Mechanisms underlying developmental programming of elevated blood pressure and vascular dysfunction: evidence from human studies and experimental animal models

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ABSTRACT

Cardiovascular-related diseases are the leading cause of death in the world in both men and women. In addition to the environmental and genetic factors, early life conditions are now also considered important contributing elements to these pathologies. The concept of ‘fetal’ or ‘developmental’ origins of adult diseases has received increased recognition over the last decade, yet the mechanism by which altered perinatal environment can lead to dysfunction mostly apparent in the adult are incompletely understood. This review will focus on the mechanisms and pathways that epidemiological studies and experimental models have revealed underlying the adult cardiovascular phenotype dictated by the perinatal experience, as well as the probable key causal or triggering elements. Programmed elevated blood pressure in the adult human or animal is characterized by vascular dysfunction and microvascular rarefaction. Developmental mechanisms that have been more extensively studied include glucocorticoid exposure, the role of the kidneys and the renin–angiotensin system. Other pathophysiological pathways have been explored, such as the role of the brain and the sympathetic nervous system, oxidative stress and epigenetic changes. As with many complex diseases, a unifying hypothesis linking the perinatal environment to elevated blood pressure and vascular dysfunction in later life cannot be presumed, and a better understanding of those mechanisms is critical before clinical trials of preventive or ‘deprogramming’ measures can be designed.

INTRODUCTION

The prevalence of obesity, Type 2 diabetes and hypertension, which are major risk factors for cardiovascular-system-related morbidity and mortality, has increased substantially in many countries over the last two decades. Cardiovascular-related diseases are the leading cause of death in the world (World Health Organization at http://www.who.int/mediacentre/factsheets/fs310/en/index.html), and are the cause of death in nearly 50 % of all women and men in the United States. In developing countries, cardiovascular diseases cause twice as many deaths in women as childbirth and HIV/AIDS combined. Importantly, in developing countries, the death toll is high in adults of working age (for example, <10 % of adults who die from stroke and heart disease...
are <65 years of age in the United States compared with 28% in Brazil; www.world-heart.org). Risk factors such as heredity and lifestyle habits are important contributing elements, but do not account for all cases [1]. It has now been well-established that events occurring during early life significantly impact on the incidence of cardiovascular-related complications [2–5].

It has long been known that intrauterine and postnatal environments can significantly influence subsequent health (such as growth and neurodevelopment). It is the concept that diseases that manifest in adulthood can also be programmed by fetal and neonatal events or environmental cues that has received the most attention over the last 15 years. On the basis of the well-documented observations of health visitors collecting data in the early 20th century on newborns, their mothers and the family living conditions, Barker and Bagby [6] demonstrated an inverse relationship between birthweight and adult BP (blood pressure), incidence of cardiovascular-related mortality and morbidities, as well as Type 2 diabetes. Early postnatal life can also result in programming of adult-onset diseases through further modulation of the impact of an altered antenatal life environment [as illustrated by the role of infancy growth trajectories of SGA (small-for-gestational-age) babies on later life BP] or in itself (through nutrition or prematurity for example). The present review will focus on the mechanisms studied to date underlying developmental programming of elevated BP and vascular dysfunction, leaving aside, however, analysis of the heart programming literature [7].

DEVELOPMENTALLY PROGRAMMED ELEVATED BP

The association between birthweight and adult BP has now been demonstrated in many parts of the world, in industrialized as well as in more developing countries, in both men and women [4,8–10]. The association is first noted in early childhood (but not at birth) and appears to increase with age [11]. Even though some studies have failed to show an inverse relationship between birthweight and BP [10], most reveal associations with other disorders such as glucose intolerance, endothelial dysfunction and obesity, all of which impact on BP and cardiovascular diseases [12–14]. Careful analysis of published findings clearly shows that there is a continuum of risk across the birthweight strata, with both extremes of birthweight associated with the highest risk of hypertension, meaning that programming can occur without apparently affecting birthweight.

Despite these large and numerous studies, the concept of developmental programming of adult-onset diseases has remained controversial and has been challenged mainly on the relatively small impact (in terms of changes in mmHg/kg of birthweight increase, which ranges from −0.6 in the largest studies to −4 mmHg in smaller studies) on adult BP [15]. Furthermore, considering that birthweight is positively associated with current body weight and that current body weight is also positively correlated with current BP, it is argued that adjustment of BP with current weight might create an artefactual, or at least exaggerated, inverse relationship between birthweight and current BP [15,16]. Therefore it can also be argued that the important element is the change in weight categories (or percentile crossing) between birth and adulthood, emphasizing the determinant role of postnatal life and especially nutrition on adult outcomes [17]. Studies of twins, especially monozygotic twins, also show that the smaller twin has a slightly higher BP as an adult; however, considering that, on average, twins weigh 1 kg less than singletons at birth, it is of note that large population studies have not reported an increased incidence of cardiovascular-related diseases in twins [15]. However, considering that birthweight alone is an oversimplified marker of ‘healthy’ antenatal life and when disease outcome (rather than sole measure of BP) is considered, the impact of perinatal life (both ante- and post-natal) is clear for both men and women [18,19].

The concept of developmental programming of cardiovascular-related diseases is supported by animal models. Fetal and early postnatal nutritional manipulation (such as, but not solely, restriction of dietary protein or a global restriction in nutrients during pregnancy), maternal hypoxia, uterine artery ligation and exogenous administration of glucocorticoids to a pregnant animal give rise to offspring with elevated BP and vascular dysfunction, as well as glucose intolerance and insulin resistance [20–24]. Excellent and thorough papers have been published reviewing the epidemiological evidence and the various animal models used to study cardiovascular and metabolic syndrome programming, as well as the observations these models allowed us to make (the reader is referred to the particularly extensive review by McMillen and Robinson [20], as well as [6,10,25,26]).

