Chapter 12

Early Life Stress and Predisposition to Cardiovascular Disease

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Abstract

Cardiovascular disease (CVD) is a major health concern globally, as it is the greatest cause of death, produces substantial long-term morbidity and brings enormous humanitarian and economic cost. No longer thought to result from a combination of genetic predisposition and an unhealthy adult lifestyle, CVD prevalence is rising rapidly in developing societies. Recent research has revealed how CVD risk can be set in early life, particularly as a result of an unbalanced maternal diet, materno-fetal exposure to excess glucocorticoids or reduced oxygenation, risks that are amplified across the lifecourse in terms of reduced compensatory responses to later challenges. Developmental challenges, transduced by the mother and the placenta, can be viewed as stressors of the developing offspring’s physiological phenotype, even though they may operate within the normal range of the developmental environment. Such stressors induce integrated responses which modulate the offspring’s cardiovascular phenotype to maximise potential later fitness but can increase CVD risk, especially with unhealthy lifestyle and ageing. More severe challenges disrupt development. We discuss the processes underlying the adaptive responses, with particular emphasis on the interaction between blood flow and tissue and organ growth, and the role of reactive oxygen species in mediating these effects. At the molecular level, epigenetic processes by which environmental stressors can affect gene expression and alter offspring phenotype without changing the fixed genetic sequence, are thought to underlie the adaptive processes.

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These new insights hold promise for the early detection of individuals and groups at greater risk of later CVD and for devising and monitoring early life interventions to reduce this risk.

**Keywords:** Cardiovascular disease, epigenetic, glucocorticoids, maternal diet, hypoxia, reactive oxygen species, stress

**Introduction**

Cardiovascular disease is the greatest killer in the world today, imposing a staggering burden on every nation’s health and wealth (World Health Organization, 2012). Worldwide, 1 in 3 people die of heart disease per year. Furthermore, the economic costs are tremendous in terms of treatment, family income and workforce productivity, amounting to over £30 billion in the United Kingdom (European Heart Network, 2012) and well over US$100 billion in the United States per year (Heidenreich et al., 2011). Therefore, to ameliorate the increasing clinical, social and economic burden of treating cardiovascular disease once established, there is an urgent need to understand the science behind its earliest origin to give possible insight into preventative therapy.

Traditionally, it was widely believed that individual variations in genetic makeup interact with adult lifestyle risk factors, such as smoking, obesity and lack of exercise to determine the risk of cardiovascular disease (Agarwal et al., 2005). Only comparatively recently, has it also become widely established that gene-environment interactions during early life may be just as, if not more important in predisposing to later cardiovascular dysfunction (Barker, 1994, Gluckman et al., 2008). Different types of environmental stressors during early life may induce pathological changes in important organs and systems, such as in the heart and the circulation that persist until, or are amplified, in later life, expressing themselves as dysfunction in adulthood. Alternatively, early life environmental stressors may alter adaptive responses to later challenges such as an unhealthy lifestyle, making physiological changes maladaptive, and this can progress to overt cardiovascular disease. It is also clear that exposure to stress during different critical time windows of development, including the pre-conceptional, peri-conceptional, embryonic, fetal and neonatal periods can increase the risk of later cardiovascular dysfunction, that the magnitude and duration of the developmental stress can modify the time of onset and severity of later cardiovascular disease, that developmental stress can affect later cardiovascular function in men and women differentially, and that phenotypes with an increased risk of cardiovascular disease can be intergenerational, being passed on from parent to offspring. Therefore, there is no question regarding the importance and far-reaching implications that the early environment has in shaping our cardiovascular health and that of our children and their offspring. Likewise, it is now becoming obvious that the opportunity for correction of a change in the developmental trajectory moulding our cardiovascular health diminishes drastically from early life to adulthood. Hence, the concept of developmental origins of disease creates an exciting opportunity to halt the development of heart disease at its very origin, through preventative medicine in the womb and even prior to conception, or by instituting interventions post-natally following a pregnancy which conveyed high risk to the offspring in order to diminish and control the progression of disease. The mechanisms underlying developmental origins of cardiovascular dysfunction
following development complicated by stress are beginning to emerge, making potential clinical therapy with rational interventional strategies a realistic possibility in the near future.

