabolic alterations in the offspring. Fewer studies have considered the effects of early-onset hypoxia, but these have demonstrated similar findings in terms of the vascular phenotype.

specific culpability for sFlt-1 in programming of offspring cardiovascular cannot be confirmed. Nevertheless, the results suggest that, even with normal placentation and uterine blood flow in early pregnancy, exposure to an excess of anti-angiogenic factors, specifically raised sFlt-1 and its biological sequelae, is sufficient to induce long-term changes in BP in the offspring.

Systemic endothelial dysfunction

The specific role of systemic maternal endothelial dysfunction is relatively simpler to explore. This is because targeted animal models are available that allow *in utero* development in a mother with endothelial dysfunction to be studied. These models are based either on systemic inhibition of eNOS (endothelial NO synthase) by administration of L-NAME (*N*^G-nitro-L-arginine methyl ester) or by eNOS knockout [39]. A

limitation is that these are not good models of pre-eclampsia itself as the hypertension in eNOS-knockout mice [80] is not pregnancy-specific and, indeed, one study has reported that eNOS-knockout mothers have no difference in BP during pregnancy [81]. Nevertheless, the mice consistently exhibit abnormal endothelial function and, through selective breeding of an eNOS-knockout mother with a wild-type male, an experimental scenario can be generated to study the impact of maternal endothelial dysfunction on the *in utero* development of an eNOS-heterozygous offspring (Figure 4). These offspring have higher BP during adulthood than the genetically similar offspring bred from a wild-type mother and an eNOS-knockout father, who differ only in that their *in utero* development was in a wild-type mother with normal endothelial responses [82]. Such findings highlight the potential pivotal importance of maternal

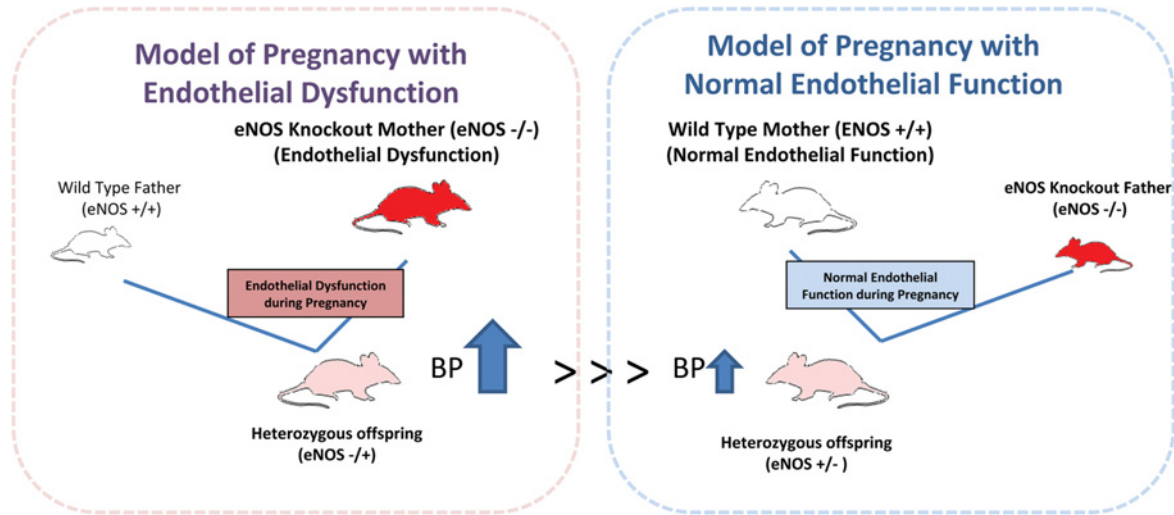


Figure 4 Effect of maternal systemic endothelial dysfunction during pregnancy on offspring BP

Experiments in eNOS-knockout mice have demonstrated the importance of the development in an environment of maternal systemic endothelial dysfunction. eNOS-knockout heterozygotes born to knockout mothers have shown some evidence of increased BP and BP variability compared with heterozygotes gestated in an environment with normal endothelial function. Second-generation offspring also show alterations in BP. Maternal systemic endothelial dysfunction during pregnancy is characteristic of the human syndrome of pre-eclampsia and these models highlight the potential importance of this *in utero* insult on later offspring vascular function [82].

endothelial dysfunction during pregnancy, even in the absence of other pre-eclamptic features, on long-term offspring outcome.

