Developmental programming of cardiovascular disease by prenatal hypoxia

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It is now recognized that the quality of the fetal environment during early development is important in programming cardiovascular health and disease in later life. Fetal hypoxia is one of the most common consequences of complicated pregnancies worldwide. However, in contrast to the extensive research effort on pregnancy affected by maternal nutrition or maternal stress, the contribution of pregnancy affected by fetal chronic hypoxia to developmental programming is only recently becoming delineated and established. This review discusses the increasing body of evidence supporting the programming of cardiac susceptibility to ischaemia and reperfusion (I/R) injury, of endothelial dysfunction in peripheral resistance circulations, and of indices of the metabolic syndrome in adult offspring of hypoxic pregnancy. An additional focus of the review is the identification of plausible mechanisms and the implementation of maternal and early life interventions to protect against adverse programming.

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Developmental programming of cardiovascular disease

Heart disease is the greatest killer in the world today, imposing a staggering burden on every nation’s health and wealth.1 Worldwide, it results in one in three deaths per year. Moreover, the economic costs are tremendous for treatment, patient care and lost workforce amounting to over £30 billion per year in the United Kingdom alone2 and over US$130 billion/year in Canada and the United States.3,4 Therefore, there is no question that cardiovascular disease is an important as well as an expensive problem to resolve. The concept that traditional lifestyle risk factors, such as smoking and obesity, interact with our genetic makeup to determine a risk of cardiovascular disease is well accepted.5 However, only comparatively recently, it has become appreciated that the interaction between our genes and the quality of the environment during early development may be just as, if not more, important in programming cardiovascular health and disease in later life.6,7 This additional concept of developmental programming of disease is supported by overwhelming evidence derived from human studies now dating back more than two decades and encompassing six continents; evidence that strongly links development under sub-optimal intrauterine conditions with fetal growth restriction, low birth weight and increased rates of coronary heart disease and the metabolic syndrome in adulthood.8–14 It is generally accepted that the more immature the individual, the greater the influence the environment will have on it. Therefore, the impact of the environment in the programming of physiology is greatest in early life and decreases from embryonic to fetal to postnatal and adult life. Similarly, the opportunity for correction of a change in this developmental programme follows a similar trajectory, drastically diminishing from early life to adulthood. Hence, the concept of programming of disease creates an exciting window of opportunity to diagnose and halt the development of heart disease at its very origin, bringing preventative medicine back into the womb, or to treat postnatally as soon as possible after diagnosis to diminish and control the progression of disease. However, the mechanisms underlying developmental origins of cardiovascular dysfunction remain elusive, precluding the identification of potential clinical therapy.

Developmental programming by different suboptimal conditions during pregnancy

Epidemiological studies relating the type of suboptimal intrauterine condition with physiological dysfunction in later life have largely focussed on human populations undergoing alterations in maternal nutrition, or on human pregnancy affected by maternal psychological stress or by exposure to stress hormones.15–17 This focus on the nutrient supply to the fetus or on materno-fetal stress in humans is supported by a large number of investigations in experimental animal models demonstrating that cardiovascular dysfunction in adulthood can be programmed in pregnancy by inappropriate nutrition or by exposure to glucocorticoid excess.7,18,19 In addition to
alterations in maternal nutrition and maternal stress, fetal hypoxia is one of, if not the most common consequence of complicated pregnancy worldwide (see Koos). Further, over 140 million people live at altitudes higher than 2500 m where lowered oxygen availability has been shown to reduce fetal growth and birth weight, thereby comprising the largest single human group at risk for fetal growth restriction. However, in contrast to the international research effort on pregnancy affected by nutrition or glucocorticoid exposure, the contribution of fetal chronic hypoxia to developmental programming has only recently become delineated and established.