**Stress in Early Life and Risk of Cardiovascular Disease in Later Life: Evidence from Human and Animal Studies**

The concept of developmental origins of cardiovascular disease is supported by overwhelming evidence derived from human studies now dating back nearly four decades and encompassing six continents; evidence that strongly links development under sub-optimal environmental conditions with alterations in fetal and/or postnatal growth and increased rates of coronary heart disease and the metabolic syndrome in adulthood (Barker et al., 1989, Fall et al., 1998, Forsdahl, 1977, Forsén et al., 1999, Gennser et al., 1988, Huang et al., 2012, Leon et al., 1998, Levitt and Lambert, 2002, Li et al., 2010, Rich-Edwards et al., 1976, Roseboom et al., 1999, Silva et al., 2012). Early evidence that linked phenotypic changes mediated by developmental plasticity with chronic cardiovascular disease in humans in the UK and the US stemmed from the association between coronary heart disease, type 2 diabetes, or hypertension in adult life and low birth weight (Barker et al., 1989, Rich-Edwards et al., 1976). Similar evidence has since been obtained in Sweden (Leon et al., 1998), Finland (Forsén et al., 1999), India (Fall et al., 1998), Australia (Huang et al., 2012), Africa (Levitt and Lambert, 2002), China (Li et al., 2010) and Latin America (Silva et al., 2012). Epidemiological and clinical studies have also related different types of suboptimal early environmental conditions not only with alterations in growth but also with physiological dysfunction in later life. For instance, there have been studies of human development affected by maternal psychological stress or by exposure to stress hormones during pregnancy or of human populations or cohorts undergoing substantial nutritional challenges (Dalziel et al., 2005, Eskenazi et al., 2007, Roseboom et al., 1999, 2011). Roseboom and colleagues (Roseboom et al., 1999) reported findings from a large cohort of middle-aged men and women, born as term singletons around the time of the Dutch famine between 1944 and 1945. These data not only revealed an association between maternal under-nutrition and adult-onset cardiovascular disease in the offspring, but the results showed that the prevalence of coronary heart disease was best related to exposure to famine in early gestation while impaired glucose tolerance was related to exposure to famine in late gestation (Roseboom et al., 2011). Furthermore, impaired maternal diet during pregnancy could predispose men but not women to later hypertension (van Abeelen et al., 2011) and offspring of prenatally undernourished fathers but not mothers during the Dutch famine were heavier and more obese than offspring of parents who had not been undernourished prenatally (Veenendaal et al., 2013). Therefore, the Dutch famine cohort provide an example of data derived from human studies linking nutritional stress during early life with later cardiovascular dysfunction in the offspring, with time of onset, sexually dimorphic as well as intergenerational effects.

