Heart Disease Link to Fetal Hypoxia and Oxidative Stress

7

Dino A. Giussani, Youguo Niu, Emilio A. Herrera, Hans G. Richter, Emily J. Camm, Avnesh S. Thakor, Andrew D. Kane, Jeremy A. Hansell, Kirsty L. Brain, Katie L. Skeffington, Nozomi Itani, F.B. Peter Wooding, Christine M. Cross, and Beth J. Allison

Abstract

The quality of the intrauterine environment interacts with our genetic makeup to shape the risk of developing disease in later life. Fetal chronic hypoxia is a common complication of pregnancy. This chapter reviews how fetal chronic hypoxia programmes cardiac and endothelial dysfunction in the offspring in adult life and discusses the mechanisms via which this may occur. Using an integrative approach in large and small animal models at the *in vivo*, isolated organ, cellular and molecular levels, our programmes of work have raised the hypothesis that oxidative stress in the fetal heart and vasculature underlies the mechanism via which prenatal hypoxia programmes cardiovascular dysfunction in later life. Developmental hypoxia independent of changes in maternal nutrition promotes fetal growth restriction and induces changes in the cardiovascular, metabolic and endocrine systems of the adult offspring, which are normally associated with disease states during ageing. Treatment with antioxidants of animal pregnancies complicated with reduced oxygen delivery to the fetus prevents the alterations in fetal growth, and the cardiovascular, metabolic and endocrine dysfunction in the fetal and adult offspring. The work reviewed offers both insight into mechanisms and possible therapeutic targets for clinical intervention against the early origin of cardiometabolic disease in pregnancy complicated by fetal chronic hypoxia.

D.A. Giussani (✉) • Y. Niu • E.J. Camm
A.S. Thakor • A.D. Kane • J.A. Hansell • K.L. Brain
K.L. Skeffington • N. Itani • F.B.P. Wooding
C.M. Cross • B.J. Allison
Department of Physiology, Development & Neuroscience, University of Cambridge, Cambridge, UK
e-mail: dag26@cam.ac.uk

E.A. Herrera
Laboratorio de Función y Reactividad Vascular, Programa de Fisiopatología, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile, Santiago, Chile

H.G. Richter
Instituto de Anatomía, Histología y Patología, Universidad Austral de Chile, Valdivia, Chile

L. Zhang and C.A. Ducsay (eds.), *Advances in Fetal and Neonatal Physiology*, Advances in Experimental Medicine and Biology 814, DOI 10.1007/978-1-4939-1031-1_7,

Keywords
Hypoxia • Oxidative stress • Antioxidants • IUGR • Programming

1 Fetal Hypoxia

Fetal hypoxia is one of the most common consequences of complicated pregnancy and it may occur by insufficiency of either uterine blood flow, increased placental vascular resistance with a consequent decrease in umbilical blood flow, or by a decrease in the maternal arterial oxygen content [1–3]. Other mechanisms such as fetal anaemia or increased fetal oxygen consumption (e.g. in pyrexia) are relatively rare in clinical practice [4]. Risk factors which predispose to the development of fetal hypoxia can be classified into maternal, intra-partum and iatrogenic. Maternal risk factors include diabetes, pregnancy induced or chronic hypertension, Rh sensitization, maternal infection, sickle cell anaemia, chronic substance abuse, asthma, seizure disorders or smoking [5–13]. Intra-partum risk factors for fetal hypoxia encompass multiple pregnancy, pre or post-term birth, prolonged labour, placental abruption, placenta praevia, prolapsed umbilical cord or abnormal presentation of the fetus [14–21]. Maternal hypotension, which may accompany epidural anaesthesia is an example of an iatrogenic risk factor for the development of fetal hypoxia [22].

2 The Fetal Defence to Hypoxia

In contrast to glucose or protein, which can be stored as reserves in our body for prolonged periods of time, there is no similar mechanism for the long-term storage of oxygen in our bodies either before or after birth. Consequently, before and after birth, our physiology is largely dependent on a constant supply of oxygenated blood. Not surprisingly, the source of this constant delivery of oxygen is drastically different between the intra- and extra-uterine environments. Therefore, it should also be unsurprising that the strategy of our cardiovascular system to defend tissues against periods of reduced oxygenation is also dramatically different during the fetal and postnatal periods. Outside the womb, there is a vast supply of oxygen from the atmosphere. In the adult period, a reduction in oxygen supply to the tissues is met with an increase in ventilation to increase the level of oxygenation in our pulmonary blood. This luxurious supply of atmospheric oxygen allows the adult cardiovascular system to increase perfusion not only to essential vascular beds but also to peripheral tissues, maintaining their oxygenation during periods of systemic hypoxia [23]. Within the womb, the supply of oxygenated fetal blood is dependent on the placenta. In contrast to pulmonary ventilator processes, intrauterine mechanisms to increase the input and output of oxygenated blood are limited. However, a number of adaptations, unique to life in the womb, permit the supply of oxygen to the fetus to exceed its metabolic needs, equipping the unborn child with a considerable margin of safety for oxygenation under basal conditions during development. For example, relative to the adult, these adaptations allow the fetus to bind greater concentrations of oxygen in its haemoglobin, to have an increased basal blood flow to most tissues, and to relinquish the bound oxygen to the fetal tissues at lower oxygen tensions [24]. In addition, shunts in the fetal circulation and preferential streaming ensure an adequate supply of oxygenated blood to tissues most at risk of damage during reductions in oxygenation [24, 25]. Finally, the fetus has a greater capacity than the adult to hinder oxygen-consuming processes [24]. Consequently, the fetal defence strategies during episodes of hypoxia capitalise on increasing the efficiency of these fetal adaptations,

