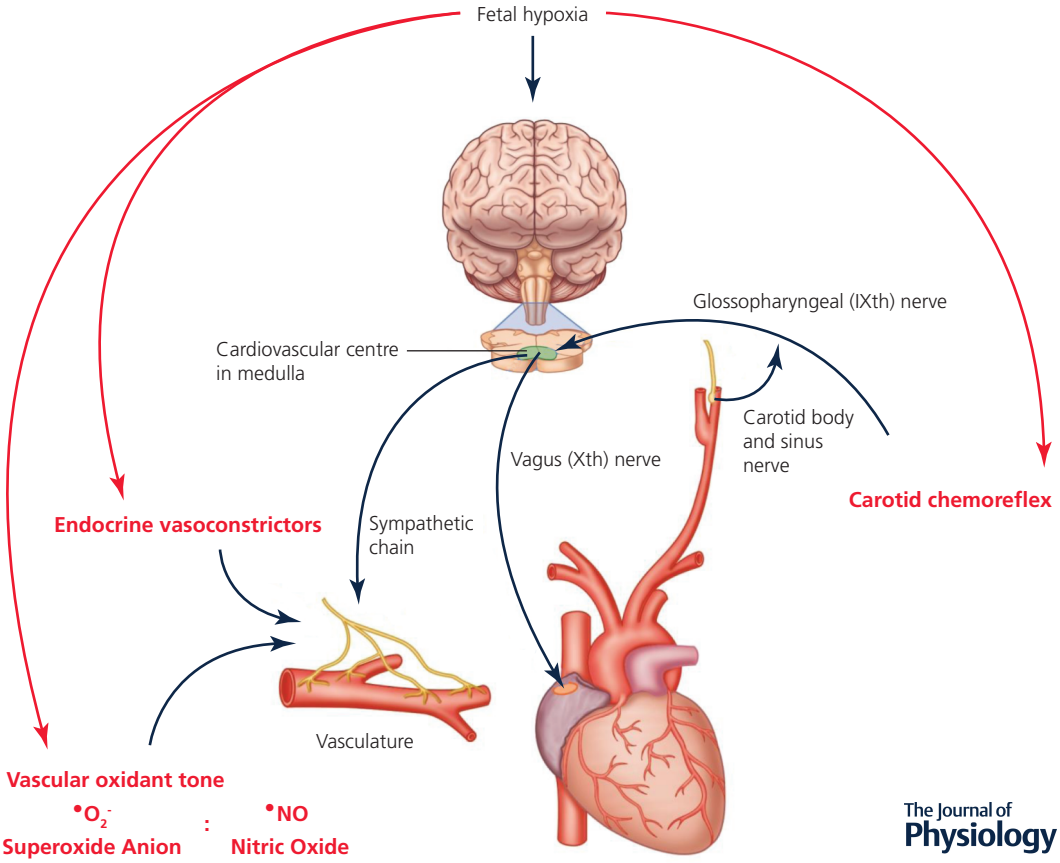

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The Fetal Brain Sparing Response to Hypoxia: Physiological Mechanisms

Dino A Giussani

Department of Physiology, Development & Neuroscience, University of Cambridge, Cambridge, CB2 3EG, UK

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Correspondence: Professor Dino A Giussani, Ph.D.
Department of Physiology Development & Neuroscience
Downing Street
University of Cambridge
Cambridge
CB2 3EG
UK
Tel: +44 1223 333894
E-mail: dag26@cam.ac.uk

ABSTRACT

How the fetus withstands an environment of reduced oxygenation during life in the womb has been a vibrant area of research since this field was introduced by Joseph Barcroft, a century ago. Studies spanning five decades have since used the chronically instrumented fetal sheep preparation to investigate the fetal compensatory responses to hypoxia. This defence is contingent on the fetal cardiovascular system, which in late gestation adopts strategies to decrease oxygen consumption and redistribute the cardiac output away from peripheral vascular beds and towards essential circulations, such as those perfusing the brain. The introduction of simultaneous measurement of blood flow in the fetal carotid and femoral circulations by ultrasonic transducers has permitted investigation of the dynamics of the fetal brain sparing response for the first time. Now we know that major components of fetal brain sparing during acute hypoxia are triggered exclusively by a carotid chemoreflex and that they are modified by endocrine agents and the recently discovered vascular oxidant tone. The latter is determined by the interaction between nitric oxide and reactive oxygen species. The fetal brain sparing response matures as the fetus approaches term, in association with the prepartum increase in fetal plasma cortisol and treatment of the preterm fetus with clinically-relevant doses of synthetic steroids mimics this maturation. Despite intense interest into how the fetal brain sparing response may be affected by adverse intrauterine conditions, this area of research has been comparatively scant but it is likely to take centre stage in the near future.