Complementing the human data, a large number of investigations in experimental animal models provide robust evidence linking different types of stress in early life with increased risk of cardiovascular disease in adulthood. These data from animal models emphasise similar considerations to the human data: they reveal effects of timing, magnitude and duration of the
stress, varying outcomes in male and female offspring and transmission of phenotypic traits between generations. Several groups have reported that fetal exposure to under- or over-nutrition, or to inappropriate concentrations of glucocorticoids can all predispose the offspring to alterations in cardiac structure and function, to endothelial dysfunction in peripheral resistance circulations and to hypertension in adult life (for reviews, see Braun et al., 2013, Cripps et al., 2007, Desai et al., 2013, Galjaard et al., 2013, Gluckman et al., 2005, McMillen and Robinson, 2005, Seckl and Meaney, 2004). For example, Seckl and colleagues (Seckl, 2001) and Langley-Evans and colleagues (Langley-Evans, 1997) have used maternal treatment with carbenoxolone to inhibit placental inactivation of maternal glucocorticoids, allowing passage to the fetal circulation in rats. Invariably, such experiments have produced IUGR, hypertension and cardiovascular dysfunction in adult offspring. Pregnancies affected by maternal stress lead to preterm onset of labour (Hoffman and Hatch, 1996). Both exposure to maternal stress and prematurity have been linked with increased susceptibility of cardiovascular disease in later life (Roggero et al., 2013). We and others have also shown that exposure to synthetic steroids in early life promotes endothelial dysfunction and cardiac remodelling with associated dysfunction in later life (Adler et al., 2010, Bal et al., 2008, Herrera et al., 2010, Niu et al., 2013). These effects are of additional clinical importance because pregnancies threatened with preterm labour and/or premature infants are routinely treated with synthetic glucocorticoids to accelerate fetal lung maturation and prevent the development of chronic lung disease (Crowley, 2006, Halliday et al., 2010, Liggins and Howie, 1972).

In addition to alterations in maternal nutrition and maternal stress hormones, fetal hypoxia is one of, if not the most common consequence of complicated pregnancy (Hutter et al., 2010). Further, over 140 million people live at altitudes higher than 2500 m where the lower oxygen availability has been shown to reduce fetal growth and birth weight (Giussani et al., 2001, Moore et al., 2011), thereby comprising the largest single human group at risk for fetal growth restriction and/or early origins of cardiovascular disease. A cluster of research groups have employed the chick embryo as a model to isolate the effects of chronic hypoxia on fetal growth and the developing cardiovascular system independent of effects on the maternal and placental physiology. In the chick, chronic hypoxia promotes 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 (Giussani et al., 2007, Lindgren and Altimiras, 2009, 2011, Rouwet et al., 2002, Ruijtenbeek et al., 2003a, Salinas et al., 2010, Sharma et al., 2006, Tintu et al., 2007, 2009, Villamor et al., 2004). The 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 (Giussani et al., 2007, Salinas et al., 2010), underlying the direct but not necessarily linked effects of isolated chronic hypoxia on fetal growth and cardiovascular development. 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 (Alonso et al., 1989, Browne et al., 1997a, 1997b, Camm et al., 2010, Gilbert, 1998, Gilbert et al., 2003, Hemmings et al., 2005, Herrera et al., 2012a, Jacobs et al., 1988, Kamitomo et al., 1992, 1994, 2002, Kim et al., 2005, Onishi et al., 2004, Thompson, 2003, Thompson et al., 1999, 2000, 2011, Williams et al., 2005a, 2005b). Fetal aortic wall thickening is particularly relevant in the clinical setting, as increased large artery stiffness predicts cardiovascular risk in humans (McEniery
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and Wilkinson, 2005), being a key component in the development of hypertension, atherosclerosis and coronary heart disease (Arnett et al., 1994). Four separate human clinical studies (Akira and Yoshiyuki, 2006, Cosmi et al., 2009, Koklu et al., 2006, Skilton et al., 2005) 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 (Mayhew et al., 1999, Miyague et al., 1997, Rizzo et al., 1995, Veille et al., 1993). A more recent clinical study reported that 3 to 6 year old children born IUGR had more globular hearts, reduced longitudinal motion, and impaired relaxation with an increase in radial function. They also had increased blood pressure and carotid intima-media thickness (Crispi et al., 2012). Several studies in rats have also 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 (Hauton, 2012, Hauton and Ousley, 2009, Li et al., 2003, 2004, Patterson and Zhang, 2010, Patterson et al., 2010, 2012, Rueda-Clausen et al., 2009, 2011, 2012, Xu et al., 2006, Xue and Zhang, 2009, Xue et al., 2011). Davidge and colleagues reported that adult offspring of hypoxic pregnancy have several cardiac structural and functional changes including in vivo evidence of elevated left ventricular end diastolic pressure (LVEDP) (Rueda-Clausen et al., 2009). Giussani and colleagues (Giussani et al., 2012) 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 (Salem et al., 2006), and sustained increases in myocardial contractility due to heightened sympathetic excitation and diminished parasympathetic reactivity have been strongly associated with cardiovascular disease, being an unsustainable condition and leading to eventual heart failure in humans (Bristow, 2002, Danson et al., 2009). A number of studies by various groups in different species have also now reported that developmental hypoxia can predispose to endothelial dysfunction in later life. Ruijtenbeek et al. (Ruijtenbeek et al., 2003b) 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 induction 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 (Giussani et al., 2012, Williams et al., 2005b). 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 (Leeson et al., 1997, 2001).