MECHANISM OF LONG-TERM PROGRAMING? THE CARDIOVASCULAR SYSTEM

The data from available animal models implicate reduced uterine perfusion, if present from early in pregnancy, as well as exposure to anti-angiogenic factors and systemic endothelial dysfunction, from at least the second trimester, as capable of inducing long-term changes in BP in offspring. The evidence for a specific effect of hypoxia is weaker in current animal models, although some changes in BP response if hypoxia occurs from early in pregnancy is observed. To understand how these insults during pregnancy might lead to long-term alterations in BP, a more in-depth study of the cardiovascular phenotype that might be relevant to BP in these experimental models is required.

Vascular structure

A key determinant of BP in humans is arterial stiffness, with increased arterial stiffness being associated with elevation of central BPs [83,84]. Therefore one possibility is that exposure to the abnormal intra-uterine milieu of pre-eclampsia leads to vascular structural remodelling that persists into post-natal life [85]. One RUPP model study has evaluated the stress-stain relationship of offspring arteries and found evidence of increased aortic

stiffness, as well as greater elastic fibre content in the vessel wall [63]. Apparently in support of the hypothesis that pre-eclamptic insults induce structural changes in blood vessels are findings from a guinea-pig model of RUPP-exposed offspring, which showed increased thickness of the aortic media [86]. Offspring of dams, hypoxic from early in gestation, were also found to have a thicker aortic media with increased smooth muscle cell density in fetal life [87,88]. However, changes in arterial stiffness are not found in studies of human offspring in adolescence (14 years of age) [11] or young adulthood (20–30 years of age) [12] and, in animal models, by adult life, 16-month-old offspring of hypoxic dams start to exhibit quite distinct vascular structural changes with oedematous and necrotic aortic endothelium and disarranged proliferative smooth muscle cells [89]. These findings are more characteristic of atherogenic vascular remodelling rather than the vascular structural matrix changes associated with abnormal arterial stiffness [84]. Other studies of rat RUPP offspring have also identified an increased propensity to develop arterial internal elastic lamina lesions, an early atherosclerotic process, when studied at 8 and 16 weeks of age [64].

These observations highlight development of an early atherogenic phenotype, independent of BP, as an additional potential link between pre-eclampsia exposure and later cardiovascular disease. Atherosclerosis in humans is characterized by thickening of the arterial intima media, which can be evaluated non-invasively by ultrasound [90], and carotid artery IMT (intima-media thickness) associates with the extent of coronary

artery disease [90,91]. Consistent with the observations in animal studies, offspring of pre-eclamptic pregnancies also show an increased IMT in their 20s [12] with aortic arterial thickening already evident at birth [92]. Therefore the features of vascular remodelling that have been observed are predominantly not those associated with the arterial stiffening that underlies BP variation. However, there appear to be significant early atherosclerosis-related changes in the vascular wall, the importance of which require further exploration.

Cardiac structure

An alternative possibility to explain the variation in BP is that the *in utero* insult alters cardiac development. The structural and functional changes, such as increased cardiac output or LVH [LV (left ventricular) hypertrophy], then predispose to development of hypertension [93]. Hypoxic exposure in pregnancy does lead to abnormalities in cardiac appearances, although these seem to be predominantly of a cardiomyopathic phenotype more commonly linked with the development of heart failure. Rat offspring exposed to hypoxia during late gestation have increased cardiac size relative to body weight [89,94–96], with reduced LV wall thickness, reduced cardiomyocyte proliferation and epicardial detachment [96,97]. It has been hypothesized that this detachment of the epicardium in fetal life [97] may be mechanistically related to the changes in the myocardium, as the epicardium is a major source of growth factors during cardiac development [96,97]. Higher levels of apoptotic proteins and induction of HIF (hypoxia-inducible factor)-1 α are also evident and may drive some of this process [98,99].

Additionally, offspring cardiac collagen content can be altered by maternal hypoxia, with rats exposed to hypoxia during late gestation having increased cardiac levels of collagen I and III [96,100]. These animals express a complex array of alterations in extracellular matrix regulation so that, by day 21, rat fetuses exposed to hypoxia have decreased MMP (matrix metalloproteinase)-1 and increased MMP-13 and TIMP (tissue inhibitor of metalloproteinases)-3 and TIMP-4. In neonatal life, both MMP-1 and MMP-13 levels are still raised and at 4 months of age MMP-2 levels are reduced [96,100]. Another potential link between *in utero* hypoxia and later cardiovascular disease is through alterations in cardiac ischaemia/reperfusion mechanisms. Specifically, rats exposed to hypoxia in late gestation have been found to have alterations in cardiac proton production and increased myocardial production of acetyl-CoA during reperfusion [94,99]. In fetal life, such animals also have a significant reduction in cardiac PKC ϵ (protein kinase C ϵ), possibly secondary to increased methylation at the PKC ϵ promoter site [101]. As PKC ϵ plays a critical role in cardioprotection during ischaemia [102], this may underlie the observed differences.