Oxygen deprivation or hypoxia is one of the most common challenges in fetal life. Short term episodes of acute hypoxia, perhaps lasting a few minutes, are associated with labour and delivery, as a result of uterine contractions and/or compressions of the umbilical cord (Huch *et al.* 1977). Oxygen deprivation to the unborn child lasting for several weeks or even months is denominated chronic fetal hypoxia. This is the most common consequence of complicated pregnancy resulting from increased placental vascular resistance, as occurs during placental insufficiency, preeclampsia or any inflammatory condition during pregnancy, such as chorioamnionitis, gestational diabetes or even maternal obesity (see Table 1). An inadequate fetal defence to acute or chronic hypoxia renders the fetal brain susceptible to injury leading to hypoxic-ischaemic encephalopathy (Low *et al.* 1985; Gunn & Bennet, 2009). The latter has long been known to be predictive of developing cerebral palsy and cognitive disability later in life (Hall, 1989). Therefore, the physiology underlying the fetal defence to hypoxia remains at the forefront of basic science and clinical interest.

Fetal versus adult defence response to hypoxia

Outside the womb, the supply of oxygen from the atmosphere is vast. Therefore, in the adult period, episodes of hypoxia trigger both ventilator and cardiovascular compensatory responses. These are designed to increase pulmonary oxygenation and cardiac output, permitting the maintenance of perfusion of oxygenated blood even to peripheral circulations during periods of systemic hypoxia (Rowell & Blackmon, 1987; Marshall, 1999). Inside the womb, the supply of oxygenated blood is comparatively finite as it is limited by the placenta. However, a number of adaptations unique to fetal life ensure that the supply of oxygen to the fetus exceeds its metabolic demands. Therefore, under basal conditions during development, the unborn child is equipped with a considerable margin of safety for

oxygenation. 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 this bound oxygen to the fetal tissues at lower oxygen tensions (Barcroft, 1935; Rudolph & Heymann, 1968; Maurer *et al.* 1970). Shunts in the fetal circulation, such as the *ductus venosus* and *ductus arteriosus* and preferential streaming further ensure an adequate supply of oxygenated blood to tissues most at risk of damage during adverse conditions (Rudolph & Heymann, 1968; Edelstone, 1980; Itskovitz *et al.* 1987; Godfrey *et al.* 2012). The fetus has also a greater capacity than the adult to hinder oxygen-consuming processes. The fetal defence strategy during episodes of acute hypoxia concentrate on increasing the efficiency of these compensatory mechanisms, thereby either consuming even less oxygen (Boyle *et al.* 1990), extracting even more oxygen from haemoglobin (Edelstone & Holzman, 1982; Gardner *et al.* 2003) or making better use of this limited supply of oxygenated blood (Rudolph, 1984; Cohn *et al.* 1974; Giussani *et al.* 1993; 1994). The responses of the fetal cardiovascular system during episodes of acute hypoxia illustrate some of these strategies.

When the late gestation fetus is exposed to acute hypoxia, fetal breathing movements cease (Boddy *et al.* 1974; Bekedam & Visser, 1985; Giussani *et al*. 1995) and the fetal heart rate decreases (Boddy *et al*. 1974), both responses favouring a fall in fetal oxygen consumption (Rurak & Gruber, 1983; Fisher *et al.* 1982). The reduction in fetal heart rate prolongs end diastolic filling time, increases end diastolic volume and thereby contributes to the maintenance of cardiac output and perfusion pressure despite bradycardia (Anderson *et al*. 1986). The resulting increases in ventricular stretch will enhance sarcomere length, tension and contractility by means of the Frank-Starling mechanism, which has been shown to