Exposure to synthetic glucocorticoids in early life in animal models could be used as an example to highlight differential effects of timing of the stress during development in predisposing to later cardiovascular disease. A remarkable finding was that treatment of pregnant sheep with dexamethasone for only two days, at the end of the first month of pregnancy but not at the end of the second month of pregnancy, produced hypertension in lambs at 19 months of age (Dodic et al., 1998). Langley Evans and colleagues reported that in rats the hypertensive effect in the offspring at weaning of a maternal low-protein diet was unaffected if the period of undernutrition occurred during the first, middle or last third of
pregnancy. However, the blood pressure increases elicited by these discrete periods of maternal under-nutrition were all lower than those induced by feeding a low-protein diet to the dams throughout pregnancy (Langley-Evans et al., 1996). Furthermore, when feeding pregnant dams diets containing a range of protein levels at the same stage of gestation, an inverse relationship between maternal protein intake and the degree of hypertension in the offspring was reported (Langley and Jackson, 1994). Combined, these studies provide experimental evidence to complement the human data reflecting how changes in the duration and the magnitude of the stress period during development can alter the cardiovascular phenotype of the adult offspring.

There is a growing body of evidence indicating sex differences in the developmental programming of cardiovascular dysfunction by stressful conditions in early life, with the weight of the evidence supporting protection or altered strategies in female offspring. Most of the experimental evidence complementing data derived from human studies comes from models of maternal alterations in nutrition, or of maternal stress or of exposure of the pregnancy to excessive glucocorticoid concentrations (see Aiken and Ozanne, 2013, Gilbert and Nijland, 2008). However, a few studies have also reported sexually dimorphic effects of prenatal hypoxia on cardiovascular dysfunction in the adult offspring, with effects being diminished in female relative to male offspring (Morton et al., 2010, Rueda-Clausen et al., 2009, Xue and Zhang, 2009).

Emerging evidence in rodents and in sheep also indicates that the environment during development can induce non-genomically phenotypic changes across generations. Studies in animal models have focussed on the intergenerational transmission by maternal behaviour, by altered nutrition or by excess glucocorticoid exposure (Aiken and Ozanne, 2014, Crudo et al., 2012, Drake et al., 2005, Francis et al., 1999, Harrison and Langley-Evans, 2009, Iqbal et al., 2012, Long et al., 2012, Torrens et al., 2008, Zambrano et al., 2005). For instance, Torrens et al. (Torrens et al., 2008) and Harrison & Langley-Evans (Harrison and Langley-Evans, 2009) reported on the intergenerational transmission of impaired nephrogenesis, peripheral vascular endothelial dysfunction and hypertension in rats following maternal protein restriction during pregnancy. There have also been reports of heritable impaired glucose tolerance in offspring across several generations in rodent models of maternal exposure during pregnancy to a high-fat diet, to undernutrition or to excess glucocorticoids (Drake et al., 2005, Dunn and Bale, 2009, Jimenez-Chillaron et al., 2009).