However, these findings need to be interpreted with caution in relation to pre-eclampsia. None of the studies have investigated low levels of hypoxia early in pregnancy and the data for long-term changes in cardiac function are variable. Offspring of Sprague–Dawley rats exposed to hypoxia (12% O₂) from days 15 to 21 of pregnancy showed no differences from controls in cardiac function at 4 months of age, as assessed by echocardiography, but, by 12 months, male offspring only demonstrated signs of LVH. In a second study, both male and female offspring had structurally similar hearts, but those exposed to hypoxia had impaired diastolic function and evidence of pulmonary hypertension in later life [95].

In humans, placental tissue from pre-eclamptic pregnancy has differential expression of genes associated with cardiovascular development [103]. However, an 18-year follow-up of children born to hypertensive pregnancies did not show differences in LV mass, as assessed by echocardiography [104]. More recently, one study in children aged 5–8 years exposed to pre-eclampsia *in utero* found they had smaller hearts, increased heart rate and increased late diastolic velocity at the mitral valve attachments [105]. Overall, a pragmatic conclusion is that more detailed work is needed to understand whether pre-eclampsia leads to cardiac changes in humans. If so, the geometric cardiac changes observed so far are likely to be a distinct pathway to disease, or secondary to BP variation, rather than the primary underlying mechanism for the hypertensive phenotype of the offspring of pre-eclamptic pregnancies.

Vascular function

One possibility is that the variation in BP is driven by underlying induced abnormalities in vascular function, rather than structural cardiovascular changes. In particular, systemic endothelial function is associated with traditional cardiovascular risk factors, including hypertension [106]. Endothelial dysfunction is also an early biological factor in the development of atherosclerotic vascular disease [107,108] and predisposes to the development of LVH [109]. Consistent with vascular function being a central factor in the association between pre-eclampsia and long-term cardiovascular health, abnormalities in vascular responses are found in nearly all offspring models that mimic pre-eclampsia, as well as the majority of human studies of offspring of pre-eclamptic pregnancies. RUPP offspring have enhanced vascular contraction in response to phenylephrine and a reduction in endothelium-dependent relaxation during early life [54,63,110]. Lower flow-mediated vasodilation is also evident up to 12 months of age in rats born to mothers housed under hypoxic conditions, even if only during late gestation (days 15–21 of pregnancy) with relatively high oxygen levels (11.5–12% O₂) [69,70]. In more specific 'pre-eclampsia' models, long-term effects on endothelial responses are the major

adverse change reported in cardiovascular phenotype of male offspring of sFlt-1-induced 'pre-eclamptic' mothers. They exhibit the same pattern of higher responses to phenylephrine and reduced vasorelaxation in response to acetylcholine [111] observed in the RUPP offspring model. Mice heterozygous for eNOS, whose mothers were eNOS-knockout and therefore who developed in an *in utero* environment of endothelial dysfunction, similarly have enhanced phenylephrine-induced contraction and reduced acetylcholine-mediated relaxation compared with heterozygous mice with wild-type mothers [112,113].

This finding is also evident in humans, with young adults (20–30 years of age) born pre-term to hypertensive pregnancies having a distinct vascular phenotype when compared with those born pre-term to normotensive pregnancies, despite both groups demonstrating a similar magnitude of increase in BP when compared with term-born controls [12]. Only those born to hypertensive pregnancies had evidence of impaired flow-mediated endothelial responses. Offspring of pre-eclamptic women also have impaired endothelial function in childhood (5–8 years of age) [114] and adolescence (14 years of age) [11]. Only one study, in children between 9 and 10 years of age, has not been able to demonstrate this difference in endothelial response [15]. Recently, it has been reported that there are endothelium-dependent differences evident within the microvasculature in offspring of pre-eclamptic pregnancies during neonatal life [115], and evidence has been found of endothelial activation at birth based on cord blood analysis [116].