be operational in the late gestation fetus (Kirkpatrick *et al*. 1976). Through this mechanism, left and right ventricular stroke volumes are relatively well-maintained in the face of increases in afterload (Hawkins *et al*. 1989), with the left ventricle having a greater reserve capacity for increases in afterload than the right ventricle (Reller *et al*. 1987). Although increases in preload and ventricular filling pressure may help maintain cardiac output during acute hypoxia, the Frank Starling mechanism may be somewhat limited to increase cardiac output above baseline during fetal life. This is partly due to the working point of the fetal circulation being close to or on the plateaux of the ventricular function curves, thereby limiting the extent to which increases in ventricular stroke volume can actually lead to elevations in end diastolic ventricular filling pressures (Gilbert 1980; Thornburg & Morton 1986). Fetal heart decelerations also slow down the passage of blood through the circulation, increasing the efficiency of gaseous exchange in essential vascular beds (Boudoulas *et al*. 1979). In addition to fetal cardiac compensatory mechanisms, the fetal blood flow is redistributed in response to acute hypoxia away from peripheral vascular beds and prioritised towards essential circulations, such as those perfusing the brain - the so called 'brain sparing effect'. Since oxygen delivery is coupled to oxygen consumption, limiting blood flow to less essential vascular beds such as the fetal intestines and fetal hind limbs also contributes to the overall decrease in oxygen consumption by the fetal tissues during acute hypoxia (Edelstone & Holzman, 1982; Boyle *et al*. 1990). Decreased oxygen delivery to the hind limbs increases lactate output, acidifying the fetal blood which facilitates the unloading of oxygen from haemoglobin to the fetal tissues as the fetal blood becomes hypoxic (Gardner *et al.* 2003). Independent seminal studies have provided evidence of the end effect of this circulatory redistribution by calculation of the resulting blood flow in the target organs using radioactive or fluorescent microspheres (Rudolph & Heymann 1968; Cohn *et al.* 1974; Peeters *et*

al. 1979; Reuss *et al.* 1982; Rudolph, 1984; Yaffe *et al.* 1987; Rudolph, 1985; Peréz *et al*. 1989; Jansen *et al.* 1989; Mulder *et al*. 1998). However, simultaneous measurement of carotid blood flow and femoral blood flow in response to acute hypoxia in the late gestation fetus by Transonic flowmetry permitted visualisation of the dynamics of this fetal brain sparing response in real time for the first time (Giussani *et al.* 1993; 1994a; Figure 1). This revealed the fast speed of onset of some of these defence responses, implicating the involvement of neural reflexes. The technique also permits calculation of the ratio of simultaneous carotid relative to femoral blood flow during basal and acute hypoxic conditions. This ratio, which I have called 'the fetal brain sparing index' clearly increases during acute hypoxia (Figure 1).

Fetal brain sparing: Neural, endocrine and local components

While vasodilatation in the fetal cerebral vascular bed during acute hypoxia occurs as result of local increases in adenosine and, to a lesser extent, nitric oxide (NO) and prostanoids (Kjellmer *et al.* 1989; van Bel *et al*. 1995; Pearce, 1995; Green *et al.* 1996; Blood *et al*. 2002; Hunter *et al*. 2003; Nishida *et al.* 2006), the fetal bradycardia and the fetal peripheral vasoconstriction are now known to be triggered by a chemoreflex. Early experiments by Dawes and colleagues in the 1960's introduced the idea of fetal peripheral chemoreflexes being active *in utero* (Dawes *et al.* 1968; 1969). Those experiments were performed in exteriorised fetuses exposed to relative hypoxia from an artificially elevated oxygenation baseline under the effects of general anaesthesia. Under such conditions, early ideas suggested that aortic chemoreceptors were more sensitive than carotid chemoreceptors to stimulants, such as hypoxia. Deriving information from experiments using chronically instrumented, un-anaesthetised fetal sheep preparations in late gestation, it is now widely accepted that the fetal bradycardia and peripheral

vasoconstrictor response to acute hypoxia are exclusively triggered by carotid and not aortic chemoreflexes. It has been shown that selective carotid (Giussani *et al*. 1993) but not aortic (Itskovitz *et al*. 1991; Bartelds *et al.* 1993) chemo-denervation completely prevents the fetal bradycardia and the initial increase in femoral vascular resistance during acute hypoxia (Figure 1). Bilateral section of the carotid sinus nerves also diminishes the increase in the fetal brain sparing ratio (Figure 1) and prevents the initial fall in renal (Green *et al*. 1997) and pulmonary arterial (Moore & Hanson, 1991) blood flow in response to acute hypoxia in the late gestation fetus. This highlights the greater contribution of the carotid chemoreceptors over the aortic chemoreceptors in mediating the redistribution of blood flow away from the periphery and towards the brain during periods of oxygen deprivation in the fetus.