**Stress in Early Life and Risk of Cardiovascular Disease in Later Life: Underlying Mechanisms and Potential Interventions**

*Altered regional blood flow to the fetus* One of the earliest mechanisms proposed to increase risk of disease in later life relates to permanent changes in the structure and thereby function of important organs. For example, if the period of stress occurs in late gestation, when defence mechanisms in the fetus have had a chance to mature, there are physiological adaptive responses which may improve viability in the short term but they may claim costs in later life. For instance, the late gestation fetal circulatory defence to hypoxic stress involves dilatation of essential vascular beds and constriction of peripheral circulations (Cohn et al.,...
Therefore, the fetal cardiac output is redistributed away from less essential vascular beds to maintain oxygen and nutrient delivery to the brain - the so-called brain sparing effect (Giussani and Davidge, 2013, Rudolph et al., 1981). The physiology underlying this response is well delineated. The fetal peripheral vasoconstriction is triggered exclusively by a carotid body chemoreflex (Giussani et al., 1993) and maintained by the release of hormones, such as catecholamines, into the fetal circulation (Jones and Robinson, 1975). More recently, we have discovered that the neuroendocrine peripheral constrictor response is further fine-tuned by a vascular oxidant tone. This is created by the interaction between vascular $\cdot{}O_{2}^{-}$ and NO during hypoxia, whereby a fall in the ratio favours dilatation and an increase enhances constriction (Herrera et al., 2012b, Kane et al., 2012, Morrison et al., 2003, Thakor et al., 2010a, 2010b). In response to chronic hypoxic stress in the fetus, the maintained redistribution of blood flow away from peripheral circulations may become problematic, triggering a number of biological trade-offs in the fetus and increasing the risk of morbidity in later life. The most described adverse side effect is asymmetric intrauterine growth restriction (IUGR), yielding offspring whose brain growth is spared, but having bodies which are thin for their length with a low ponderal index (Barker, 1994). Such infants show a greater impact of hypoxia on body growth relative to brain growth, usually represented in neonatology by an increase in the ratio of the bi-parietal diameter to the body length (Barker, 1994). In response to fetal hypoxic stress, improved shunting of blood flow through the ductus venosus into the inferior vena cava further improves preferential blood flow to the brain at the expense of oxygen and nutrient delivery to the liver, impairing its growth (Ebbing et al., 2009, Godfrey et al., 2012). Persistent redistribution of blood flow away from peripheral beds may also explain the reduced growth of the fetal kidneys and of the pancreas, and the impaired endowment of nephrons and of beta cells in the Islets of Langerhans, respectively, in infants born from chronically stressed pregnancies (Barker and Hanson, 2004, Duque-Guimarães and Ozanne, 2013, Fowden and Hill, 2001, Latini et al., 2004, Mackenzie and Brenner, 1995). In turn, a reduced nephron number at birth will limit glomerular filtration and promote excess extracellular sodium retention throughout the life course, providing a mechanistic link between stress in early life, asymmetric IUGR and risk of risk of hypertension in later life (Mackenzie and Brenner, 1995). Similarly, a reduced number of pancreatic beta cells and impaired insulin secretion at birth has been associated with compensatory increases in insulin sensitivity in target tissues, such as in skeletal muscle and fat, in the young offspring of compromised pregnancy (Duque-Guimarães and Ozanne, 2013, Fowden and Hill, 2001). Increased glucose uptake promotes fat deposition, particularly if the postnatal nutrient availability is better than predicted in utero. In turn, adipogenesis via the increased secretion of adipokines favours insulin resistance, glucose intolerance and type II diabetes, again, providing a mechanistic link between stress in early life, the thin-for-length infant phenotype and an increased risk of developing the metabolic syndrome in later life (Duque-Guimarães and Ozanne, 2013, Fowden and Hill, 2001, Phillips et al., 1994). In addition, sustained increases in fetal peripheral vascular resistance will increase fetal arterial blood pressure if cardiac output is maintained. A sustained increase in fetal cardiac afterload will overwhelm the Frank-Starling mechanism, triggering compensatory 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 (Akira and Yoshiyuki, 2006, Cosmi et al., 2009, Gilbert, 1998, Herrera et al., 2012a, Koklu et al., 2006, Skilton et al., 2005). This may, once more, provide a mechanistic link between stress in early life and the thin-for-length infant phenotype.
life, asymmetric IUGR and cardiac and aortic wall hypertrophy; first order indices of an increased cardiovascular risk in later life.