Experimental studies provide some insight into the potential underlying vascular abnormality. In arterial segments from RUPP model offspring, both basal and acetylcholine-induced NO production is reduced and arterial segments from RUPP animals do not demonstrate the normal impairment of acetylcholine-induced vasodilation after L-NAME pre-treatment [54]. This latter observation suggests there may also be a compensatory up-regulation of NO-independent pathways to maintain the vasodilation response. This loss of NO-mediated vasomodulation due to reduced NO production also underlies the vascular dysfunction demonstrated in several studies of rats exposed to hypoxia *in utero* [69–71,117] and has been observed in studies of HUVECs (human umbilical vein endothelial cells) grown from umbilical cords from pre-eclamptic pregnancies [118].

What might underlie the reduced NO synthesis? A characteristic feature of pre-eclampsia is a derangement of circulating anti-angiogenic factors. Elevated sEng in the pre-eclamptic mother, with resultant alterations in TGF (transforming growth factor)- β signalling, has been directly implicated in reduced NO bioavailability in the mother. Persistent abnormalities in anti-angiogenic factors in the offspring could therefore lead to persistent endothelial dysfunction in later life. Studies during

neonatal life demonstrate that the fetus is exposed to elevated anti-angiogenic factors *in utero*, as amniotic fluid levels of sFlt-1 are higher in pre-eclamptic pregnancies [119] and neonates born to pre-eclamptic pregnancies have significantly higher circulating sFlt-1 and sEng, as well as lower VEGF and PlGF levels [120,121]. However, to date, the only study of anti-angiogenic factors in children of 5–8 years of age has demonstrated no long-term differences [114].

One possibility is that *in utero* exposure to anti-angiogenic factors leads to altered *in utero* endothelial development and physiology. Consistent with this hypothesis, sFlt-1 administered *in vitro* inhibits endothelial cell proliferation and tubule formation [122]. HUVECs from pre-eclamptic donors also appear to differ from normal in their response to oxygen levels. Under normoxic conditions, they develop a higher numbers of connections and shorter tubule lengths, creating networks similar to those seen when control cells were grown under hypoxic conditions [118]. These findings suggest that endothelial cells from pre-eclamptic pregnancies may be fixed in a 'hypoxic' phenotype. This 'hypoxic' programming may not be endothelium-specific, as gene expression in pre-eclamptic placentae is also similar to that seen in control placental explants grown at 3% O₂ [67], and metabolomic studies have demonstrated that pre-eclamptic placental tissue grown under normoxic conditions behaves similarly to control tissue grown under hypoxia [123].

Another possibility is that the endothelial dysfunction represents a biomarker of other underlying metabolic abnormalities related to cardiovascular disease. However, there is limited evidence that pre-eclampsia leads to the metabolic syndrome in the offspring [18,124], with no convincing evidence for changes in serum lipids or glucose tolerance beyond acute transient changes in cord blood samples [12,14,15,114,125–130]. Animal studies of maternal hypoxia suggest that it is possible to induce metabolic changes in animals exposed to a high-fat diet, leading to increased serum leptin, triacylglycerols (triglycerides), non-esterified ('free') fatty acids and impaired glucose tolerance [131]. However, the need for a secondary inducer would suggest that lipid and glucose abnormalities are not fundamental features of the underlying hypertensive cardiovascular phenotype.

Alternatively, the vascular changes could be inherited between mother and child. Polymorphisms in certain genes, such as those encoding eNOS, ACE (angiotensin-converting enzyme) and angiotensin, have been proposed as potential links [132] underlying the development of the condition, although no consistent associations have been found [133]. Adverse exposures during fetal life could also lead to heritable characteristics through programming of the epigenome, as the greatest level of active programming of the epigenome occurs during fetal life [134]. This is clearly possible in humans, as placental DNA is

hypomethylated following early-, but not late-, onset pre-eclampsia [135,136] and imprinting of non-coding RNA in placental tissue also differs in studies of pre-eclamptic tissues [137]. As epigenetic changes are potentially dynamic [138,139], this mechanism is of interest as a target for intervention [140]. Transgenerational, potentially epigenetic, effects are seen in 'pre-eclampsia' models. Elevated BP is evident in the first 12 weeks of life in both male and female second-generation offspring born to RUPP offspring [141]. Such transgenerational effects are also evident in eNOS-knockout models as second-generation offspring born to the eNOS-heterozygous mothers, who developed in an *in utero* environment of endothelial dysfunction, have increased BP compared with those born to genetically similar mothers who had a normal *in utero* development.