The timing of appearance of elevated BP, as well as its persistence, varies with the animal model studied. It is important to note that many of the studies examining the programming of elevated BP in rats have used tail cuff plethysmography which entails restraint of the animal. There is therefore a potential contribution of a programmed ‘stress response’ to the increase in BP [20]. In vivo measurements obtained from an indwelling catheter in a conscious unrestrained animal [27,28] minimize this factor.

In both male and female offspring of LP (low-protein)-fed dams, some studies report the presence of a significant difference in BP already at 4–5 weeks of age, which is the age at which tail BP starts to be measurable with standard equipment, whereas in other studies elevated BP appears by 6 weeks of age, depending on the diet composition, duration of exposure to the
BP and atherosclerosis. Vasodilation is a well-recognized precursor of elevated and in early adult life [35–39]. Endothelium-dependent individuals at birth, at 3 months of age, in later childhood flow-mediated dilation is decreased in low-birthweight Several studies have shown that endothelium-dependent independent vasodilator) [36]. Endothelium-dependent vasodilation (in response to local application of ACh) was found to be positively, and in a graded manner [41], associated with birthweight in children 8–13 years of age [36,39,41]. In an older group, 315 young adults aged 20–28 years old, lower flow-mediated dilation of the brachial artery was related to lower birthweight; the impact of birthweight when comparing the top and bottom strata (fifths) was as important as current smoking [38]. Other markers associated with vascular dysfunction and hypertension have also been reported in young subjects in association with low birthweight, such as elevated uric acid levels [39] and von Willebrand factor [42]. However, it is interesting to note that the association of elevated von Willebrand factor (as well as other markers of endothelium function and inflammation) was not associated with birthweight in an older (54 years of age) cohort [43]; in that later study, however, the inverse relationship between birthweight and insulin resistance persisted. The later observation might suggest that programming is not a fixed status and that postnatal life can modulate (enhance or reverse) the influences of perinatal life.

More recent studies indicate that prematurely born infants also display metabolic (insulin resistance) and cardiovascular dysfunction (elevated BP and abnormal retinal vasculature) as adults [44–49]. In a study of 430 49-year-old Swedish men, systolic and diastolic BPs did not correlate with birthweight, but rather were inversely correlated with gestational age for those born before 38 weeks of gestation [49], independently of current BMI (body mass index), the authors estimated that every additional week of gestation at birth was associated with a decrease in adult systolic BP of 7.2 mmHg. Such an impact of early gestation, rather than or in addition to IUGR (intrauterine growth restriction), was observed by other [50,51] but not by all [52,53] studies. In women, former preterm adolescents and young adults have higher BP, abnormal retinal vascularization [46], a narrower, but however less stiff, abdominal aorta (measured by ultrasonography) and lower peripheral skin blood flow than control subjects [54,55].

Prematurity also appears to have an impact on hypertensive complications of pregnancy, which are recognized to be an early manifestation of cardiovascular dysfunction in later life [56,57]. Women born before 37 weeks of gestational age in 1966 appear to have a 2.5-fold increased risk of developing gestational hypertension compared with women born \( \geq 37 \) weeks [57], whereas the fact of being born SGA had no impact on the incidence of hypertensive complications of pregnancy. Interestingly, in those studies, SGA subjects were not different from appropriate-for-gestational age ones, both within the premature and the term groups, suggesting an important role of preterm birth itself in the programming of cardiovascular function in later life. In fact, it can be argued that the multiple studies examining cardiovascular outcome of low birthweight are in fact, at least in part, examining

**CHARACTERIZATION OF VASCULAR DYSFUNCTION**

**Human studies**

Several studies have shown that endothelium-dependent and -independent vasodilation can be impaired and that flow-mediated dilation is decreased in low-birthweight individuals at birth, at 3 months of age, in later childhood and in early adult life [35–39]. Endothelium-dependent vasodilation is a well-recognized precursor of elevated BP and atherosclerosis.

Martin et al. [40] reported that, at 3 days old, forearm skin vasodilation response to local application of ACh (acetylcholine) in SGA newborns was significantly attenuated compared with normal birthweight babies (240% compared with a 650% increase respectively, in blood flow measured by laser Doppler). Former low-birthweight children at 9 years of age had impaired endothelium-dependent vasodilation (in response to local application of ACh), but not in response to the NO donor SNP (sodium nitroprusside; an endothelium-independent vasodilator) [36]. Endothelium-dependent vasodilation (measured by flow-mediated vasodilation or by skin application of ACh) was found to be positively, and in a graded manner [41], associated with birthweight in children 8–13 years of age [36,39,41]. In an older group, 315 young adults aged 20–28 years old, lower flow-mediated dilation of the brachial artery was related to lower birthweight; the impact of birthweight when comparing the top and bottom strata (fifths) was as important as current smoking [38]. Other markers associated with vascular dysfunction and hypertension have also been reported in young subjects in association with low birthweight, such as elevated uric acid levels [39] and von Willebrand factor [42]. However, it is interesting to note that the association of elevated von Willebrand factor (as well as other markers of endothelium function and inflammation) was not associated with birthweight in an older (54 years of age) cohort [43]; in that later study, however, the inverse relationship between birthweight and insulin resistance persisted. The later observation might suggest that programming is not a fixed status and that postnatal life can modulate (enhance or reverse) the influences of perinatal life.