Oxidative stress The idea that common pathways might mediate responses to suboptimal environments during early life has regained support recently as the phenotypic outcomes of various environmental stressors during development are so similar. Converging lines of evidence suggest that oxidative stress in early life might be one such common link (Davidge et al., 2008, Giussani et al., 2012, Nuyt, 2008, Patterson et al., 2012, Simmons, 2012, Thompson and Al-Hasan, 2012). Various groups have reported that common adverse intrauterine conditions, including under-nutrition, over-nutrition, excess glucocorticoid exposure, infection and hypoxia all lead to increased markers of oxidative stress in the fetal cardiovascular system (Davidge et al., 2008, Giussani et al., 2012, Nuyt, 2008, Patterson et al., 2012, Simmons, 2012, Thompson and Al-Hasan, 2012). Similarly, there are several reports of increased placental and fetal oxidative stress in pregnancies complicated by IUGR and/or pre-eclampsia (Biri et al., 2007, Burton et al., 2009, Karabulut et al., 2005, Negi et al., 2012). Giussani et al. (Giussani et al., 2012) tested the hypothesis that oxidative stress in the fetal cardiovascular system links chronic fetal hypoxia with cardiovascular dysfunction in later life, by an interventional study using antioxidants. They reported that chronic fetal hypoxia promoted fetal aortic wall thickening and molecular markers of oxidative stress in the fetal heart and vasculature by the end of gestation. By adulthood, these effects resolved but chronic fetal hypoxia set a dysfunctional phenotype 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 (Giussani et al., 2012). Thompson and colleagues have also reported that treatment with N-acetyl cysteine of pregnant guinea pigs inhibited the adverse effects on the fetal liver of chronic prenatal hypoxia (Thompson and Al-Hasan, 2012). To better mimic the clinical situation and treat IUGR offspring of hypoxic pregnancy following diagnosis, the Davidge and Dyck laboratories used the natural polyphenolic antioxidant resveratrol. Their studies demonstrated that early postnatal administration of resveratrol in the diet of weanling rats born IUGR from hypoxic pregnancy and fed an obesogenic diet also prevented features of cardiovascular dysfunction and of the metabolic syndrome in later life (Dolinsky et al., 2011, Rueda-Clausen et al., 2012). In a series of studies, we have now also reported that treatment of neonatal rat pups with glucocorticoids, modelling steroid therapy for the treatment of chronic lung disease in premature infants, also induces oxidative stress and alterations in structure and function of the cardiovascular system of the offspring (Adler et al., 2010, Herrera et al., 2010, Niu et al., 2013). Excess glucocorticoid exposure promotes oxidative stress with consequent decreases in NO bioavailability via several pathways (Iuchi et al., 2003, Wallwork et al., 2003, Zhang et al., 2004). Consequently, combined neonatal glucocorticoid with either antioxidant vitamins or with statins restored NO bioavailability, improved postnatal survival, protected the developing brain and prevented overt cardiac dysfunction at adulthood (Adler et al., 2010, Herrera et al., 2010, Niu et al., 2013, Tijsseling et al., 2013). Such data have raised the question of whether it is finally time to review current perinatal clinical practice and fine-tune it to maintain beneficial effects on the developing lung but to diminish adverse side-effects of synthetic steroid therapy on the developing brain and heart in perinatal medicine (Bonanno and Wapner, 2012, Nijland, 2003, Wapner and Jobe, 2011). Combined neonatal
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glucocorticoid and antioxidant therapy may provide one such effective strategy and be a safer clinical intervention in the treatment of the premature infant. The groups of Torrens, Hanson and Clough have further reported that in mice and in rats either maternal intake of a low protein diet or of fat during pregnancy could perturb redox status, impair endothelial NO-mediated vasorelaxation in peripheral circulations and induce hypertension in the adult offspring (Rodford et al., 2008, Torrens et al., 2012). Furthermore, statin administration to the dams during pregnancy or to the offspring after weaning could protect against the adult onset cardiovascular dysfunction triggered by either suboptimal environment during early life (Elahi et al., 2013, Torrens et al., 2009). Combined, therefore, there is mounting evidence to support oxidative stress being a mechanistic link between various stressors in early life with increased risk of cardiovascular disease in later life.