thereby either consuming even less oxygen, extracting even more oxygen from haemoglobin and ultimately making better use of this finite supply of oxygenated blood [25]. The defence responses to episodes of hypoxia of the fetal cardiovascular system exemplify some of these strategies. In the late gestation fetus, an episode of acute hypoxia triggers fetal bradycardia [26]. Deceleration of the fetal heart has several advantages as it permits maintenance of normal levels of myocardial oxygen consumption despite hypoxic conditions [27] and prolongs the beat-to-beat interval, thereby increasing end diastolic filling volume, which helps maintain fetal cardiac output [28]. Reducing the velocity of blood flow through the fetal coronary circulation will also prolong diffusion time, an effect likely to increase the efficiency of myocardial blood gas exchange. During episodes of acute hypoxia, the fetal cardiac output is also redistributed due to differential vasomotion. For instance, circulations perfusing the fetal brain undergo active vasodilatation and those perfusing peripheral circulations undergo active vasoconstriction [25, 26]. Therefore, the fetal cardiac output follows the path of least resistance, becoming diverted from less essential vascular beds towards those perfusing the brain—the so called brain sparing effect. The fetal bradycardia and brain-sparing circulatory responses to acute hypoxia have been conserved across all species studied to date, from the chick embryo to the sheep fetus, the non-human primate and the human fetus (see [29]).

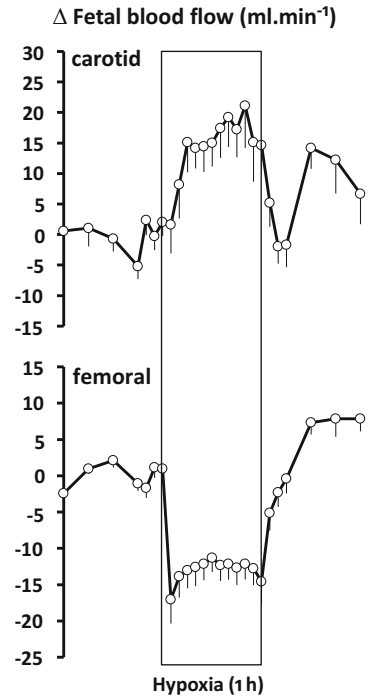

Fig. 7.1 Fetal brain sparing effect during acute hypoxia in real time *in vivo*. Values are mean ± S.E.M. of change from baseline in carotid blood flow and femoral blood flow measured simultaneously in 14 fetal sheep between 118 and 125 days gestation (term ~145 days) during basal conditions and in response to a 1 h period of acute hypoxia (*box*). Fetal descending aortic PO_2 during acute hypoxia was reduced from 23.4 ± 0.7 to 13.2 ± 0.3 mmHg. Redrawn from [30]

3 The Physiology Underlying the Fetal Cardiovascular Defence to Hypoxia

More than two decades ago, for the first time, Transonic flow probes were surgically implanted around the carotid and femoral arteries of late gestation fetal sheep in long-term preparations to be able to visualise the fetal brain sparing response to acute hypoxia in real time ([30]; Fig. 7.1). The data revealed that the fetal peripheral circulatory response occurs within seconds of the onset of hypoxia, suggesting a neurally-triggered response. It is now established that while the increase in cerebral blood flow during acute hypoxia results from an increase in nitric oxide (NO; [31]), both the bradycardia and the peripheral vasoconstriction in the fetus are initially triggered by the same carotid body chemoreflex, as bilateral section of the carotid sinus nerves prevents them from occurring [30]. Once triggered by the carotid chemoreflex, fetal peripheral vasoconstriction is maintained by the release of constrictor agents into the fetal circulation, such as catecholamines, vasopressin and neuropeptide Y [32–34].

We now know that this neuro-endocrine peripheral constrictor drive is fine-tuned by opposing dilator influences, such as an increase in NO production in fetal peripheral vasculature during acute hypoxia [35]. Data to support this comes from studies using fetal *in vivo* treatment with a NO clamp. The latter is a technique that permits blockade of *de novo* synthesis of NO during acute hypoxia while maintaining basal cardiovascular function [36, 37]. Application of the NO clamp to the late gestation sheep fetus markedly enhanced the magnitude of the femoral vasoconstrictor response, revealing the full strength of the unopposed chemoreflex and endocrine constrictor responses [35]. Most recently, we have discovered that the bioavailability of NO in the fetal peripheral vascular beds is itself limited by the generation of reactive oxygen species, such as the superoxide anion ($^{\bullet}O_2^-$), during acute hypoxia. Therefore, it is the actual interaction between $^{\bullet}O_2^-$ and NO that provides a local oxidant tone to the fetal vasculature, whereby a fall in the ratio favours dilatation and an increase promotes vasoconstriction. We have shown that this vascular oxidant tone is operational in fetal life and that it can be easily manipulated [29, 38–40]. For instance, fetal treatment with antioxidants or with statins markedly diminished the magnitude of the femoral vascular resistance response to acute hypoxia. This effect was by increasing the bioavailability of NO, as fetal treatment with antioxidants or with statins during NO blockade with the NO clamp restored the full magnitude of the femoral vasoconstrictor response [29, 39]. Combined, therefore, data show that the peripheral vasoconstrictor response to acute hypoxia in the fetus is an aggregate of mechanisms, resulting from carotid chemoreflex stimulation, enhanced endocrine vasoconstrictors and a local vascular oxidant tone determined by the interaction between $^{\bullet}O_2^-$ and NO.