The fetal cardiovascular defence to hypoxia

The fetal cardiovascular responses to short-term episodes of acute hypoxia include a redistribution of the cardiac output away from peripheral circulations to maintain perfusion to the brain. This redistribution of blood flow is aided by peripheral vasoconstriction, the latter significantly also decreasing oxygen consumption in less essential vascular beds. The physiology underlying this circulatory response is well established and involves the activation of a selective carotid body chemoreflex. Once initiated, the neurally triggered vascular response is maintained by the release of constrictor agents into the fetal circulation, such as catecholamines, vasopressin and neuropeptide Y. More recently, it has become clear that these neuroendocrine constrictor influences on the fetal peripheral circulation can be further influenced by the release of local vascular agents during acute hypoxia. Studies have shown that nitric oxide (NO) bioavailability in the fetus can increase during acute hypoxia. Further, increased generation of reactive oxygen species (ROS), such as the superoxide anion (O$^\cdot_2$), during acute hypoxia can interact with NO, providing an oxidant tone to the fetal vasculature. Therefore, the fetal peripheral vasoconstrictor response to acute hypoxia is an aggregate of carotid chemoreflex activation, humoral constrictor influences and a local oxidant tone determined by the ratio of NO: O$^\cdot_2$ in the fetal circulation.

There is now a considerable evidence that this homeostatic circulatory defence to hypoxia is maintained, should the duration of the hypoxic challenge persist. In response to fetal chronic hypoxia, the maintained redistribution of blood flow away from peripheral circulations can become maladaptive, triggering a number of unwanted side effects in the fetus. The most described consequence is asymmetric fetal growth restriction, yielding offspring whose brain and heart growth is spared, but having bodies that are thin for their length with a low ponderal index. In addition, sustained increases in fetal peripheral vascular resistance because of sustained prenatal hypoxia will increase fetal arterial blood pressure if cardiac output is maintained. An increase in fetal cardiac afterload may trigger changes in the morphology and function of the fetal heart. In turn, remodelling of the walls of the fetal aorta may occur in response to the greater pressure generated by the fetal heart. Therefore, depending on the timing, duration and severity of the challenge, chronic fetal hypoxia is not only an immediate threat to fetal survival, but it is also an important environmental influence triggering intrauterine growth restriction (IUGR) and the developmental programming of cardiovascular disease.

Chronic hypoxia and fetal origins of cardiovascular disease

Over the years, various animal models have been created to induce adverse intrauterine conditions. Many of these techniques have involved impairing utero-placental perfusion, which reduces nutrient as well as oxygen delivery to the fetus, and these studies have been the subject of several excellent reviews. Other studies have concentrated on determining the contribution of fetal chronic hypoxia alone in promoting fetal growth restriction and early origins of cardiac and peripheral vascular dysfunction; it is these studies that will be the focus of this review.