**Epigenetics** Recent advances in the field of epigenetics are beginning to explore the mechanistic link between stress in early life with cardiovascular disease in later life, and have also refuelled the once popular heretical idea that ancestral environment could affect the physiology of future generations. During development, epigenetic processes can alter gene expression without a change in DNA sequence through several processes including DNA methylation and/or histone modification and/or changes to small non coding RNAs. In a study series in rats, Zhang and colleagues linked the reduced expression of the cardio-protective gene protein kinase C epsilon (PKCe) with increased cardiac susceptibility to ischaemic/reperfusion (I/R) injury in adult offspring (Li et al., 2003, 2004, Patterson and Zhang, 2010, Patterson et al., 2010, 2012, Xue and Zhang, 2009, Xue et al., 2011). Not only was the expression of PKCe reduced in hearts of offspring of hypoxic pregnancy, 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 (Li et al., 2003, 2004, Patterson and Zhang, 2010, Patterson et al., 2010, 2012, Xue and Zhang, 2009, Xue et al., 2011). The mechanism via which hypoxic pregnancy caused heightened offspring cardiac susceptibility to I/R injury was shown to be epigenetic, as they also reported 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 (Li et al., 2003, 2004, Patterson and Zhang, 2010, Patterson et al., 2010, 2012, Xue and Zhang, 2009, Xue et al., 2011). Patterson et al. (Patterson et al., 2012) confirmed a role for prenatal hypoxia-derived oxidative stress in programming cardiac dysfunction in adulthood and expanded this idea by linking it to epigenetic mechanisms, reporting that maternal treatment in rats with the antioxidant N-acetyl-cysteine inhibited the hypoxia-induced increase in methylation of the SP1-binding sites, reversed the decreased SP1 binding to the PKCe promoter, restored PKCe mRNA and protein abundance and abrogated the hypoxia-induced increase in susceptibility of the heart to I/R injury in adult offspring. Recently, they have further shown that noradrenaline causes the epigenetic repression of the PKCe gene in rodent hearts by activating Nox1-dependent ROS generation (Xiong et al., 2012). Developmental hypoxia may therefore increase the risk of a sympathetically dominant cardiac phenotype by catecholamine-induced ROS, which in turn causes the epigenetic repression of cardiac PKCe, thereby enhancing cardiac susceptibility to I/R injury in adult offspring.

Accordingly, treatment with the selective PKCe activator peptide ψ-εRACK of fetal hearts isolated from hypoxic pregnancy markedly improved their recovery from an I/R challenge (Patterson et al., 2010).
Epigenetic pathways may also represent the molecular substrate for the influence of the early environment in the regulation of vascular function (Fish et al., 2010, Krause et al., 2013, Yan et al., 2010). For example, epigenetic mechanisms have been reported to play a key role in the control of vasodilatation by altering the expression of key genes involved in endothelial-dependent relaxation, such as NOS3 and ARG2 (Yan et al., 2010). Using human umbilical vein endothelial cells (HUVEC), Marsden and colleagues have reported that hypoxia causes a rapid decrease in the transcription of the eNOS/NOS3 gene, accompanied by decreased acetylation and lysine 4 (histone H3) methylation of eNOS proximal promoter histones (Fish et al., 2010). Recently, Casanello and colleagues have also reported the epigenetically regulated expression of eNOS and arginase-2 in HUVEC derived from IUGR pregnancies. They also reported that the altered eNOS expression in IUGR-derived endothelial cells could be reversed by transient silencing of the DNA methylation machinery (Krause et al., 2013).