Since the seminal study of López-Barneo *et al.* (1998), discussion of the cellular processes within the carotid body that give sensitivity of this tissue to stimulants, such as fall in oxygenation, is still vibrant even for the adult individual; this being the topic of several elegant reviews and editorials (Kumar and Prabhakar, 2012; Vilares Conde and Peers, 2013). It is generally accepted that ion channels are critical to this process, involving inhibition of potassium currents, depolarization of cell membranes and elevation in intracellular calcium. However, more recent evidence suggests that gasotransmitters may also be involved (Prabhakar and Semenza, 2012; Kemp and Telezhkin, 2014). Compared to the physiology of oxygen sensing in the adult period, research on cellular mechanisms mediating carotid body sensitivity in fetal life are almost absent. A comprehensive report by Koos (2011) put forward the idea that adenosine A_{2A} receptors mediate fetal chemoreflex responses, suggesting that oxygen sensing in the carotid bodies in fetal life is critically linked to activation of 5'nucleotidase. Work in our group

support this idea, since treatment of the late gestation sheep fetus with the adenosine receptor antagonist 8-(p-sulfophenyl)-theophylline prevented fetal bradycardia and fetal femoral vasoconstriction during acute hypoxia in a manner similar to bilateral section of the carotid sinus nerves (Giussani *et al.* 2001). A report by the laboratory of Lagercrantz stated that selective down-regulation of HIF-1α may be involved in the postnatal maturation of carotid body responsiveness to hypoxia (Roux *et al*. 2005).

In fetal life, carotid chemoreflex influences on the fetal brainstem lead to an increase in both sympathetic and vagal outflow to the fetal heart, however vagal influences predominate leading to a fall in fetal heart rate (Court & Parer, 1984). Therefore, fetal treatment with the muscarinic antagonist atropine not only blocks bradycardia but leads to an increase in fetal heart rate, unveiling the unopposed increased cardiac sympathetic drive during acute hypoxia (Court & Parer, 1984; Giussani *et al.* 1993). Carotid chemoreflex influences on the fetal brainstem also increase sympathetic outflow to peripheral vascular beds. Vasoconstriction in fetal peripheral circulations is prevented by chemical sympathectomy (Iwamoto *et al.* 1983; Booth *et al.* 2012), denervation of sympathetic efferent pathways (Robillard *et al.* 1986, Booth *et al.* 2012) or by fetal treatment with alpha adrenergic receptor antagonists, such as phentolamine or phenoxybenzamine (Lewis *et al*. 1980; Reuss *et al.* 1982; Giussani *et al*. 1993). Once triggered by the carotid chemoreflex, the fetal heart and circulatory responses are modified by the release of chemicals into the fetal circulation. Measureable increases in plasma catecholamines, vasopressin, angiotensin II and neuropeptide Y occur by 15 minutes from the onset of acute hypoxia (Broughton-Pipkin *et al.* 1974; Jones & Robinson, 1975; Rurak, 1978; Giussani *et al*. 1994b; Fletcher *et al.* 2000; 2006). The increase in fetal plasma adrenaline and noradrenaline oppose vagal inputs to the heart, returning fetal heart

rate to baseline values by 30 minutes from the onset of acute hypoxia (Jones & Ritchie, 1983). Accordingly, fetal treatment with the beta blocker propranolol prolongs the fetal bradycardic response to acute hypoxia (Court & Parer, 1984). The increase in catecholamines and other constrictor agents in the fetal circulation also maintain the neurally-triggered peripheral vasoconstrictor response, not only prolonging redistribution of the fetal cardiac output but helping maintain perfusion pressure as the episode of acute hypoxia continues. Therefore, fetal treatment with alpha-adrenergic or vasopressin receptor antagonists decrease the ability of the fetus to maintain peripheral vascular resistance and arterial blood pressure during acute hypoxia (Peréz *et al*. 1989; Giussani *et al*. 1993). There is some evidence that during periods of fetal oxygen deprivation the carotid chemoreflex is also involved in affecting the release of catecholamines from the adrenal medulla (Jensen & Hanson, 1995) and in sensitising the adrenal cortex to ACTH (Giussani *et al*. 1994a), enhancing the release of cortisol into the fetal circulation. By contrast, the carotid chemoreceptors are not involved in the fetal plasma vasopressin or angiotensin II response to acute hypoxia (Giussani *et al.* 1994b; Green *et al*. 1998). Some studies have implicated calcitonin gene related peptide (cGRP) as having an important role in the activation of the sympathetic nervous system during acute hypoxia in the late gestation fetus. It has been reported that treatment of fetal sheep with cGRP antagonists markedly diminished the fetal femoral vasoconstrictor and the plasma NPY and catecholamine responses to acute hypoxia (Thakor *et al*. 2005).