A potential stimulus for epigenetic programming is hypoxia, which has been shown to induce a global decrease in transcriptional activity in the vascular endothelium [142]. Alternatively, the systemic maternal oxidative stress evident during pre-eclampsia could alter the delicate balance between the production of ROS and cellular defences in the neonate. Oxidative stress has been suggested to influence histone acetylation and phosphorylation [143], telomere length [144] and vascular gene expression. Some oxidative stress is required for normal fetal development [145], but the ischaemic pre-eclamptic placenta has significantly decreased total antioxidant capacity compared with the normal placenta, and there is a correlation between levels of oxidative stress in maternal peripheral blood and cord blood in normotensive and pre-eclamptic pregnancies [146,147], with levels significantly higher in pre-eclampsia [127,147]. The levels of oxidative stress induced by pre-eclampsia itself are sufficient to cause damage to the fetus, as placental tissue samples have higher levels of oxidative DNA damage in pre-eclamptic pregnancies, whether or not there has been fetal growth restriction [148]. This independent effect of pre-eclampsia is not surprising, as fetal antioxidant capacity generally only increases in the last trimester after the increase in oxidative stress observed in early-onset pre-eclampsia [127,149]. Mitochondrial oxidative species may be particularly relevant to this fetal programming mechanism as, following pre-eclampsia, fetal-derived HUVECs have impaired mitochondrial function [150] associated with increased endothelial mitochondrial oxidative species generation.

OTHER POTENTIAL MECHANISMS FOR LONG-TERM PROGRAMING

Altered sympatho-adrenal function

It is also possible that the increased BP following *in utero* exposure to pre-eclampsia is explained, in part, by non-

vascular alterations. The sympathetic nervous system has a key role in regulating BP in humans and overactivity may be associated with development of hypertension [151]. Heart rate variability, a marker of autonomic nervous system function, has been associated with and precedes the development of several cardiovascular risk factors, as well as being a predictor of mortality in high- and low-risk populations [152]. Furthermore, alterations in adrenal function have been associated with features of the metabolic syndrome in adults [153]. Tenhola et al. [14,154] have suggested that, in childhood, children born to a pre-eclamptic mother have increased serum adrenaline, but were not able to demonstrate differences in other adrenal hormones. Despite this lack of human data, there is some supportive evidence from animal models. In particular, hypoxia studies have considered sympatho-adrenal function in both fetal and post-natal life. A chick embryo that has developed at high altitude, and therefore has been exposed to significant hypoxia throughout gestation, has elevated plasma corticotrophin, decreased plasma corticosterone and elevated adrenal adrenaline and noradrenaline compared with those incubated at sea level [155]. Fetuses of pregnant ewes who undergo gestation at high altitude similarly had reduced adrenal mRNA for CYP (cytochrome P450) 17, CYP11A1 and ACTH (corticotropin) [156], and higher eNOS expression in the inner adrenal cortex [157]. There is some evidence that these alterations in sympatho-adrenal programming also persist through post-natal life, as the offspring of Sprague–Dawley rats exposed to hypoxia from pre-natal days 5–20 demonstrate persistent alterations in the levels and utilization of catecholamines in the heart and stellate ganglion at 12 weeks of age [72]. Even shorter exposures later in pregnancy may effect adrenal function, as exposure of rat fetuses to 12% O₂ in the last 3 days of gestation led to altered aldosterone levels at 7 days of age [158]. Further studies will be required to elucidate whether this mechanism is significant in humans.

Renal dysfunction

Another possibility is that changes in renal function are central to the programming of hypertension in offspring of pre-eclamptic pregnancies. Offspring of the rat RUPP model have inhibition of the RAS (renin-angiotensin system), and bilateral renal denervation normalizes their BP without influencing BP in control animals [61,62]. RUPP offspring also have reductions in glomerular number [57,65,159], increased glomerular collagen deposition [86], increased glomerular volume [57,159,160], altered renal VEGF expression [161] and alterations in intrarenal renin and angiotensin expression [61], although no difference in plasma renin activity has been demonstrated [59,60,162]. At 18 months of age, female RUPP offspring have increased plasma creatinine and renal expression of TGF- β , MMP-9 and type VI

collagen [65]. Additionally, RUPP offspring have reduced renal expression of the glucocorticoid receptor at day 21 after birth and increased mineralocorticoid receptor expression at birth compared with control animals [160]. Similar findings have never been demonstrated in humans, but conceivably these changes would be relevant to BP control and a further understanding of the potential role of pre-eclampsia-induced renal changes in humans subjects requires investigation [163].