Therefore, accumulating evidence of the epigenetic regulation of key genes, important in the control of cardiac and vascular function, is beginning to provide another mechanistic link between stress in early life and increased cardiovascular risk in later life, indentifying other possible avenues of potential clinical therapy.

It is clear that the three examples described above of underlying mechanisms linking stress in early life with risk of cardiovascular disease in later life do not constitute an exhaustive list; accelerated cellular ageing and more rapid telomere shortening comprising another possibility (Martin-Gronert and Ozanne, 2012). It is also clear that a number of these mechanisms are likely to be inter-linked. For instance, environmental stressors in early life may promote oxidative stress. Higher levels of ROS are not only associated with telomere shortening but with epigenetic repression of key genes involved in cardiovascular function, such as NOS3 (Fish et al., 2010, Krause et al., 2013, Martin-Gronert and Ozanne, 2012). Reduced expression of eNOS and consequent impaired NO bioavailability may tip the vascular oxidant tone towards sustained constriction in peripheral vascular beds, thereby altering regional blood flow. Sustained redistribution of the fetal cardiac output favours the thin-for-length baby phenotype with increased cardiac afterload, altered autonomic control of the cardiovascular system, decreased nephron number and reduced pancreatic β-cell endowment, all of which increase cardiovascular risk in later life.

**Stress in Early Life and Risk of Cardiovascular Disease in Later Life: Bench to Hospital Bedside and Health Policy**

The challenge posed by cardiovascular disease is on a global scale and necessitates a lifecourse strategy as part of NCD prevention (WHO Global Action Plan for the Prevention and Control of Non-communicable Diseases 2013-20). It is now widely recognised that interventions to reduce risk need to commence in early life. This requires action to promote health literacy and healthy lifestyle in young people if they are to become healthy adults. In addition this may reduce the transmission of risk to subsequent generations. Whilst cardiovascular disease constitutes a major medical problem, the solution will involve broader
social interventions and, whilst culturally specific, need to engage the widest range of the population possible.

A large body of research, in animal models, human cohorts and clinical settings, has identified processes by which the developmental environment induces changes in the developing phenotype of the offspring. Environmental challenges may disrupt development altogether if severe. Of more relevance to the lifecourse aetiology of CVD, however, are the range of stressors which alter CV development within the normal range. The responses to a variety of stressors, e.g. unbalanced materno-fetal nutrition or oxygenation may operate via similar effect pathways, e.g. involving oxidative stress, glucocorticoids, the sympathetic nervous system etc., to induce stereotypical adaptive responses. These may induce pathological changes in important organs and systems that persist until, or are amplified, in later life. Alternatively, they may affect the ability of an individual to respond to later stresses to the CV system, especially those associated with aspects of lifestyle such as diet, physical activity and psychological stress. The degree of response to such stressors sets the trajectory of the individual’s increasing risk of CVD over time.

The developmental processes influenced by environment operate at several levels. By affecting organ and tissue perfusion, they influence growth and subsequent functional capacity. At the tissue level, many of these processes involve changes in the balance of pro- and anti-oxidant systems which are also intimately involved in growth and a range of signalling and response pathways. At the molecular level, the processes may involve epigenetic processes which can affect the developing phenotype through altering gene expression without fixed genetic changes. There is accumulating data that such effects can be passed across generations to influence the inheritance of CVD risk.

Increasing knowledge of the mechanistic pathways underlying the developmental origins of later CV risk is leading to the discovery of biomarkers which may be measured in early life to give a prediction of later risk. In addition, the knowledge of underlying processes, for example those involving oxidative stress, is likely to lead to novel preventative strategies. There is encouraging evidence in this respect from the use of anti-oxidant therapy in animal models.

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