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prematurely born rather than SGA individuals. Considering that the 50th percentile birthweight at 36 weeks was 2.6 kg (or 5.7 lbs) 40 years ago [58], it is indeed plausible that a significant number of newborns were labelled ‘SGA’ (or ‘IUGR’) when in fact were mildly premature. This element is important to bear in mind as studies have revealed cardiovascular disorders associated with what is considered relatively mild prematurity. The mechanisms linking prematurity and later cardiovascular disorders are not known, but may comprise impaired nephrogenesis (since nephrogenesis is completed relatively late in gestation by 34–36 weeks) [59,60], impaired vasculogenesis and neonatal/perinatal oxidative stress (see below).

**Animal studies**

Vascular dysfunction in adult programmed animals is characterized by enhanced constrictive response to vasoactive agents and impaired endothelium-mediated vasodilation, both of which can differ according to vessel and animal model studied (Figure 1).

**Vasoconstriction**

*In vivo*, programmed elevated BP has been associated with increased pressor response to AngII (angiotensin II) in anaesthetized female and in conscious male adult offspring of LP-fed dams [61–63]. *Ex vivo*, exaggerated vasoconstriction specifically to AngII [24,63] is present in carotid arteries from LP adult offspring and is secondary to enhanced production of superoxide by vascular NADPH oxidase with concomitant increase in the AT$_1$ receptor (AngII type 1 receptor) subtype on vascular smooth muscle cells [24]. The fact that others have reported unchanged mRNA [64] allows post-transcriptional and/or post-translational changes in the regulation of AT$_1$ receptor expression in LP offspring to be considered. In hypertension associated with a 50% global nutrient restriction of the pregnant dam, AT$_1$ receptor mRNA expression was found to be unchanged in mesenteric arteries of adult offspring (but vasomotor response to AngII was not reported) [64]. In these models, the ontogeny of vascular dysfunction and AT$_1$ receptor expression is unknown.

Vasomotor response to phenylephrine is increased in aortic rings of 4-, 8- and 12-week-old growth-retarded offspring of dams with reduced uterine perfusion [30], which is related, at least in part, to decreased endothelium-dependent vasorelaxation. Comparing two models of programmed elevated BP, Williams et al. [65] report, in femoral arteries of 1-day-old pups, that the constriction to phenylephrine is increased after antenatal hypoxia, but reduced after exposure to 40% restriction of maternal caloric intake. The carotid response to phenylephrine is also decreased after the caloric restriction, but unchanged after hypoxia. However, the mechanisms underlying these observations were not elucidated.

In the LP rat model, studies have reported no modification in the $E_{max}$ response to phenylephrine, as well as to the TXA$_2$ (thromboxane A2) analogue U46619, in pial microvessels, mesenteric and carotid arteries [24,32,66,67], although Brawley et al. [32] reported a slight increase in pEC$_{50}$ (negative log of EC$_{50}$) to U46619 in mesenteric arteries at 12 but not 23 weeks. The response to endothelin has been examined sporadically: it is increased in the coronary arteries of newborn lambs after early exposure to dexamethasone at 27 days of gestation (term is 145 days) [68], and is reduced in the carotid arteries, but unchanged in the femoral arteries, of 1-day-old pups exposed to hypoxia [65].

In summary, enhanced or unchanged responses to vasoconstrictive agents are reported in most models of developmental programming of elevated BP. These vary.

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**Figure 1** Demonstrated and probable mechanisms of vascular dysfunction associated with developmentally programmed elevated BP

EC, endothelial cell; GC, guanylate cyclase; O$_2^\cdot$, superoxide anion; VSMC, vascular smooth muscle cell.
significantly with the vascular bed studied, but also with the triggering insult, its timing and the age at which the offspring are studied. Mechanisms underlying many of these observations require further studies.

**Vasodilation**

Endothelium-dependent vasodilation is impaired in nearly all models of developmental programming of elevated BP where this has been studied [20]. Decreased vasorelaxation to the NO donor SNP was reported in pial microvessels and mesenteric arteries of LP adult offspring [32,66]. However, in carotid arteries, we found that LP offspring have an impaired response to ACh, but not to the NO donor SNP [69]. Similar observations to the latter study have been reported in mesenteric arteries from offspring of 50%-nutrient-restricted dams [29], of dams fed a high-fat (lard-enriched) diet [70] and of dams subjected to hypoxia from day 15–21 of pregnancy (term is 21 days). In the offspring of hypoxic dams, this decreased endothelium-dependent vasodilation was present at 4, but not 7, months of age [21].

Mechanisms proposed to underlie impaired NO-mediated vasodilation include enhanced generation of superoxide and decreased expression of soluble guanylate cyclase in the presence or not of decreased eNOS [endothelial NOS (nitric oxide synthase)] expression and NO production, depending on the vascular bed and the animal model studied [22,29,66,71–73]. Adult offspring of dams subjected to a 50% caloric restriction during gestation have increased vascular superoxide anion concentration and decreased levels of SOD (superoxide dismutase) activity and of glutathione synthetic enzyme; in these adult rats, SOD mimetic or antioxidant improved endothelium-dependent vasorelaxation [29,71,74]. Mechanisms leading to these changes are incompletely understood, but it is known that ROS (reactive oxygen species) can scavenge NO as well as decrease soluble guanylate cyclase expression [75,76]. Indeed, whether vascular dysfunction and increased superoxide generation are present early in development in programmed animals and perhaps precede elevation of BP is unknown.