A cluster of research groups have employed the chick embryo model, which isolates the effects of chronic hypoxia on fetal growth and the developing cardiovascular system independent of effects on the maternal and placental physiology. It is now established that exposure of the chick embryo to chronic hypoxia promotes asymmetric fetal growth restriction, cardiac and aortic hypertrophic growth, altered cardiac function and sympathetic hyper-innervation of peripheral resistance arteries by the end of the incubation period. The asymmetric growth restriction and cardiac and aortic wall remodelling that develops in sea level chick embryos incubated at high altitude no longer occurs in sea level embryos incubated at high altitude with oxygen supplementation, underlying the direct effects of isolated chronic hypoxia on fetal growth and cardiovascular development. Asymmetric growth restriction, aortic wall thickening, cardiac and vascular dysfunction have also been reported in the chronically hypoxic fetus of mammalian species, such as in sheep, rodents and guinea pigs. Fetal aortic wall thickening is particularly relevant in the clinical setting, as increased large artery stiffness independently predicts cardiovascular risk in humans, being a key component in the aetiology of hypertension, atherosclerosis and coronary heart disease. In the aorta, in particular, an increase in wall thickness has been proposed as the first physical sign in the development of atherosclerosis. Aortic pulse-wave velocity measurements, rather than systolic blood pressure measurement, better predict later cardiovascular disease, including impaired coronary artery flow and left ventricular dysfunction. A comprehensive series of studies by Gilbert and colleagues in fetal sheep subjected to high altitude from day 30 of gestation to term (ca. 145 days) reported that in the chronically hypoxic ovine fetus, cardiac output was decreased secondary to a decrease in myocardial cell contractile function. The intracellular mechanisms responsible for these reductions included reduced myofibrillar Mg$^{2+}$-activated
ATPase and a decrease in β1-adrenergic receptor stimulated influence on myocardial contraction. An overproduction of cAMP by β1-adrenergic receptor stimulation, promoting over-phosphorylation of troponin I may also contribute to reduced calcium binding by troponin C. Similarly, Sharma et al. reported that chronic hypoxia in the chick embryo decreased maximum ventricular + dP/dt and peak pressure, increased ventricular end-systolic volume, and decreased ventricular ejection fraction, consistent with depressed systolic function. In the same study, it was shown that arterial afterload increased and steady-state hydraulic power decreased in response to hypoxic incubation. Four separate human clinical studies have now reported that babies born from pregnancies complicated by placental insufficiency show aortic thickening with increased vascular stiffness and reduced distensibility. Additional reported abnormalities in cardiovascular morphology and function of the human IUGR fetus include an increase in relative heart weight and ventricular wall hypertrophy, a decrease in ventricle and myocyte volume, and compromised biventricular ejection force and diastolic filling. Since fetal hypoxia alone can trigger fetal aortic wall thickening and fetal cardiac dysfunction in experimental animal models, a reasonable assumption is that fetal hypoxia alone may be responsible for triggering similar cardiovascular defects in the human fetus in pregnancies complicated by placental insufficiency.

**Chronic hypoxia and programming cardiovascular disease in adulthood**

With an established contribution of chronic hypoxia to a fetal origin of cardiovascular dysfunction, experimental research has expanded to determine the long-term consequences of developmental hypoxia on adult health. Investigation has focussed on the effects of chronic prenatal hypoxia in programming cardiac dysfunction, alterations in peripheral vascular reactivity and indices of the metabolic syndrome. Several studies in rats have now reported cardiac dysfunction and an increased susceptibility to an episode of ischaemia and reperfusion (I/R) injury in hearts isolated from adult offspring of hypoxic pregnancy, particularly in males and in those fed an obesogenic diet postnatally. Zhang and colleagues, in an elegant series of investigations, linked the reduced expression of cardio-protective genes, such as protein kinase C epsilon (PKCe) with programming an increased cardiac susceptibility to I/R injury in male offspring, as not only was the expression of PKCe reduced in hearts of hypoxic offspring, but treatment of hearts from adult offspring of normoxic pregnancy with a PKCe translocation inhibitor mimicked the defects in hearts of offspring from hypoxic pregnancy. A later study by the same group demonstrated that the mechanism via which hypoxic pregnancy caused heightened offspring cardiac susceptibility to I/R injury was epigenetic, reporting both an increase in the promoter methylation and the reduced expression of the PKCe gene in fetal pup hearts of hypoxic pregnancy, and the prevention of both effects by treatment with a DNA methylation inhibitor. In addition to an increased risk of I/R injury, diastolic dysfunction and sympathetic dominance appear to be variables common to this cardiac phenotype in offspring of hypoxic pregnancy. Davidge and colleagues also demonstrated that adult offspring of hypoxic pregnancy have several cardiac structural and functional changes including increased expression of collagen type I and III and altered β/α myosin heavy chains ratio along with in vivo evidence of elevated left ventricular end diastolic pressure (LVEDP). Giussani et al. additionally reported reciprocal changes in β1-adrenergic and muscarinic receptor responsiveness in hearts from rat adult offspring of hypoxic pregnancy. Both effects are of further clinical relevance, as elevated LVEDP is associated with increased mortality, and sustained increases in myocardial contractility due to heightened sympathetic excitation and diminished parasympathetic reactivity have been strongly associated with cardiovascular disease and eventual heart failure in humans.