4 Fetal Chronic Hypoxia

In fetal physiology, it is a well accepted view that should the duration of the period of fetal oxygen deprivation become prolonged, then the fetal circulatory defence response to acute hypoxia persists. Therefore, in response to chronic fetal hypoxia, such as may occur during preeclampsia or placental insufficiency, persisting redistribution of blood flow away from peripheral circulations is believed to maintain oxygen and nutrient delivery to the fetal brain. The biological trade-off is asymmetric fetal growth restriction, whereby babies are not only small but they are thin for their length with a low ponderal index [41]. These infants also show a greater impact of hypoxia on body growth relative to brain growth, usually represented in neonatology by an increase in the bi-parietal diameter to the body length ratio of the infant [41]. Persisting redistribution of blood flow away from vascular beds considered less essential in fetal life, such as those perfusing the fetal kidneys or the fetal pancreas, may also explain the reduced endowment of kidney nephrons and of beta cells in the islet of Langerhans in intrauterine growth-restricted (IUGR) offspring [42, 43]. Data to support persisting redistribution of blood flow away from less essential vascular beds towards the fetal brain in pregnancy complicated by chronic fetal hypoxia is difficult to obtain, but the group of Professor Longo at Loma Linda University has been able to just do that. Exploiting the natural hypobaric hypoxia of pregnancy at high altitude and combining it with expertise to measure regional blood flow changes with radioactively labelled microspheres, they reported that during long-term high altitude hypoxia in sheep, blood flow to the fetal brain is maintained and there is a sustained decrease in blood flow to the fetal carcass ([44]; Fig. 7.2). In comparison to asymmetric IUGR, the less well described side effect of persisting redistribution of blood flow away from peripheral circulations may be endothelial dysfunction leading to an increase in fetal peripheral vascular resistance, which will increase fetal cardiac afterload if cardiac output is maintained. Persisting increases in fetal cardiac afterload may overwhelm the Frank-Starling mechanism and trigger changes in the morphology and function of the fetal heart and aorta [28]. Accordingly, several studies in chick embryos and fetuses of mammalian species have now reported that developmental hypoxia promotes cardiac and aortic wall hypertrophic growth, altered cardiac function, pulmonary

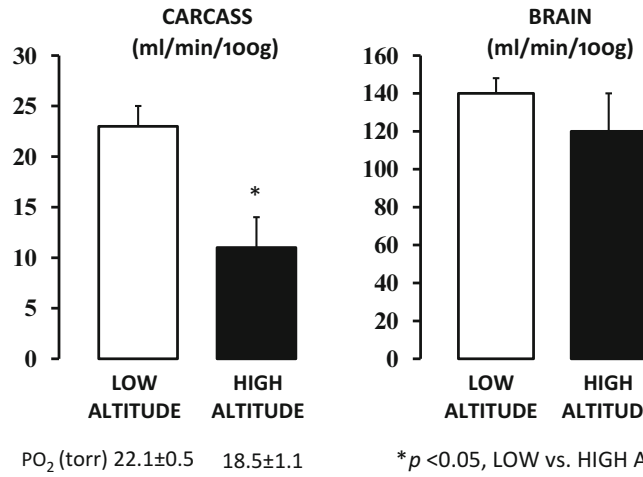

Fig. 7.2 Fetal brain sparing effect during chronic hypoxia. Values are mean ± S.E.M. of brain and carcass basal blood flow in late gestation fetal sheep from control low altitude pregnancy or pregnancy exposed to high altitude from 30 to 135 days of gestation. Blood flow was measured at 135 ± 1 days of gestation (term ~146 days). *P < 0.05, low altitude *vs.* high altitude hypoxia. Redrawn from reference [44] with permission

hypertension, sympathetic hyper-innervation of peripheral resistance arteries and NO-dependent peripheral vascular endothelial dysfunction (see [45] for review). The cardiac and aortic wall remodelling and asymmetric growth restriction that occurs in sea level chick embryos incubated at high altitude is no longer observed in sea level embryos incubated at high altitude with oxygen supplementation [46, 47], underlying the direct effects of chronic hypoxia on fetal growth and on cardiovascular development. In the clinical setting, fetal aortic wall thickening is particularly relevant as, in humans, an increase in large artery stiffness independently predicts cardiovascular risk [48], being a key component in the aetiology of cardiovascular diseases including hypertension, atherosclerosis and coronary heart disease [49]. An increase in wall thickness, in particular, in the aorta has also been proposed as the first physical sign of atherosclerosis [50]. In human perinatal clinical studies, there have been four additional reports linking babies born from pregnancies complicated by placental insufficiency with aortic wall thickening, increased aortic stiffness and reduced distensibility [51–54]. In addition, developmental hypoxia in animals and humans induces neonatal pulmonary hypertension with a marked cardiopulmonary remodelling [55–57]. A remarkable number of unique studies by the groups of Gilbert, Pearce and Longo have reported that in fetal sheep subjected to high altitude from day 30 of gestation to term (*ca.* 145 days), there is evidence of a fetal origin of cardiac dysfunction. They reported an inability of the chronically hypoxic ovine fetus to maintain cardiac output, secondary to a decrease in the contractile function of myocardial cells (see [45, 58] for review). Similarly, Keller and colleagues using the chick embryo reported that sustained hypoxia during incubation decreased ventricular pressures and ventricular ejection fraction, consistent with depressed systolic function [59]. Therefore, sustained increases in fetal cardiac afterload may not only overwhelm the Frank-Starling mechanism but also the compensatory ventricular hypertrophic growth, eventually leading to hallmarks of heart failure. Therefore, chronic fetal hypoxia is not only an immediate threat to fetal survival, but it is also an important environmental influence triggering asymmetric IUGR and a fetal origin of cardiac as well as pulmonary and peripheral vascular disease.