**Vascular structure**

In addition to vascular function and reactivity, changes in vascular structure have also been linked to perinatal elements and low birthweight [77].

Arterial stiffness is a key factor in increased systolic and pulse pressure in adults. Increased arterial stiffness imposes a higher work on the left ventricle, leading to hypertrophy, and is a major contributor to small vessel disease and atherosclerosis; all of these elements result in a higher risk of coronary disease and infarction, as well as end-organ damage [78]. The proportion of elastin compared with rigid collagen is a major determinant of arterial stiffness. Elastin synthesis in the vessels peaks in the prenatal period, mostly approaching term, decreases rapidly after birth and is minimal in the adult aorta [79]. Elastin has a very long half-life and a very slow turnover. In rats, experimentally induced fetal growth inhibition results in a lower elastin content of the aorta and persistent changes in aortic wall components [80]. Composition of the aortic wall and other large conduit arteries is, therefore, determined relatively early during development, with little possibilities of ‘catch-up’ or compensation later on. Indeed, in a group of young adults, premature birth (at less than 37 weeks of gestation) has been correlated with increased arterial stiffness measured by pulse wave velocity [81]. Increased pulse wave velocity and wave reflections (both measures of arterial stiffness) are correlated with lower birthweight in children and adolescents [36,82], as well as in adults in their fifties [53]. Pulse pressure was found to be greater and aortic vessel wall diameters to be smaller (even after correction for present body surface area) in a group of SGA compared with appropriate-for-gestational-age children [83]. Aorta of offspring of high-fat diet-fed dams have reduced endothelial cell volume, decreased smooth muscle cell number and increased arterial stiffness [84].

Structural anomalies associated with hypertension also comprise reduced density of arterioles and capillaries (termed ‘rarefaction’) [85]. Rarefaction is an important common characteristic of various microvascular beds in hypertension in both human and animal models and is generally considered a consequence, rather than a cause, of increased BP [86]. Retinal vascularization is impaired in young adults born preterm [46] or SGA at term [87]. In animal studies, we and others have found no arterial remodelling in rats with programmed hypertension [32,63]; however, we have reported muscle capillary rarefaction in a major site of peripheral resistance (the striated muscle) in 7- and 28-day-old, but not in fetal, rats with programmed elevated BP associated with exposure to an LP diet in utero [63]. These findings suggest an early postnatal disruption in normal microvessel development. Whether BP is elevated in newborn LP offspring is unknown, but studies of newborns of larger animals and humans demonstrate that BP at birth correlates mainly with actual birthweight, independently of adverse intrauterine environment conditions [88]. It can be postulated from our findings that microvascular rarefaction is a primary event in the development of programmed elevated BP. Supporting this are reports of microvessel rarefaction at very early stages in (and even prior to) the development of hypertension in high-risk individuals [89–92]. Therefore it can be postulated that the increase in arterial BP [resulting mostly from activation of the RAS (renin–angiotensin system), see below] could be an adaptive mechanism allowing sufficient capillary recruitment to maintain adequate oxygen delivery to the peripheral tissues [93].

Microvascular rarefaction can result from decreased formation (impaired angiogenesis) or active
disappearance [94]. We have found [63] decreased angiogenic potential of aortic ring explants from offspring from LP-fed dams at birth; however, muscle protein expression on the last day of gestation and at 7 days of life of the following factors involved in angiogenesis were unaltered by antenatal diet exposure: AT1 receptor subtype, eNOS, angiopoietin-1 and -2, Tie 2 receptor, VEGF (vascular endothelial growth factor), VEGF receptor-2 and PDGF-C (platelet derived growth factor-C). It has also been suggested that undernutrition during pregnancy may modify the impact of elements such as flow and angiogenic factors on vascular endothelial and smooth muscle cell development [26,95].

**MECHANISMS**

Despite the significant number of studies published, examining tens of thousands of human subjects and many animal models, the mechanisms linking fetal life and adult diseases are contrastingly incompletely understood. A large number of studies have been conceived or interpreted with the framework proposed by the ‘thrifty phenotype hypothesis’ [10]. This hypothesis suggests that, when a fetus is developing under suboptimal conditions, it adapts to guarantee immediate survival and to preserve vital functions at the expense of other less immediately critical functions, for example by inducing peripheral insulin resistance to preserve glucose for vital organs while halting the development of other organs such as the kidneys or the pancreas. The resulting phenotype will depend on the type of ‘insult’, but also of its timing, as different organs present different critical windows of susceptibility to developmental modulation. This fetal metabolic ‘economy’ mode that ensures survival in response to prenatal adverse conditions becomes maladaptive in a postnatal world characterized by an abundance of nutritional resources. Supporting this theory is the observation that the cardiovascular outcome is notably worse in subjects who are born short and thin and have catch-up growth that exceeds target weight and, therefore, results in above-average BMI compared with individuals who remain short but thin into adulthood [96,97]. However, this theory does not satisfactorily explain all situations of developmental origins of adult diseases, including the transgenerational transmission of a given phenotype (see below) [98].

Mechanistic pathways that have been extensively studied include glucocorticoid exposure and the role of the kidneys and the RAS. Other pathophysiological pathways have been explored, such as the role of the brain and the SNS (sympathetic nervous system), oxidative stress and epigenetic changes (Figure 2)

**Nutrition**

The low-birthweight infants–future hypertensive adults examined in the first epidemiological studies were living in poor conditions, and insufficient and poor nutrition
was initially proposed to be the main trigger element leading to programming of adult diseases through alteration of developing organs and function. Accordingly, many animal models were developed using global caloric or specific nutrient restriction in the diet of the pregnant animals.