It is also known that fetal carotid chemoreflex and endocrine constrictor influences on the fetal peripheral circulations are opposed by hypoxia-induced increases in the dilator gas nitric oxide (NO). Therefore, fetal treatment with the NO clamp, a technique that permits *de novo* synthesis of NO to be blocked while maintaining

basal cardiovascular function (Gardner *et al*. 2001; 2002b; Gardner & Giussani, 2003), revealed a significantly greater femoral vasoconstrictor response to acute hypoxia in the late gestation fetus (Morrison *et al.* 2003). More recently, in the last few years, our group has made the discovery that the influence of NO at the level of the fetal vasculature is itself limited by the generation of reactive oxygen species (ROS) during acute hypoxia (Thakor *et al*. 2010b). This, in essence, creates a vascular oxidant tone that contributes to the regulation of blood flow in the fetal circulation and one that can be manipulated in favour of constriction or dilatation by altering the numerator or denominator of the fraction. Hence, treatment of the sheep fetus with antioxidants, such as vitamin C (Thakor *et al*. 2010b), allopurinol (Kane *et al*. 2014), melatonin (Thakor *et al.* 2015) or agents that increase NO, such as statins (Kane *et al*. 2012), all shift the vasoactive balance in favour of dilatation, thereby diminishing the fetal peripheral vasoconstrictor response to acute hypoxia. Therefore, we now know that the magnitude of the fetal peripheral vasoconstrictor response to acute hypoxia, part of the fetal brain sparing response, represents the result of the combined influences of carotid chemoreflexes, endocrine responses and a vascular oxidant tone acting at the level of the fetal vasculature, the latter being determined itself by the interaction between NO and ROS (Figure 2). Of related interest are reports that fetal treatment with antioxidants can also increase blood flow above basal levels in NO sensitive circulations, such as in the umbilical vascular bed, via quenching ROS and increasing NO bioavailability (Thakor *et al*. 2010a Derks *et al*. 2010; Herrera *et al*. 2012). This finding is of significance because, clinically, it is generally believed that basal placental and umbilical blood flow in late gestation is maximal. This is clearly not true.

Since fetal treatment with antioxidants diminishes the fetal peripheral vasoconstrictor response to acute hypoxia, it has been suggested that fetal

exposure to antioxidants, for instance via maternal antioxidant supplementation, might weaken the fetal brain sparing response to acute hypoxia (Thakor *et al*. 2010b; Kane *et al*. 2012; 2014; Thakor *et al.* 2015; Peebles *et al.* 2012). However, while enhanced NO bioavailability in the fetal circulation as a result of antioxidant treatment might oppose constriction in fetal peripheral circulations, it may maintain or even enhance blood flow in the cerebral circulation in which NO contributes to the local vasodilator response (Thakor *et al.* 2015; Peebles *et al.* 2012). Hence, under conditions of fetal exposure to antioxidants, maintenance of cerebral blood flow might not be necessarily compromised. However, the fetal cardiovascular strategy to defend itself against acute hypoxia may need to change to increase cardiac output and maintain perfusion in the presence of a generalised dilator response, akin, interestingly, to the response to acute hypoxia in the adult period (Rowell & Blackmon, 1987; Marshall, 1999). Furthermore, several studies have dissociated the fetal peripheral vasoconstrictor and cerebral vasodilator responses to acute hypoxia. For example, the magnitude of the increase in carotid blood flow during acute hypoxia is similar in intact fetuses and in carotid sinus denervated fetal sheep, despite an attenuated peripheral vasoconstriction and fetal brain sparing ratio in the latter group (Giussani *et al*. 1993; Figure 1). Similarly, the magnitude of the increase in carotid blood flow (Giussani *et al*. 1993) and of the decrease in the vascular resistance in the cerebral (Reuss *et al*. 1982) vascular beds during acute hypoxia was similar in untreated fetuses and in fetuses treated with α-adrenergic receptor antagonists, despite abolition of peripheral vasoconstriction in the latter groups. Therefore, while the fetal peripheral vasoconstrictor response aids the redistribution of blood flow away from less essential vascular beds, it is not indispensable at least to the maintenance of carotid blood flow during acute hypoxia in the late gestation fetus.