5 Programming of Cardiovascular Dysfunction in Adulthood by Fetal Chronic Hypoxia

Over the last 5 years, there has been a surge of studies reporting programming of cardiovascular dysfunction in adult offspring of hypoxic pregnancy. The laboratory of Zhang, also at Loma Linda University, in an exceptional series of

investigations has consistently reported that hypoxic pregnancy in rats increases the susceptibility of cardiac ischaemic-reperfusion (I/R) injury at adulthood (see [45, 60] for review). The programming of this cardiac defect at adulthood is associated with the decreased expression of the cardio-protective gene protein kinase C epsilon (PKCε). Further, repression of cardiac PKCε gene expression in fetal rat pup hearts of hypoxic pregnancy is epigenetically regulated [60]. They reported that the increase in the promoter methylation and the reduced expression of the PKCε gene in fetal rat pup hearts of hypoxic pregnancy could be prevented by treatment with a DNA methylation inhibitor. Conversely, treatment of hearts from adult offspring of normoxic pregnancy with a PKCε translocation inhibitor could mimic the defects in hearts of offspring from hypoxic pregnancy. The laboratory of Davidge reported that hypoxia-induced IUGR is associated with the development of chronic cardiopulmonary dysfunction during ageing, identifying a mismatch in glucose metabolism, leading to proton accumulation in the post-ischaemic myocardium of offspring born IUGR as a potential mechanism involved (see [45, 61] for review). Further, they reported that a postnatal high fat diet accelerated cardiac dysfunction in IUGR offspring of hypoxic pregnancy. In addition, Niu and colleagues have reported that hearts of adult offspring of hypoxic pregnancy have in addition a sympathetic dominant phenotype, being more responsive to β_1 adrenergic agonists and less responsive to muscarinic agonists [62]. The latter is of further clinical relevance, as heightened sympathetic excitation with diminished parasympathetic reactivity is an unsustainable situation to maintain cardiac output, and such a cardiac phenotype has been strongly associated with eventual heart failure in humans [63, 64]. Accordingly, exposure of chick embryos, mice and rat pups to prolonged developmental hypoxia promotes dilated cardiomyopathy, with evidence of pump dysfunction that is demonstrable in the offspring and maintained into adulthood (see [45] for review). Other studies in chickens and rodents have consistently reported that hypoxic development can also programme peripheral vascular dysfunction in adulthood, showing defects in the capacity of peripheral vascular resistance vessels to relax during stimulation with NO-dependent dilator agonists, such as with acetylcholine (see [45] for review). Data are also beginning to surface to suggest the programming of an insulin resistant phenotype in adult offspring of hypoxic pregnancy [65–67].

6 Intervention Against Programming of Cardiovascular Dysfunction in Hypoxic Pregnancy

With accumulating evidence pointing towards the programming of a metabolic syndrome by development complicated by fetal chronic hypoxia, which encompasses cardiac sympathetic dominance with increased susceptibility to I/R injury, peripheral endothelial dysfunction and insulin resistance, there is intensifying interest in identifying potential clinical therapy. In recent publications, using an integrative approach at the *in vivo*, isolated organ, cellular and molecular levels, our group has proposed the hypothesis that the molecular basis underlying the programming of cardiovascular dysfunction in the adult offspring of hypoxic pregnancy is through the generation of oxidative stress *in utero* [62]. This offers the exciting prospect that maternal supplementation with antioxidants in pregnancy complicated by fetal chronic hypoxia may protect against the development of cardiovascular disease in the offspring, even before they are born. Accordingly, in a longitudinal study in rats, we reported on the effects of maternal treatment of hypoxic pregnancy with the antioxidant vitamin C on the cardiovascular system of the offspring at the end of gestation and at adulthood. In the fetus, pregnancy under hypoxic conditions promoted aortic thickening with enhanced nitrotyrosine staining and an increase in cardiac HSP70 expression, both robust measures of vascular and cardiac oxidative stress, respectively [62]. By adulthood, offspring of hypoxic pregnancy had *in vivo* evidence of altered baroreflex function,

markedly impaired NO-dependent vasorelaxation in peripheral resistance arteries and a cardiac sympathetic dominant phenotype. Maternal treatment with vitamin C prevented these cardiovascular defects in fetal and adult offspring of hypoxic pregnancy [62, 68]. In addition to antioxidant effects on the fetal cardiovascular system, additional protective effects of maternal treatment with vitamin C on the chronically hypoxic fetus may be due to alterations in the vascular oxidant tone at the level of the placental circulation. We have previously reported that vitamin C enhances umbilical blood flow via NO-dependent mechanisms [38]. Maternal treatment with vitamin C may therefore quench free radicals and increase NO bioavailability, shifting the placental vascular oxidant tone towards dilatation. Antioxidants may thus further protect fetal oxygen delivery by increasing in umbilical blood flow in complicated pregnancy [69, 70].