The importance of the maternal diet composition (by opposition to simple restriction) is supported by studies in populations from the U.K., Jamaica and India, reporting increased BPs in children and adults born to mothers who had a lower proportion of caloric intake from animal protein during pregnancy [99], or who were iron-deficient during pregnancy, which is reflective of a poor nutritional status [8,100,101]. Findings from the Dutch famine cohort show that people who were small at birth do have high BPs in later life, but could not demonstrate an effect of prenatal exposure to famine on BP [102]. A more detailed analysis of the cohort, however, revealed that BP of the offspring was inversely associated with the protein/carbohydrate ratio of the average ration during the third trimester of pregnancy, whereas it was not associated with any absolute measure of intake during pregnancy [102,103]. Children whose mothers ate little protein in relation to carbohydrate during the third trimester of pregnancy had higher BPs as adults. This may imply that BP is not so much linked to absolute amounts of nutrients, but to variations in the balance of macronutrients in the maternal diet during gestation, and that programming might have occurred without significant modification of birthweight.

Lucas [104,105] proposed the concept of postnatal nutritional programming, reviewing the evidence his team and others generated demonstrating the impact of nutrition during the very first weeks or months of life on neurodevelopment and later life bone mineral mass and obesity. Regarding BP, this group recently showed [106] a slight, but significant increase, in diastolic BP in 6–8-year-old children born at term but who were SGA (birthweight <10th percentile) and who were randomly assigned to receive a nutrient-enriched (compared with standard) formula for the first 9 months of life, suggesting that faster weight gain in infancy might have an adverse cardiovascular impact later in life. In that study [106], children who had been breast-fed had BP values intermediate between the two formula-fed groups. Breast feeding has been associated previously with lower adult BP in preterm neonates [107] and marginally so in term neonates [108]. The mechanisms underlying this observation are not well known, but Lucas and co-workers [106] suggest it is related to a relatively slower growth observed in their cohort of breast-fed infants: among the SGA breast-fed children, those with the fastest weight gain had higher BP values.

Interesting observations can be derived from studies of SHRs (spontaneously hypertensive rats) and from eNOS-KO (eNOS-knockout) mice. SHRs are considered a genetic model of hypertension in which, however, a role for neonatal environmental factors in hypertension development is suggested, as cross-fostering of SHR pups with normotensive Wistar–Kyoto control mothers lowers their BP as adults [109]. Heterozygous offspring (eNOS-KO+/−) born to an eNOS-KO+/− mother had greater vascular wall tension, vasoconstriction to phenylephrine and impaired endothelium-dependent vasodilation compared with eNOS-KO+/− offspring born to an eNOS-KO+/+ mother [110]. The latter studies underline the importance of antenatal and post-natal environment/nutrition in modulating genetic predisposition to hypertension in addition to being a susceptible period in itself for developmental programming of adult diseases.

**Reduced nephron number**

Growth-restricted infants, as well as rats exposed to an LP diet during the whole pregnancy or to a uterus with reduced perfusion (partial artery ligation), have smaller kidneys with fewer nephrons [31,111–116]. Nephron number is decreased in adult patients with primary hypertension [117]. These observations led Brenner and co-workers [59,118] to suggest that impairment of renal growth with resulting fewer nephron counts could play a role in the development of hypertension. The role of a reduced nephron number in developmental programming of elevated BP has been the subject of recent review articles [119,120]. The mechanism by which altered environment in early life might result in decreased nephron number is not totally understood. Interestingly, factors implicated in impaired nephrogenesis include the perturbation in the expression of components of the RAS during nephrogenesis [121], exposure of the fetus to elevated levels of glucocorticoids at critical periods and mild vitamin A deficiency [122], which is an antioxidant. Studies demonstrate increased expression of key mitochondrial pro-apoptotic proteins [123] and increased apoptosis [119]. Even though the role of impaired kidney development is undeniable, programming of elevated BP can occur ‘despite’ the absence of significant changes in nephron number [63,124].

**Glucocorticoids**

Developmental programming of elevated BP has been associated with excess exposure to glucocorticoids during fetal life [125,126]. The glucocorticoid-inactivating enzyme 11β-HSD2 (type 2 isofrom of 11β-hydroxysteroid dehydrogenase), present in abundance in the placenta, is decreased in human pregnancies complicated by IUGR [127,128], as well as in pregnant rats fed an LP diet [129], allowing excess exposure of the fetus to maternal glucocorticoids [130]. In humans, fetal cortisol levels in IUGR are increased [131]. Dexamethasone (which is not inactivated by 11β-HSD2 and crosses the placenta)
administered to pregnant sheep or rat leads to increased BP in the adult offspring [132,133]. Elevated BP in LP offspring is abolished by maternal adrenalectomy and is restored by corticosterone replacement during pregnancy [134]. Prenatal excess of glucocorticoids modifies (for some permanently) the development of several organs, the most studied being the lung and the central nervous system [125]. Prenatal dexamethasone exposure or 11β-HSD2 inhibition permanently programmes the HPA (hypothalamic–pituitary–adrenal) axis and increases basal plasma corticosterone levels in adulthood [135,136].