A significant number of studies by various groups in different species have also now reported that the adverse effects of developmental hypoxia on the offspring peripheral vasculature persist into adulthood, expressing themselves as endothelial dysfunction and the emergence of a peripheral vasoconstrictor phenotype. Ruijtenbeek et al. first reported that isolated femoral arteries of adult chickens following hypoxic incubation were more sensitive to electrical stimulation and pharmacological stimulation of peri-arterial sympathetic nerves, while showing reduced NO-dependent vasorelaxation. The developmental programming of NO-dependent endothelial dysfunction in peripheral resistance circulations has now been confirmed in adult offspring of mammalian species by the groups of Davidge and Giussani. Interestingly, two reports have shown a significant inverse relationship between low birth weight and endothelial dysfunction in children in the first decade of life and in early adulthood. In contrast to effects on cardiac and vascular function, studies addressing the developmental programming of indices of the metabolic syndrome by prenatal hypoxia have been restricted to three reports. Camm et al. provided molecular evidence linking developmental hypoxia to impaired hepatic and muscle insulin signalling in adult rats, suggesting that Akt may represent a pharmaceutical target for clinical intervention against the developmental programming of metabolic disease resulting from prenatal hypoxia. The Davidge and Dyck laboratories reported that when combined with a postnatal obesogenic diet, adult offspring of hypoxic pregnancy showed a relative increase in intra-abdominal fat deposition and adipocyte size, an increase in fasting plasma concentrations of leptin, triglyceride and free fatty acids, and an increased concentration of triglycerides and...
Ceramides in both liver and skeletal muscle. These changes in lipid homeostasis were accompanied by in vivo insulin resistance and impaired glucose tolerance, effects that were also associated with decreased activation of Akt in the liver and skeletal muscle in response to insulin.

**Intervention against programming of cardiovascular disease by prenatal hypoxia**

The mechanisms via which developmental hypoxia programmes cardiovascular and metabolic diseases remain uncertain, slowing the development of clinical therapy. Several groups have raised the hypothesis that programming of cardiovascular disease by adverse developmental conditions may be secondary to oxidative stress. Giussani et al. tested this hypothesis in relation to developmental programming by prenatal hypoxia with the first interventional study using antioxidants. The work reported that chronic prenatal hypoxia, leading to a significant increase in fetal haematocrit, promoted fetal aortic wall thickening and oxidative stress in the fetal heart and vasculature by the end of gestation. By adulthood, these effects resolved but prenatal chronic hypoxia set a functional deficit in both the heart and the peripheral circulation. Maternal treatment with vitamin C during pregnancy prevented the adverse effects in fetal offspring and reversed the enhanced myocardial contractility due to sympathetic dominance and the NO-dependent endothelial dysfunction in peripheral resistance vessels in adult offspring (Fig. 1). In that study, it was of additional interest to note that the balance of redox modulation of vascular tone, imposed by the $\text{O}_2^\bullet^{-}$:NO ratio, may be tipped in either direction to promote disequilibrium, as maternal treatment with vitamin C in normoxic pregnancy also promoted endothelial dysfunction (Fig. 1c). Maternal antioxidant supplementation may therefore only restore the offspring vascular dysfunction in pregnancy conditions associated with increased $\text{O}_2^\bullet^{-}$ generation and vascular oxidative stress, such as during chronic prenatal hypoxia. Conversely, antioxidant treatment in healthy conditions where the offspring vascular physiology is already replenished with an appropriate redox balance may, in fact, lead to excess NO bioavailability, tipping the balance in the opposite direction. Excess NO bioavailability is known to promote peroxynitrite generation, thereby triggering mechanistic side effects resembling those of vascular oxidative stress. The implications of these data are that maternal treatment with antioxidants may provide possible therapy against the programming effects on vascular dysfunction in complicated pregnancy, however, they clearly show that excessive vitamin C supplementation in healthy pregnancy is potentially dangerous. Patterson et al. confirmed a role for prenatal hypoxia-derived oxidative stress in programming cardiac dysfunction, reporting that maternal treatment in rats with another antioxidant, N-acetyl-cysteine, inhibited the hypoxia-induced increase in methylation of the SP1-binding sites, reversed the decreased SP1 binding to the PKCɛ promoter, restored PKCɛ mRNA and protein abundance and abrogated the hypoxia-induced increase in susceptibility of the heart to ischaemic injury in adult offspring. Recently, they have further reported that noradrenaline causes the epigenetic repression of the PKCɛ gene in rodent hearts by activating Nos1-dependent ROS production. Therefore, it is possible that programming of developmental hypoxia of a sympathetic dominant cardiac