7 Antioxidant Protection in Hypoxic Pregnancy of Large Mammalian Species

With the perspective of translating the findings in rodent species to the human situation, the British Heart Foundation requested evidence that maternal treatment with antioxidants was protective in fetal and adult offspring of hypoxic pregnancy in larger mammalian species, such as in sheep. In contrast to rodent species, sheep permit surgical instrumentation of the mother and fetus for long-term recording, allowing *in vivo* real time evaluation of the safety of antioxidant therapy on the maternal and fetal physiology as the chronic hypoxic pregnancy is occurring. At the Barcroft building of the University of Cambridge, we have now created four isobaric hypoxic chambers able to maintain chronically-instrumented maternofetal ovine preparations for the duration of pregnancy under controlled hypoxic conditions. The chambers are supplied with nitrogen and air provided by nitrogen generators and air compressors from a bespoke nitrogen generating system (Domnick Hunter Gas Generation, Gateshead, Tyne & Wear, UK). We have also created a wireless data acquisition system (CamDAS, Maastricht Instruments, The Netherlands), which is able to record maternal and fetal arterial blood pressure and blood flow in four circulations, such as the uterine, umbilical, fetal carotid and fetal femoral vascular beds. Exteriorised maternal and fetal catheters and flow probe leads terminate in miniaturised pressure and flow boxes, which are housed in a bespoke jacket worn by the ewe. Electronic signals are then transmitted via bluetooth technology to a laptop sitting outside the chambers, thereby recording continuous maternal and fetal cardiovascular function without interruption of the hypoxic exposure. In this set up, we have taken great care to minimise maternal stress. An additional advantage of this system is that the degree of fetal chronic hypoxia induced is not restricted to the level achieved by ovine pregnancy at 3,500–4,000 m above sea level, which results in fetal arterial PO_2 values of *ca.* 18 mmHg [44]. Consequently, one is able to determine the effects of significant chronic fetal hypoxia, resulting in fetal arterial PO_2 of *ca.* 11–13 mmHg, akin to values reported in human pregnancy complicated by severe IUGR [71]. Using these hypoxic chambers for the first time, our investigations revealed that hypoxic pregnancy, reducing the maternal arterial PO_2 from 107.0 ± 1.5 to 46.8 ± 0.4 and the fetal arterial PO_2 from 22.1 ± 1.1 to 12.0 ± 0.2 mmHg during the last third of gestation in sheep, promoted asymmetric fetal growth restriction, systolic and diastolic cardiac dysfunction in the fetus and significant hypertension at adulthood. Intravenous treatment of pregnant ewes with vitamin C for the last third of gestation prevented these defects in fetal and adult offspring of hypoxic pregnancy (unpublished data).

8 Future Perspectives

While our studies in rodent and ovine pregnancy offer insight to mechanism and thereby closer targets for human clinical intervention against developmental origins of cardiac and peripheral vascular dysfunction in offspring of risky pregnancy, there is a caveat. In both our rodent and

ovine studies, only maternal treatment with vitamin C in very high doses, incompatible with human treatment, are effective. The reason is because the kinetics of the reaction between $^{\bullet}O_2^-$ and NO is so fiercely fast, that for vitamin C to be able to compete *in vivo* with NO for any given concentration of $^{\bullet}O_2^-$, its concentrations must exceed that of NO by a factor of 100,000. Therefore, for vitamin C to be able to work as an antioxidant *in vivo*, its concentrations would need to be *ca.* 10,000 μmol l^{-1} or 10 mmol l^{-1} to prevent the interaction between $^{\bullet}O_2^-$ and NO [29, 62]. This may explain the inability of maternal treatment with vitamin C to protect against placental vascular oxidative stress in preeclamptic pregnancy in all reported clinical trials to date [72, 73]. Interestingly, in all trials, the dose of maternal vitamin C administration was 1 g per pregnant woman per day, yielding circulating maternal concentrations of vitamin C of ~120 μmol l^{-1}, far short of the 10,000 μmol l^{-1} range required for the antioxidant vitamin to be effective *in vivo*. Since excessive doses of vitamin C promote oxaluria leading to the formation of kidney stones and several studies have suggested pro-oxidant activity of ascorbic acid with pharmacological doses even in healthy pregnancy [74, 75], there is increasing interest in alternative antioxidant therapies, compatible with human treatment. One interesting candidate is a mitochondrial-targeted antioxidant. Mitochondria are a major site of reactive oxygen species (ROS) production, therefore protecting them from oxidative damage should be one of the most effective antioxidant therapeutic strategies. However, conventional antioxidants are ineffective because they cannot penetrate the mitochondria [76]. Part of the problem relates to the difficulty of delivering antioxidants to mitochondria *in situ*. Dr Mike Murphy from the Mitochondrial Biology Unit, Addenbrookes Hospital, Cambridge and colleagues have developed the mitochondrial targeted antioxidant MitoQ that overcomes the problem. MitoQ consists of a quinone moiety covalently attached to a triphenylphosphonium (TPP) cation. The TPP cation is lipophilic, and its positive charge results in the 100–1,000-fold accumulation of MitoQ in the mitochondrial matrix, driven by the negative trans-membrane potential. Once inside the matrix, MitoQ is reduced by complex II in the respiratory chain to the active quinol form of the antioxidant. This will react with any ROS present, recycling MitoQ back into the quinone form and removing the ROS. Initial experiments with MitoQ demonstrated its ability to prevent lipid peroxidation in cell culture [77]. The benefits of MitoQ have now been revealed in a range of *in vivo* studies in rats and mice and in two phase II human trials [76, 78]. For instance, Dominiczak and colleagues showed elegantly that in vivo treatment with MitoQ of stroke-prone spontaneously hypertensive rats reduced arterial blood pressure, cardiac hypertrophy and improved NO-dependent endothelial function [78]. In contrast to vitamin C and other conventional antioxidants, MitoQ demonstrates no pro-oxidant activity and long-term administration to mice for 28 weeks revealed no toxic effects [76, 79]. However, the antioxidant benefits of MitoQ in risky pregnancy of any species await investigation. Future studies in our laboratory will determine the protective effects of MitoQ against the programming of cardiovascular disease in pregnancy complicated by fetal chronic hypoxia in small and large animal models. This offers exciting potential for human clinical intervention.