In humans, adult plasma cortisol concentrations are inversely proportional to birthweight [137] and could, in turn, contribute directly to hypertension [138]. This mechanism could play a role in the microvascular rarefaction observed in LP offspring. Indeed, glucocorticoid-induced hypertension is associated with increased endothelial cell death and capillary structural rarefaction [139], in part through increased transcription of apoptotic mitochondrial proteins [140]. Excess exposure to maternal glucocorticoids is unlikely, however, to be the sole mechanism leading to increased BP associated with a modified intrauterine environment, as some studies failed to show an association between programmed elevated BP and alteration of the HPA axis [141,142].

RAS

The major tonic role of the RAS in programming, as well as maintaining programmed, elevated BP is clearly established; however, it is still debated whether it is a causal factor [143,144] or an early associated event [145,146]. In humans, cord blood renin activity and renal renin-containing cells are increased in growth-retarded newborns [147–149]. Studies in fetal sheep indicate that the role of the RAS in maintaining BP and in the pressor response to AngII infusion is greater in growth-restricted than in control animals [145,146]. Elevated BP of the adult LP offspring is normalized by both peripheral and central (see below) blockade of AngII effects [28,143,150]. Hypertension is prevented by blockade of AngII formation or losartan administration (AT1 receptor antagonist) from 2–4 weeks of age in LP offspring; this long-term effect upon BP is not observed when adult offspring are treated [143,144,151]. These observations suggest increased levels of AngII and/or AT1 receptor expression in these first weeks of life in LP-programmed animals. Indeed, circulating plasma renin activity and ACE (angiotensin-converting enzyme) levels are increased in 4-, as well as in 13-, week-old LP offspring [63,143].

In the kidneys, Woods et al. [152] have shown reduced renal renin and AngII levels in 1-day-old LP offspring. At 4 weeks of age, renal AT1 receptor protein expression (as determined by Western blotting and binding analyses) is increased in male LP offspring [153,154]. AT1 receptor mRNA expression is also reported to be increased at 4 weeks (gender not specified) [154] or unchanged in female LP offspring at 10 weeks of age [61]. A deficient or prematurely stimulated (by glucocorticoids for example) intrarenal RAS might result in a nephron number deficit and participate in elevation of BP in later life. Whether the circulating/vascular RAS presents a pattern of expression and activity similar to the renal expression (i.e. relative suppression in the immediate neonatal period, followed by overexpression by 4 weeks) remains to be demonstrated.

The developmental pattern and the underlying factors leading to enhanced vascular (carotid) AT1 receptor expression observed in adult LP offspring are unknown. One of the most potent elements affecting AngII receptor expression is AngII itself. In vitro and in vivo, AngII decreases vascular smooth muscle cell AT1 receptor expression [155,156]. Therefore, if the decreased renal AngII levels observed in the newborn also apply to circulating AngII, it could permanently programme enhanced vascular AT1 receptor expression. Factors leading to enhanced expression of AT1 receptor could also comprise corticosteroids [126–130,132,157–159]. An exaggerated response to AngII, along with increased expression of AT1 receptors, is observed in coronary arteries, but not in mesenteric arteries, of 4-month-old lambs with elevated BP associated with early antenatal dexamethasone exposure [23]; however, this enhanced constriction to AngII was not observed in the coronary arteries from 1-week-old dexamethasone-exposed lambs [68].

Brain

It is well known that cardiovascular-regulating areas in the brain participate in the maintenance of hypertension, mainly through overactivity of the brain RAS [160–162]. The brain RAS can increase BP through increased efferent sympathetic activity to the vessels and the kidneys (increasing renin secretion and sodium reabsorption) and modulation of the arterial baroreflex. The brain RAS is also up-regulated in models of developmental programming of elevated BP, such as the LP diet model and in sheep exposed to glucocorticoids early in gestation [28,163]. In these animals, BP is normalized by intracerebroventricular injection of an ACE inhibitor or AT1 receptor antagonist, and the arterial baroreflex control of heart rate is shifted towards higher pressure (without changes in gain or sensitivity). These observations are associated with increased expression of AT1 receptors in the medulla oblongata and forebrain structures involved in cardiovascular control [28,164].

Experimental evidences also support a role for the SNS in programmed elevated BP. In a cohort of 449 adults, a direct relationship was found between adult pulse rate and birthweight [165]. The authors concluded that, although the resting pulse rate is an imperfect index of activity of the SNS, elevated SNS activity established in utero may be one mechanism linking small size with increased BP in adult life. Enhanced SNS
activity is also suggested by the enhanced BP variability of adult LP rats [28], the fact that denervation of renal sympathetic activity normalizes BP of growth-restricted rats (associated with uterine artery ligation) [166] and the increased hypothalamic tissue levels of noradrenaline in adult IUGR female rats [13]. The mechanistic pathways leading to enhanced sympathetic activity most probably include the brain RAS, but whether this is the sole mechanism remains to be demonstrated.

**Oxidative stress**

The oxygen-derived molecules superoxide (O$_2^-$), H$_2$O$_2$, hydroxyl anion (OH$^-$), NO and peroxynitrite (ONO0$^-$) are biologically important through their redox potential and are involved in numerous pathways of cellular homoeostasis. For clarity purposes, these molecules will be grouped under ROS, realizing that this will also include reactive nitrogen species (NO and ONOO$^-$) [167]. When production of ROS overwhelms endogenous antioxidant defence mechanisms and is implicated in processes in which biological macromolecules are oxidized, such as DNA, proteins, carbohydrates and lipids [168], the condition is referred to as oxidative stress.