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**Fig. 1.** Cardiovascular effects of chronic developmental hypoxia with and without maternal antioxidant treatment on fetal and adult offspring. Effects on fetuses at the end of gestation (top row): (a) individual examples of fetal aortic sections; (b) mean ± S.E.M. of the fetal aortic wall-to-lumen area; (c) fetal aortic nitrotyrosine staining, index of peroxinitrite generation; (d) fetal cardiac expression of HSP70, index of cardiac stress. Effects on adult offspring at 4 months of age (bottom row): (e) dilator response to methacholine expressed as overall area under the curve (AUC) in isolated femoral arteries using in vitro wire myography. Black histogram represents the AUC of the NO component and white histograms of the NO-independent component; (f) myocardial contractility of isolated heart in Langendorff preparation; (g and h) chronotropic responsiveness to muscarinic and β1-agonists, respectively. Groups are all $n = 8$ for normoxia (N), hypoxia (H), hypoxia with vitamin C (HC) and normoxia with vitamin C (NO). * v. N or all; † H v. HC ($P < 0.05$, ANOVA). Modified from Giussani et al.
phenotype is mediated by catecholamine-induced ROS, which in turn causes the epigenetic repression of cardio-protective genes, such as PKCδ, thereby enhancing future cardiac susceptibility to I/R injury in adult offspring. Accordingly, treatment of fetal hearts isolated from hypoxic pregnancy with a selective PKCδ activator peptide ε-eRACK, markedly improved their recovery from I/R injury.39 Hashimoto et al.117 have reported that treatment with N-acetyl cysteine of pregnant guinea pigs also inhibited the adverse effects on the fetal liver of chronic prenatal hypoxia.

It is becoming clear that enhanced oxidative stress during adverse intrauterine conditions may have a significant impact on circulations, which are highly dependent on NO, such as the utero-placental vascular bed, promoting an increase in placental vascular resistance, thereby leading to slowing of fetal growth and a reduction in birth weight. In support of this idea, it has been shown in ovine and rodent species that maternal treatment with antioxidants enhances viability and birth weight in hypoxic pregnancy.118–120 Two other studies have shown that maternal supplementation with melatonin protects against IUGR in a rodent and ovine model of undernourished pregnancy.121,122 Stanley et al.123,124 have further reported that maternal treatment with sildenafil or tempol can rescue pup growth and improve abnormal uterine and umbilical Doppler waveforms in different knockout mice models of IUGR. The protective effects of antioxidants on fetal growth in adverse pregnancy are likely secondary to replenished NO bioavailability and improved NO-mediated umbilical perfusion, as treatment of chronically instrumented late gestation fetal sheep with either melatonin or vitamin C produced a significant increase in umbilical blood flow and vascular conductance and in vivo blockade of NO prevented the vasodilator effect.31