Acknowledgements Dino Giussani is Professor of Cardiovascular Physiology & Medicine at the Department of Physiology Development & Neuroscience at the University of Cambridge, Professorial Fellow and Director of Studies in Medicine at Gonville & Caius College, a Lister Institute Fellow and a Royal Society Wolfson Research Merit Award Holder. He is supported by the British Heart Foundation, The Biotechnology and Biological Sciences Research Council and the Isaac Newton Trust.

References

1. Yaffe H, Parer JT, Block BS, Llanos AJ. Cardiorespiratory responses to graded reductions of uterine blood flow in the sheep fetus. J Dev Physiol. 1987;9:325–36.
2. Itskovitz J, LaGamma EF, Rudolph AM. The effect of reducing umbilical blood flow on fetal oxygenation. Am J Obstet Gynecol. 1983;145:813–8.

3. Parer JT. The effect of acute maternal hypoxia on fetal oxygenation and the umbilical circulation in the sheep. Eur J Obstet Gynecol Reprod Biol. 1980; 10:125–36.
4. Parer JT, Livingston EG. What is fetal distress? Am J Obstet Gynecol. 1990;162:1421–5.
5. Macfarlane CM, Tsakalakos N. Evidence of hyperinsulinaemia and hypoxaemia in the cord blood of neonates born to mothers with gestational diabetes. S Afr Med J. 1985;67:81–4.
6. Galanti B, Kaihura CT, Ricci L, Bedocchi L, Rossi T, Benassi G, et al. Perinatal morbidity and mortality in children born to mothers with gestational hypertension. Acta Biomed Ateneo Parmense. 2000;71:361–5.
7. Thilaganathan B, Salvesen DR, Abbas A, Ireland RM, Nicolaides KH. Fetal plasma erythropoietin concentration in red blood cell-isoimmunized pregnancies. Am J Obstet Gynecol. 1992;167:1292–7.
8. Kendall G, Peebles D. Acute fetal hypoxia: the modulating effect of infection. Early Hum Dev. 2005; 81:27–34.
9. Manzar S. Maternal sickle cell trait and fetal hypoxia. Am J Perinatol. 2000;17:367–70.
10. Mukherjee AB, Hodgen GD. Maternal ethanol exposure induces transient impairment of umbilical circulation and fetal hypoxia in monkeys. Science. 1982; 218:700–2.
11. Witlin AG. Asthma in pregnancy. Semin Perinatol. 1997;21:284–97.
12. Tomson T, Danielsson BR, Winbladh B. Epilepsy and pregnancy. Balancing between risks to the mother and child. Lakartidningen. 1997;94:2827–35.
13. Socol ML, Manning FA, Murata Y, Druzin ML. Maternal smoking causes fetal hypoxia: experimental evidence. Am J Obstet Gynecol. 1982;142:214–8.
14. Maier RF, Bialobrzeski B, Gross A, Vogel M, Dudenhausen JW, Obladen M. Acute and chronic fetal hypoxia in monochorionic and dichorionic twins. Obstet Gynecol. 1995;86:973–7.
15. Stubblefield PG, Berek JS. Perinatal mortality in term and post-term births. Obstet Gynecol. 1980;56:676–82.
16. Salafia CM, Minior VK, Lopez-Zeno JA, Whittington SS, Pezzullo JC, Vintzileos AM. Relationship between placental histologic features and umbilical cord blood gases in preterm gestations. Am J Obstet Gynecol. 1995;173:1058–64.
17. Leszczynska-Gorzelak B, Poniedzialek-Czajkowska E, Oleszczuk J. Fetal blood saturation during the 1st and 2nd stage of labor and its relation to the neonatal outcome. Gynecol Obstet Invest. 2002;54:159–63.
18. Yamada T, Yamada T, Morikawa M, Minakami H. Clinical features of abruptio placentae as a prominent cause of cerebral palsy. Early Hum Dev. 2012; 88:861–4.
19. Kovalovszki L, Villanyi E, Benko G. Placental villous edema: a possible cause of antenatal hypoxia. Acta Paediatr Hung. 1990;30:209–15.
20. Faiz SA, Habib FA, Sporrong BG, Khalil NA. Results of delivery in umbilical cord prolapse. Saudi Med J. 2003;24:754–7.
21. Mukhopadhyay S, Arulkumaran S. Breech delivery. Best Pract Res Clin Obstet Gynaecol. 2002;16:31–42.
22. Preston R, Crosby ET, Kotarba D, Dudas H, Elliott RD. Maternal positioning affects fetal heart rate changes after epidural analgesia for labour. Can J Anaesth. 1983;40:1136–41.
23. Marshall JM. The Joan Mott Prize Lecture. The integrated response to hypoxia: from circulation to cells. Exp Physiol. 1999;84:449–70.
24. Rudolph AM, Heymann MA. The fetal circulation. Annu Rev Med. 1968;19:195–206.
25. Rudolph AM, Itskovitz J, Iwamoto H, Reuss ML, Heymann MA. Fetal cardiovascular responses to stress. Semin Perinatol. 1981;5:109–21.
26. Cohn HE, Sacks EJ, Heymann MA, Rudolph AM. Cardiovascular responses to hypoxemia and acidemia in fetal lambs. Am J Obstet Gynecol. 1974;120:817–24.
27. Fisher DJ, Heymann MA, Rudolph AM. Fetal myocardial oxygen and carbohydrate consumption during acutely induced hypoxemia. Am J Physiol. 1982; 242:H657–61.
28. Kirkpatrick SE, Pitlick PT, Naliboff J, Friedman WF. Frank-Starling relationship as an important determinant of fetal cardiac output. Am J Physiol. 1976; 231:495–500.
29. Thakor AS, Richter HG, Kane AD, Dunster C, Kelly FJ, Poston L, et al. Redox modulation of the fetal cardiovascular defence to hypoxaemia. J Physiol. 2010; 588:4235–47.
30. Giussani DA, Spencer JA, Moore PJ, Bennet L, Hanson MA. Afferent and efferent components of the cardiovascular reflex responses to acute hypoxia in term fetal sheep. J Physiol. 1993;461:431–49.
31. van Bel F, Sola A, Roman C, Rudolph AM. Role of nitric oxide in the regulation of the cerebral circulation in the lamb fetus during normoxemia and hypoxemia. Biol Neonate. 1995;68:200–10.
32. Jones CT, Robinson RO. Plasma catecholamines in foetal and adult sheep. J Physiol. 1975;248:15–33.
33. Fletcher AJ, Edwards CM, Gardner DS, Fowden AL, Giussani DA. Neuropeptide Y in the sheep fetus: effects of acute hypoxemia and dexamethasone during late gestation. Endocrinology. 2000;141:3976–82.
34. Perez R, Espinoza M, Riquelme R, Parer JT, Llanos AJ. Arginine vasopressin mediates cardiovascular responses to hypoxemia in fetal sheep. Am J Physiol Regul Integr Comp Physiol. 1989;256:R1011–8.
35. Morrison S, Gardner DS, Fletcher AJ, Bloomfield MR, Giussani DA. Enhanced nitric oxide activity offsets peripheral vasoconstriction during acute hypoxaemia via chemoreflex and adrenomedullary actions in the sheep fetus. J Physiol. 2003;547:283–91.
36. Gardner DS, Fowden AL, Giussani DA. Adverse intrauterine conditions diminish the fetal defense to acute hypoxia by increasing nitric oxide activity. Circulation. 2002;106:2278–83.
37. Gardner DS, Giussani DA. Enhanced umbilical blood flow during acute hypoxemia following chronic umbilical cord compression: a role for nitric oxide. Circulation. 2003;108:331–5.