Accumulating evidence show that oxidative stress is present in, and most probably is a pathogenic element of, cardiovascular diseases, such as atherosclerosis, vascular dysfunction and vascular remodelling [167,169]. ROS are also considered to be a central factor in major complications of prematurity, including chronic lung disease, necrotizing enterocolitis, periventricular leukomalacia and retinopathy of prematurity [170]. Many adverse fetal and neonatal conditions are associated with oxidative stress or are pro-oxidant in nature, such as gestational hypertension and pre-eclampsia, gestational diabetes, smoking, clinical and subclinical infection or inflammation, malnutrition or overnutrition and prematurity [171–180].

In models of developmental programming, elevated BP and vascular dysfunction are characterized by enhanced vascular superoxide production generated, at least in part, by NADPH oxidase [24,26,29,63,66,71]. Indeed, the SOD mimetic tempol normalized the enhanced vasoconstriction to AngII in LP offspring, suggesting an increased vascular production of the superoxide anion [24]. This was supported by lucigenin-enhanced chemiluminescence revealing a marked increase in the aortic production of the superoxide anion in the presence of AngII only in the LP group. The nearly complete inhibition of superoxide production in the presence of apocynin indicated that NADPH oxidase is the main source of superoxide in LP offspring aorta, both in baseline conditions and after stimulation by AngII. These results are in agreement with many reports showing that, in adults with chronic hypertension, membrane-bound NADH and NADPH oxidases are the most significant source of ROS in the vascular wall [181,182] and that AngII enhances the vascular production of ROS (mostly superoxide) essentially through the activation of NADH and NADPH oxidases [183–185]. In another model of fetal programming of elevated BP, AngII increased superoxide production through NAPDH oxidase in mesenteric arteries [64]; however, this study did not examine the vasomotor response to AngII, but did show that apocynin normalized defective vasodilation to bradykinin and ACh.

The hypothesis that oxidative stress may be the initiating trigger for developmental programming of elevated BP is supported by the following experimental evidence. In a recent study [69], the ubiquitous cellular antioxidant glutathione was found to be decreased in the LP-exposed rat fetus, and the peroxidation inhibitor lazaroizid administered to the pregnant dams concomitantly with the LP diet prevented elevated BP and vascular dysfunction in the offspring. LP offspring also exhibited an increase in kidney nitrotyrosine staining early in life; treatment of the young rats with the SOD mimetic tempol for 3 weeks prevented the increased renal nitrotyrosine staining and elevated BP [186]. Supplementation of SHR dams with l-arginine (which favours formation of NO) and antioxidant vitamins during gestation and the early postpartum weeks resulted in a persistent reduction in BP in the adult offspring [187].

Increased oxidative stress early in life could also underlie postnatal microvessel rarefaction [63] through endothelial cell necrosis, enhanced apoptosis and/or perturbation of specific angiogenic factors [188–191]. The peroxidation product isoprostane causes greater microvessel vasoconstriction in young compared with adult animals [192]. A combination of vasoconstriction and endothelial cell damage leads to degenerative processes in terminal arterioles and capillaries, inducing anatomical rarefaction [193,194].

**Potential sources of ROS in the vasculature in relation to developmental programming of elevated BP**

Vascular ROS are produced in all cell layers and derive from NADPH oxidases, uncoupled NOS, xanthine oxidase and the mitochondrial electron transport chain [195].

As discussed above, vascular NADPH oxidase has been shown to be a major source of superoxide in some models of developmentally programmed elevated BP. Vascular NADPH oxidase is regulated by multiple factors, including humoral molecules and mechanical forces [195,196]. Of these factors, AngII appears to be one of the most important, via AT$_1$ receptor activation [197]. AngII can also increase NADPH oxidase expression [198].

NOS may contribute to superoxide generation in conditions where L-arginine or BH$_4$ (tetrahydrobiopterin), both essential for NO synthesis, are deficient [199]; NOS is then described as ‘uncoupled’. BH$_4$ corrected impaired endothelium-dependent vasodilation, enhanced
NO production and decreased superoxide generation in microvessels of adult offspring of dams subjected to a 50% reduction in their diet during gestation [200]. All NOS isoforms can become uncoupled, and iNOS (inducible NOS) appears to be particularly sensitive [201]. In SHRs, inhibition of iNOS during pregnancy and lactation reduced BP in the adult, whereas BH₄ supplementation in the adults decreased aortic superoxide production and expression of iNOS, suggesting a role specifically for iNOS uncoupling as a source of superoxide [187].

Mitochondria can be an important source of superoxide [167]. Reciprocally, mitochondria are also themselves targets of ROS, with damage to mitochondrial lipids, enzymes and DNA, leading to mitochondrial dysfunction and greater ROS production [202,203]. Studies have underlined the prevalence of vascular cells mitochondrial dysfunction in Type 2 diabetes, heart failure and hypertension [204–211]. Interestingly, there appears to be reduced mitochondrial DNA content in liver and muscle of adult LP offspring [20,212,213]. There is also reduced mitochondrial DNA content in kidneys and aorta of hypertensive adult offspring of high-fat diet-fed dams [214].