Maturation of the fetal brain sparing response to acute hypoxia

Prior to *ca.* 110 days of approximately a 150 day gestation, the sheep fetus has an immature cardiovascular defence to acute hypoxic stress. This includes tachycardia rather than bradycardia and an inability to increase peripheral vascular resistance and maintain arterial blood pressure (Boddy *et al*. 1974; Iwamoto *et al.* 1989). After *ca.* 120 days of gestation, the pattern and the magnitude of the fetal heart and circulatory responses to acute hypoxia change as the fetus approaches term, in close temporal association with the prepartum increase in fetal plasma cortisol. As the fetus approaches term, the bradycardic response to acute hypoxia switches from being transient to becoming sustained and more pronounced (Fletcher *et al*. 2006). In addition, the femoral vasoconstrictor response to acute hypoxia becomes much greater with advancing gestation (Fletcher *et al*. 2006; Figure 3). The physiology underlying the enhanced and sustained bradycardia in the term compared with the preterm fetus includes maturation of carotid body chemoreflexes and reciprocal changes in the responsiveness of fetal heart to autonomic agonists. While fetal cardiac reactivity to muscarinic agonists increases, it decreases to β_1-adrenergic stimulation, thereby promoting cardiac vagal dominance with increased maturation (Fletcher *et al*. 2005; 2006). Similarly, the greater increment in fetal femoral vascular resistance during acute hypoxia with advancing gestational age results, in part, from maturation of the carotid body chemoreflex and, in part, from greater plasma catecholamine, vasopressin and neuropeptide Y responses (Fletcher *et al*. 2006). During episodes of acute hypoxia, the adrenal output of catecholamines may be stimulated both by the direct effects of hypoxia on the gland and by neuronal stimulation (Jones *et al*. 1988), especially following the establishment of splanchnic nerve synapses with adrenal chromaffin cells at around 130 days of gestation (Boshier *et al*. 1989). The relative contribution of these two stimuli in promoting adrenal catecholamine secretion during acute hypoxia also

changes with advancing gestation, reported both in the acutely-exteriorised, anaesthetised fetal sheep preparation (Comline & Silver, 1961) and in the un-anaesthetised chronically instrumented fetal sheep preparation (Cheung, 1990). Comline & Silver (1961) studied the effects of splanchnic nerve section on the outputs of adrenaline and noradrenaline from the adrenal gland in response to asphyxia in pentobarbitone-anaesthetised fetal sheep from 80 days to term. The degree of attenuation of the noradrenaline and adrenaline outputs following splanchnic nerve section increased with advancing gestational age, suggesting an increasing dependence of the adrenal medulla on innervation to respond to acute oxygen deprivation. In addition, it was shown that the adrenal outputs of adrenaline and noradrenaline evoked by electrical stimulation of the splanchnic nerves increased with advancing gestational age. The timing of the increase in fetal adrenal noradrenaline content is coincident with an increase in adrenal tyrosine hydroxylase mRNA levels and the onset of splanchnic innervation of the fetal adrenal gland (80-120 days; see McMillen *et al*. 1997). The main increase in adrenaline content occurs after 130 days, in close temporal association with an increase in phenylethanolamine N-methyltransferase (PNMT) mRNA and the prepartum increase in adrenal glucocorticoid production (McMillen *et al.* 1997). Using the un-anaesthetised chronically instrumented fetal sheep preparation in combination with hexamethonium blockade, Cheung (1990) detected both direct and neuronal release of catecholamines during hypoxia at 110 and 120 days, and showed that the adrenal medullary responses to hypoxia were solely under neuronal control by 130 days, again coinciding with completion of functional innervation (Boshier *et al*. 1989; see Cheung, 1990). However, if the adrenal gland is separated from its splanchnic innervation and is perfused *in vitro*, it can also respond directly to hypoxia even after 135 days (Adams *et al*. 1996). While information is available on the effects of gestational age on chemoreflex and

endocrine responses to acute hypoxia in the fetus, the contribution of alterations in the vascular oxidant tone to the fetal brain sparing response during hypoxia with advancing gestational age awaits investigation.

It is now also established that exposure of the preterm ovine fetus to synthetic glucocorticoids, such as dexamethasone or betamethasone, administered in human clinically-relevant doses, can switch the pattern and magnitude of the fetal heart and circulatory responses to acute hypoxia in similar fashion to advancing gestational age. Therefore, either fetal intravenous infusion with dexamethasone for 48h to yield human clinically relevant circulating doses of the synthetic steroid, or maternal intramuscular injection with a single course of dexamethasone of 2 x 12 mg 24h apart at 0.7-0.8 of gestation both switch the fetal heart and femoral constrictor responses to acute hypoxia from the immature to the mature phenotype (Fletcher *et al*. 2000b; 2003, 2006; Jellyman *et al*. 2005; 2009; Figure 4). As with advancing gestational age, synthetic steroids sensitise carotid chemoreflex function, they promote cardiac vagal dominance and enhance the fetal vasoconstrictor hormone responses to acute hypoxia (Fletcher *et al*. 2000b; 2003, 2006; Jellyman *et al*. 2005; 2009). Therefore, in obstetric practice, it should be now known that antenatal glucocorticoid therapy not only accelerates fetal lung maturation but also the capacity of the fetal cardiovascular system to respond to acute hypoxic stress.