38. Thakor AS, Herrera EA, Serón-Ferré M, Giussani DA. Melatonin and vitamin C increase umbilical blood flow via nitric oxide-dependent mechanisms. J Pineal Res. 2010;49:399–406.
39. Kane AD, Herrera EA, Hansell JA, Giussani DA. Statin treatment depresses the fetal defence to acute hypoxia via increasing nitric oxide bioavailability. J Physiol. 2012;590:323–34.
40. Herrera EA, Kane AD, Hansell JA, Thakor AS, Allison BJ, Niu Y, et al. A role for xanthine oxidase in the control of fetal cardiovascular function in late gestation sheep. J Physiol. 2012;590:1825–37.
41. Barker DJP. Mothers, babies and disease in later life. London: BMJ Publishing Group; 1994.
42. Brenner BM. The etiology of adult hypertension and progressive renal injury: an hypothesis. Bull Mem Acad R Med Belg. 1994;149:121–5.
43. Phillips DI, Hirst S, Clark PM, Hales CN, Osmond C. Fetal growth and insulin secretion in adult life. Diabetologia. 1994;37:592–6.
44. Kamitomo M, Alonso JG, Okai T, Longo LD, Gilbert RD. Effects of long-term, high-altitude hypoxemia on ovine fetal cardiac output and blood flow distribution. Am J Obstet Gynecol. 1993;169:701–7.
45. Giussani DA, Davidge ST. Developmental programming of cardiovascular disease by prenatal hypoxia. J Dev Orig Health Dis. 2013;4:328–37.
46. Giussani DA, Salinas CE, Villena M, Blanco CE. The role of oxygen in prenatal growth: studies in the chick embryo. J Physiol. 2007;585:911–7.
47. Salinas CE, Blanco CE, Villena M, Camm EJ, Tuckett JD, Weerakkody RA, et al. Cardiac and vascular disease prior to hatching in chick embryos incubated at high altitude. J Dev Orig Health Dis. 2010;1:60–6.
48. McEniery CM, Wilkinson IB. Large artery stiffness and inflammation. J Hum Hypertens. 2005;19:507–9.
49. Arnett DK, Evans GW, Riley WA. Arterial stiffness: a new cardiovascular risk factor? Am J Epidemiol. 1994;140:669–82.
50. Crispi F, Figueras F, Cruz-Lemini M, Bartrons J, Bijnens B, Gratacos E. Cardiovascular programming in children born small for gestational age and relationship with prenatal signs of severity. Am J Obstet Gynecol. 2012;207:121.e1–9.
51. Skilton MR, Evans N, Griffiths KA, Harmer JA, Celermajer DS. Aortic wall thickness in newborns with intrauterine growth restriction. Lancet. 2005;365:1484–6.
52. Koklu E, Kurtoglu S, Akcakus M, Koklu S, Buyukkayhan D, Gumus H, et al. Increased aortic intima-media thickness is related to lipid profile in newborns with intrauterine growth restriction. Horm Res. 2006;65:269–75.
53. Akira M, Yoshiyuki S. Placental circulation, fetal growth, and stiffness of the abdominal aorta in newborn infants. J Pediatr. 2006;148:49–53.
54. Cosmi E, Visentin S, Fanelli T, Mautone AJ, Zanardo V. Aortic intima media thickness in fetuses and children with intrauterine growth restriction. Obstet Gynecol. 2009;114:1109–14.
55. Keyes LE, Armaza JF, Niermeyer S, Vargas E, Young DA. Intrauterine growth restriction, preeclampsia, and intrauterine mortality at high altitude in Bolivia. Pediatr Res. 2003;54:20–5.
56. Herrera EA, Reyes RV, Giussani DA, Riquelme RA, Sanhueza EM, Ebensperger G, et al. Carbon monoxide: a novel pulmonary artery vasodilator in neonatal llamas of the Andean altiplano. Cardiovasc Res. 2008;77:197–201.
57. Gassmann M, Ogunshola OO, Tissot van Patot M. The impact of hypoxia on cells, mice, and men. High Alt Med Biol. 2012;13:63–4.
58. Gilbert RD. Fetal myocardial responses to long-term hypoxemia. Comp Biochem Physiol A Mol Integr Physiol. 1998;119:669–74.
59. Sharma SK, Lucitti JL, Nordman C, Tinney JP, Tobita K, Keller BB. Impact of hypoxia on early chick embryo growth and cardiovascular function. Pediatr Res. 2006;59:116–20.
60. Patterson AJ, Zhang L. Hypoxia and fetal heart development. Curr Mol Med. 2010;10:653–66.
61. Davidge ST, Morton JS, Rueda-Clausen CF. Oxygen and perinatal origins of adulthood diseases: is oxidative stress the unifying element? Hypertension. 2008;52:808–10.
62. Giussani DA, Camm EJ, Niu Y, Richter HG, Blanco CE, Gottschalk R, et al. Developmental programming of cardiovascular dysfunction by prenatal hypoxia and oxidative stress. PLoS One. 2012;7:e31017.
63. Danson EJ, Li D, Wang L, Dawson TA, Paterson DJ. Targeting cardiac sympatho-vagal imbalance using gene transfer of nitric oxide synthase. J Mol Cell Cardiol. 2009;46:482–9.
64. Bristow MR. Beta-adrenergic receptor blockade in chronic heart failure. Circulation. 2002;101:558–69.
65. Camm EJ, Martin-Gronert MS, Wright NL, Hansell JA, Ozanne SE, Giussani DA. Prenatal hypoxia independent of undernutrition promotes molecular markers of insulin resistance in adult offspring. FASEB J. 2011;25:420–7.
66. Rueda-Clausen CF, Dolinsky VW, Morton JS, Proctor SD, Dyck JR, Davidge ST. Hypoxia-induced intrauterine growth restriction increases the susceptibility of rats to high-fat diet-induced metabolic syndrome. Diabetes. 2011;60:507–16.
67. Dolinsky VW, Rueda-Clausen CF, Morton JS, Davidge ST, Dyck JRB. Continued postnatal administration of resveratrol prevents diet-induced metabolic syndrome in offspring born growth restricted. Diabetes. 2011;60:2274–84.
68. Kane AD, Herrera EA, Camm EJ, Giussani DA. Vitamin C prevents intrauterine programming of in vivo cardiovascular dysfunction in the rat. Circ J. 2013;77:2604–11.
69. Richter HG, Camm EJ, Modi BN, Naeem F, Cross CM, Cindrova-Davies T, et al. Ascorbate prevents placental oxidative stress and enhances birth weight in hypoxic pregnancy in rats. J Physiol. 2012;590:1377–87.
70. Richter HG, Hansell JA, Raut S, Giussani DA. Melatonin improves placental efficiency and birth