Potential role of impaired antioxidant capacity in developmental programming of elevated BP

Although the role of oxidative stress in cardiovascular diseases is well established, the protective role of antioxidant mechanisms remains poorly defined. During the last trimester of a normal pregnancy antioxidant enzyme levels increase. Preterm newborns are, therefore, relatively antioxidant-deficient when confronted with the massive surge in PO₂ (partial pressure of oxygen) at birth. Indeed, virtually all studies of premature, as well as growth-retarded, infants demonstrate lower and less inducible antioxidant defences, such as SOD, catalase and glutathione peroxidase [170,178,215–219]. Interestingly, gender differences prevail in the antioxidant capacity of premature babies [220–222], which would need to be correlated with the differential incidence of morbidities in newborns and in adults, as well as the differing mechanisms of hypertension programming between the genders [223]. Therefore the combination of decreased antioxidant capacity (through nutrient restriction [224,225], exposure to excess glucocorticoids [226–229] or simply because of prematurity) and increased exposure to ROS during a pathological pregnancy can increase the susceptibility of the newborn baby to further significant oxidative stress at birth when tissue PO₂ increases dramatically.

Epigenetic changes

One of the main criticisms of the concept of developmental programming of hypertension and adult cardiovascular diseases is that the association between low birthweight (which is the variable most studied) and high BP is in fact created by confounding factors among parental characteristics, such as low socioeconomic status or a specific genotype that causes both low birthweight and coronary heart diseases in the offspring [230]. Accordingly, some reports showing that complications of pregnancy linked to low birthweight are associated with an increased incidence of subsequent ischaemic heart disease in the mother have alluded to common genetic factors explaining the link between birthweight and risk of heart disease in both the individual and the mother [231]. To explain this, it has been argued that programming extends across generations through the oocytes [232–234], which are submitted to the same deleterious intrauterine environment while developing in the ovaries of the fetus, thereby programming the grandchildren of the current pregnant mother. However, this latter hypothesis could support the observations made in an F2 but not in an F3 generation. Another possibility is that the programmed mother, who also has vascular dysfunction, will provide a deprived intrauterine environment to her offspring, thus perpetuating the cycle of fetal (mal)adaptation. Pregnant dams which were themselves exposed to an LP diet during fetal life have decreased endothelium-dependent vasodilation of their uterine and mesenteric arteries, via a reduction in NO production [26]. As mentioned previously, low birthweight and premature girls appear to be at increased risk of pregnancy-related complications, such as gestational hypertension, pre-eclampsia and gestational diabetes [57,235–238]; their own offspring therefore suffering from, and consequently might be programmed by, a deleterious intrauterine environment.

An alternative possibility is that epigenetic modification of the germ line by stable methylation caused by the prenatal exposure will transmit the prenatal experience of one generation to future generations. Although epigenetic modifications are proposed in the literature as probable mechanisms of cardiovascular and metabolic programming, there are relatively few findings to date to support this possibility. Many candidate factors and confirmed players able to induce developmental programming of elevated BP are also known to be able to modify gene methylation. Rees et al. [239] have shown hypermethylation in the liver of fetuses from LP-fed dams. PPARα (peroxisome-proliferator-activated receptor α) and glucocorticoid receptor genes are hypomethylated and their expression increased in the liver of young LP offspring [240]. More recently, Bogdarina et al. [241] have reported significant undermethylation of one element of the RAS, a promoter of the AT₁b receptor subtype, associated with its increased expression in the adrenal glands of LP offspring.

DNA methylation is greatest during fetal life. Glucocorticoids have a demethylating action [242], which might modulate expression of genes involved in vascular structure and functional development. ROS can modify methylation leading to changes in gene transcription and protein expression [243–245]. Glycine and its metabolites...
are precursors for the synthesis of nucleotide bases and methylation of DNA. It also is a component in the composition of collagen and the antioxidant glutathione. Glycine exerts a protective role against methionine and homocysteine; a lack of glycine leads to increased levels of methionine and homocysteine, which will ultimately influence DNA methylation. The potential importance of these pathways has been suggested by studies showing that supplementation of an LP-fed dam with glycine prevented elevated BP and impaired ACh vasodilation in the adult offspring [246,247].

Epigenetic changes, in contrast with genetic changes, are potentially reversible. Thus this introduces the concept of change to the fixed world of genetics and offers a hope of intervention to remove deleterious epigenetic modifications. There is some evidence that epigenetic programming by early life events is reversible by pharmacological manipulations later in adulthood [248–250]. This challenges the notion that these changes are induced during fetal life and are immutable thereafter; it is critical to take this possibility into account when interpreting epigenetic changes observed in animal models of developmental programming of hypertension or when making associations in humans between perinatal factors and epigenetic changes of key genes.

CONCLUSIONS

As with many complex diseases, one cannot presume a unifying pathway linking perinatal environment to hypertension and vascular dysfunction in later life. Many mechanisms explored to date still require further studies. The ontogeny of the circulating compared with renal compared with brain RAS and the factors modulating their expression need to be established. The role of an oxidative insult as the triggering element and the role of a possible impaired antioxidant capacity also need to be clearly demonstrated. Identification of epigenetic changes that could be caused by perinatal insults is a field vastly under explored. Importantly, the mechanisms by which ante- and/or post-natal nutritional perturbation can ultimately lead to elevated BP and vascular dysfunction are very poorly understood. The interaction of these mechanistic pathways also needs to be established. Finally, all studies should carefully take into consideration gender differences that might prevail. A better understanding of those mechanisms is critical before clinical trials of preventive or ‘deprogramming’ measures can be designed.

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130 Levitt, N. S., Lindsay, R. S., Holmes, M. C. and Seckl, J. R. (1996) Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. Neuroendocrinology 64, 412–418


191 Sirinyan, M., Sennlaub, F., Dorfman, A. et al. (2006) Hyperoxic exposure leads to nitrosative stress and ensuing microvascular degeneration and diminished brain mass and function in the immature rat. Stroke 37, 2807–2815


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