An important point of related scientific and clinical interest is that the practise of intra-partum electronic fetal monitoring (EFM) has been implemented worldwide for several decades. Intra-partum EFM attempts to use changes in fetal heart rate patterns to identify fetuses with a suspected hypoxic or asphyxic compromise in order to deliver them before fetal cardiovascular collapse. Surprisingly, despite the knowledge that advancing gestational age as well as antenatal glucocorticoid

therapy affects the magnitude and pattern of the fetal heart rate, fetal endocrine and fetal metabolic responses to hypoxia (Fletcher *et al*. 2003, 2006; Jellyman *et al*. 2005; 2009), all of which contribute to alterations in fetal heart rate variability, how any of these factors influence EFM is not taken into account in the clinic. Clearly, investigation of the effects of gestational age and of antenatal glucocorticoid therapy on the mechanisms mediating changes in fetal heart rate variability is a highly significant area of urgently needed future clinical research.

The fetal brain sparing response during acute hypoglycaemia

Whether the carotid body can sense stimuli in addition to alterations in PO_2, PCO_2 and pH, such as glucose concentration, has been a matter of scientific interest for some time. Carotid body glomus cells have been found to detect hypoglycaemia in several non-primate mammals as well as humans in adult life (Alvarez-Buylla & de Alvarez-Buylla, 1988; for review, see Gao *et al*. 2014). Pardal and Lopez Barneo (2002) proposed a new glucose-sensing role for the carotid body that serves to integrate information about blood glucose and O_2 levels in adult animals. Consequently, there has been accruing interest in whether the carotid chemoreceptors might also contribute to a brain sparing response during acute episodes of glucose in addition to oxygen deprivation in fetal life. Insulin-induced fetal hypoglycaemia, without fetal hyperinsulinaemia, does promote a fall in basal heart rate and redistributes the fetal cardiac output in favour of essential vascular beds at the expense of peripheral circulations. However, the cardiovascular responses are not marked or rapid in onset and they take time to develop (Burrage *et al*. 2009; Cleal *et al*. 2010). Burrage *et al*. (2008) reported that carotid body denervation has subtle effects on differential perfusion, slowly influencing organ growth responses to maternal undernutrition in late gestation fetal sheep. Therefore, combined, current evidence suggests that the carotid bodies do not

trigger immediate neural compensatory cardiovascular responses to acute hypoglycaemia that may spare the fetal brain in a manner akin to fetal hypoxia. Rather, the fetal carotid bodies likely play a role in the longer term redistribution of blood flow, which may contribute to differential organ growth in pregnancy compromised by maternal undernutrition (Burrage *et al*. 2008).

The fetal brain sparing response in chronic hypoxic pregnancy

What happens to the fetal brain sparing response during chronic fetal hypoxia has been comparatively more difficult to study because of the technical difficulty to record fetal cardiovascular function *in vivo* in pregnancies complicated by chronic fetal hypoxia. However, slowly accumulating evidence is beginning to suggest that the fetal brain sparing response persists during chronic fetal hypoxia (Kamitomo *et al*. 1993; Richardson *et al.* 1993; Richardson & Bocking, 1998; Gardner *et al*. 2001; Morrison, 2008; Poudel *et al*. 2015; Allison *et al*. 2015). While persistent redistribution of the fetal cardiac output may serve to maintain oxygen and nutrient delivery to the brain during sub-optimal pregnancy, sustained reductions in oxygen and nutrient delivery to peripheral organs trigger a number of unwanted adverse side-effects. Amongst the best described is that fetuses are not only small but they are asymmetrically intrauterine growth restricted, being thin for their length or having a relatively normal sized head with a shorter body length (Barker, 1998; McMillen *et al*. 2001; Halliday, 2009; Giussani *et al*. 2007; Soria *et al*. 2013). Persistent redistribution of oxygen and nutrient delivery away from peripheral circulations may also help explain the reduced endowment of kidney nephrons (Dorey *et al*. 2014) and of pancreatic beta cells in offspring of compromised pregnancy (Snoeck *et al*. 1990; Limesand *et al*. 2005). Further, persistent increases in fetal peripheral vascular resistance in adverse pregnancy will increase fetal cardiac afterload, enforcing alterations in cardiomyocytes, remodelling of the

walls of the heart and major vessels (Veille *et al.* 1993; Skilton *et al*. 2005; Salinas *et al*. 2010) and resetting of the arterial baroreflex function (Fletcher *et al*. 2002). Not surprisingly, adverse intrauterine conditions have been consistently associated with an increased risk of cardiovascular, metabolic and renal diseases in the adult offspring (Barker, 1998; Fowden *et al.* 2006; Gluckman *et al.* 2008). Programmed cardiovascular disease in later life linked specifically to chronic fetal hypoxia has been recently reviewed by Giussani & Davidge (2013).