Altered immune function and inflammation

Finally, evidence for a further non-vascular non-hypertension pathway of relevance to cardiovascular disease development in the offspring of pre-eclamptic pregnancy has recently emerged. Integration of data from genome-wide gene expression studies of pre-eclamptic decidua basalis tissue with tissue from subjects with a history of familial heart disease has identified commonality between disease processes and several differentially expressed genes relevant to immune function and inflammatory responses [164]. These include NOD1 [NOD (nucleotide-binding and oligomerization domain)-like receptor 1], which plays a key role in intracellular innate immunity [165], and SPPL2 (signal peptide peptidase-like 2), which has been shown to play a role in the release of pro-inflammatory cytokines [166]. Pre-eclampsia involves a range of key immune responses at different stages of the syndrome [34], with the final symptomatic phase characterized by an intense maternal systemic inflammatory response [34]. Women who suffer from autoimmune conditions are at an increased risk of developing pre-eclampsia [167], and in animal models the transfer of activated Th1 cells or infusion of inflammatory cytokines induces pre-eclampsia-like symptoms [168–170]. Inflammation has a key role in cardiovascular disease, with inflammatory cell infiltration of the vessel wall an early pathophysiological stage in the development of atherosclerotic cardiovascular disease [171,172]. Interestingly, neonates born following pre-eclamptic pregnancies have elevated IL (interleukin)-8 and greater percentages of natural killer cells, reduced number of T-cells and altered expression of the Fas/Fas ligand in their umbilical cord blood [173–176]. However, without longer term follow-up it is difficult to determine whether these changes reflect acute changes associated with the pregnancy or long-term changes relevant to disease. A single study of 5–8-year-old children born to women who experienced pre-eclampsia showed no difference in general systemic inflammation, assessed by high-sensitivity CRP (C-reactive protein) [114], but more sophisticated immunological or inflammatory markers were not considered. Nevertheless, given the growing understanding of the importance of inflammation and immune function in the pathophysiology of pre-eclampsia

and cardiovascular disease, further investigation of these factors may prove useful in understanding the links between pre-eclampsia and offspring cardiovascular health.

Gender differences and the effect of hormonal status

In addition to other potential programming mechanisms, some studies have suggested the association between exposure to pre-eclampsia and alteration of offspring phenotype may be sex-specific. Animal work suggests that hormonal status may play some role in phenotypic modulation. Lu et al. [78] have demonstrated that the male offspring of sFlt-1-induced ‘pre-eclamptic’ mothers demonstrated a sustained increase in BP from the first day of life, which was not shown in female offspring [78]. The hormonal status of RUPP offspring may play a significant role, as castration of male RUPP offspring has been demonstrated to normalize BP [162], whereas ovariectomy induced hypertension in otherwise normotensive female RUPP offspring [60]. Such findings suggest that gender-specific hormone expression may mediate or alter the phenotype associated with exposure to adverse intra-uterine conditions. Some human studies of BP in children born to pre-eclamptic mothers have also demonstrated gender differences; however, these differed with some showing elevation in males and some in females [177–179]. A recent meta-analysis of data considering BP in children born to pre-eclamptic pregnancies has suggested that the phenotype is similar in both males and females [18]. Despite this, the findings from animal models arguably suggest that gender-related changes in biology may require closer investigation in relation to other aspects of the phenotype in human populations.

CONCLUSIONS

Pre-eclampsia has a long-term impact on the cardiovascular health of both mother and offspring. The pre-eclamptic *in utero* environment is characterized by reduced uterine perfusion, hypoxia, raised anti-angiogenic factors and systemic oxidative stress, leading to maternal endothelial dysfunction. Animal studies support the potential relevance of these insults to programming of offspring BP and, in combination with human experimental studies, have demonstrated a range of potential mechanisms for the hypertensive phenotype. In particular, endothelial dysfunction is consistently observed in offspring of both animal and human studies well into adult life. The underlying programming of vascular function may relate to ‘structural’, ‘hypoxic’ or ‘epigenetic’ programming of endothelial development and physiology. In addition, complementary pathways to disease development, including changes in cardiac

function, accelerated atherogenesis or alterations in renal and immune function, may emerge as relevant to later disease in offspring of pre-eclamptic pregnancies. Greater understanding of all of these mechanisms will provide the basis for future novel strategies to prevent cardiovascular disease in this high-risk group.

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