Rescue against programming of cardiovascular disease by prenatal hypoxia

While converging evidence from several laboratories points to an important role of oxidative stress and decreased NO bioavailability during hypoxic pregnancy in promoting fetal growth restriction and programming cardiovascular and metabolic dysfunction in adult life, diagnosis of fetal hypoxia and antenatal treatment with an appropriate antioxidant in a timely efficient manner may prove difficult. Therefore, the focus of research has shifted to considering how to rescue the low birth weight infant. Importantly, therapeutics for individuals diagnosed with IUGR are completely lacking. Early intervention is a potential opportunity to prevent the future development of metabolic and cardiovascular diseases, however, ethical issues exist with interventions in paediatric populations. One approach that has been raised is an intervention using Resveratrol (Resv, 97, 111), which is a natural polyphenolic antioxidant produced by plants in response to environmental stress and has demonstrated protective effects against stress and disease.125 Although there are multiple mechanisms of action for Resv, it is known to activate AMP-activated protein kinase (AMPK) as well as having antioxidant properties. AMPK is a protein kinase pathway that is involved in the control of oxidative metabolism and lipid homeostasis as well as decreasing fatty acid and triacylglycerol synthesis. Activation of AMPK in skeletal muscle also improves glucose uptake independent of insulin. Resveratrol has been shown to protect against the development of diet-induced insulin resistance in aged rodents.126 The Davidge and Dyck laboratories111 have demonstrated that early postnatal administration of Resv in the diet of weaning rats prevented features of the metabolic syndrome that are observed in offspring born from hypoxic pregnancy and fed a high fat diet. In addition, Resv prevented the diet-induced increase in plasma lipids and reduced the abdominal fat mass (Fig. 2). Moreover, Resv reduced the susceptibility to diet-induced metabolic alterations in glucose disposal and insulin resistance. The data suggest that this may be attributed to insulin sensitization of the peripheral tissues through the prevention of impaired Akt signalling as well as by activation of AMPK, thereby improving glucose utilization via an insulin-independent mechanism. In addition, postnatal administration of a fat-rich diet has been shown to be particularly deleterious to cardiac function of offspring exposed to a prenatal hypoxic insult; Resv reduced this enhanced cardiac susceptibility97 (Fig. 2). Thus, intervention in a vulnerable paediatric patient population may reduce the risk to developing an adverse metabolic phenotype and prevent long-term cardiovascular susceptibility to disease. Additional studies are necessary to address the impact of interventions (either with Resv or other approaches) on aortic alterations and the sympathetic dominant phenotype as discussed earlier in this review.

Conclusions and considerations

It is now overwhelmingly clear that pregnancy complicated by fetal hypoxia can programme long-term adverse consequences on the cardiovascular health of the offspring in adult life. Interestingly, in some cases, the adverse programmed cardiovascular and metabolic phenotypes may only become evident when the offspring are exposed to additional stressors such as an obesogenic diet or ageing. Other important considerations are differences in susceptibility to disease between the sexes. While some adverse phenotypes in the adult offspring in response to prenatal hypoxia are consistent between males and females, it is important to note that sexual dimorphisms in the long-term effects of prenatal hypoxia on the cardiovascular system are evident, whereby female offspring can exhibit some degree of protection.79 Therefore, the mechanism through which developmental insults can be modulated by sex differences is an important consideration when developing therapeutic strategies. Furthermore, the projected prevalence of cardiovascular pathophysiology in the global population may be grossly underestimated. We speculate that in the female offspring population of hypoxic pregnancy, particularly of reproductive age, programmed adverse cardiovascular and metabolic phenotypes may become significantly exacerbated when challenged.
by the stress of their own pregnancy. Other sensitizers to the projected prevalence of cardiovascular disease clearly include the intergenerational programming of cardiovascular pathology by prenatal hypoxia. Offspring of hypoxic pregnancy may yield offspring programmed with an increased risk of cardiovascular pathology even following normoxic pregnancy. Additional insight to mechanisms to define early interventions in pregnancy complicated by fetal hypoxia will reduce the burden not only of IUGR, but also of developmental origins of cardiovascular disease, thereby having a major clinical, economic and social impact on health.

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Statement of Interest
None.

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