weight and increases the placental expression of antioxidant enzymes in undernourished pregnancy. J Pineal Res. 2009;46:357–64.
71. Hecher K, Snijders R, Campbell S, Nicolaides K. Fetal venous, intracardiac, and arterial blood flow measurements in intrauterine growth retardation: relationship with fetal blood gases. Am J Obstet Gynecol. 1995;173:10–5.
72. Poston L, Briley AL, Seed PT, Kelly FJ, Shennan AH. Vitamins in Pre-eclampsia (VIP) Trial Consortium. Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial. Lancet. 2006; 367:1145–54.
73. Rumbold A, Duley L, Crowther CA, Haslam RR. Antioxidants for preventing pre-eclampsia. Cochrane Database Syst Rev. 2008;1, CD004227.
74. Massey LK, Liebman M, Kynast-Gales SA. Ascorbate increases human oxaluria and kidney stone risk. J Nutr. 2005;135:1673–7.
75. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: University Press; 2004.
76. Smith RA, Murphy MP. Animal and human studies with the mitochondria-targeted antioxidant MitoQ. Ann N Y Acad Sci. 2010;1201:96–103.
77. Kelso GF, Porteous CM, Coulter CV, Hughes G, Porteous WK, Ledgerwood EC, et al. Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties. J Biol Chem. 2001;276:4588–96.
78. Graham D, Huynh NN, Hamilton CA, Beattie E, Smith RA, Cochemé HM, et al. Mitochondria-targeted antioxidant MitoQ10 improves endothelial function and attenuates cardiac hypertrophy. Hypertension. 2009;54:322–8.
79. Rodriguez-Cuenca S, Cochemé HM, Logan A, Abakumova I, Prime TA, Rose C, et al. Consequences of long-term oral administration of the mitochondria-targeted antioxidant MitoQ to wild-type mice. Free Radic Biol Med. 2010;48:161–72.