How adverse intrauterine conditions affect the capacity of the fetal cardiovascular system to unleash a brain sparing response during an acute hypoxic insult has been even less well studied because of the increasing layers of technological complexity modelling these situations. In adverse pregnancy, such as during placental insufficiency or preeclampsia, it is generally accepted that the chronically compromised fetus may not necessarily experience sustained, clamped reductions in oxygenation but that it may be exposed to progressive hypoxia or repeated periods of hypoxia and re-oxygenation or ischaemia and reperfusion, which are of varying magnitude and duration. Consequently, a fetus in late gestation may be exposed to acute hypoxia on a background of sustained reductions in fetal oxygenation, what is commonly denominated acute-on-chronic hypoxia. Alternatively, a fetus in late gestation may be exposed to acute hypoxia after a period of chronic hypoxia has resolved, following normalisation of fetal oxygenation, denominated acute-after-chronic hypoxia. How acute-on-chronic hypoxia or acute-after-chronic hypoxia affect the fetal brain sparing response has always been of intense clinical and scientific interest, however the breadth and depth of investigation to date have not matched this level of interest and these questions remain to be systematically addressed.

The few experimental studies modelling the effects on fetal cardiovascular function of acute-on-chronic hypoxia include studies by Robinson *et al.* (1983) who studied the effects of acute hypoxia on placentally-restricted fetuses, by Block *et al*. (1984) who induced chronic hypoxia by embolization of the uteroplacental vascular bed, by Kamitomo *et al*. (1993) and Tissot van Patot *et al.* (2012) who investigated fetal cardiovascular responses to acute hypoxia in sheep exposed to high altitude pregnancy, by Gagnon *et al*. (1997) who investigated redistribution of the fetal cardiac output in response to acute fetal placental embolization superimposed on chronic fetal placental embolization, and by Gardner *et al*. (2002a) who studied the fetal brain sparing response to acute hypoxia in fetuses which were chronically hypoxic after surgery. Four of these studies concluded that redistribution of the fetal cardiac output during acute hypoxia was maintained in the chronically hypoxic fetus (Block *et al.* 1984; Kamitomo *et al.* 1993; Gagnon *et al.* 1997; Gardner *et al.* 2002a) with some evidence of a sensitised cardiovascular defence to acute hypoxia by pre-existing chronic hypoxia (Block *et al.* 1984; Gardner *et al.* 2002a). Robinson *et al*. (1983) and Tissot van Patot *et al.* (2012) reported respectively a blunted bradycardic or even reversed cardiac response to acute hypoxia in the chronically hypoxic fetus, which could represent a desensitised carotid body chemoreflex. However, Robinson *et al*. (1983) favoured greater resting plasma catecholamine concentrations opposing the hypoxia-induced increase in vagal tone as a likely cause for the reduced bradycardic response in the placentally-restricted fetus. Gardner *et al*. (2002a) reported that the femoral vasoconstrictor and plasma vasopressin and catecholamine responses to acute hypoxia were significantly greater in chronically hypoxic relative to normoxic fetuses. These are similar sensitising effects on cardiovascular and endocrine responses to acute hypoxia as those determined in the fetal llama, a species adapted to the chronic hypoxia of life at high altitude (Giussani *et al*. 1999). It is also recognized that chronic hypoxia in

the fetus rarely occurs in isolation, and that, in adverse pregnancy, it is often accompanied by acidaemia and/or hypoglycaemia (Nicolaides *et al.* 1989). Some studies have reported that prevailing fetal acidaemia (Gardner *et al*. 2002a; Thakor & Giussani, 2009) or chronic fetal hypoglycaemia (Gardner *et al*. 2002a; Cleal *et al*. 2010) can also sensitise the fetal cardiovascular responses to acute hypoxia. Clearly, the degree and duration of fetal acidosis is of paramount importance since the development of severe fetal acidaemia (pH < 7.05) has been deemed as a key turning point after which a large proportion of fetuses are unable to maintain cardiovascular defence mechanisms to superimposed challenges, rendering them at risk of asphyxial brain injury (Gunn *&* Bennet, 2009).

Only one study to date appears to have investigated the effects on fetal cardiovascular function of acute-after-chronic hypoxia. In contrast to acute-on-chronic hypoxia, the technical advantage of the acute-after-chronic hypoxia model is that both groups of control fetuses and fetuses pre-exposed to chronic hypoxia have normal resting partial pressures of oxygen. Consequently, an acute hypoxic challenge of similar magnitude and starting from a similar baseline can be induced in both groups, facilitating experimental comparison. Gardner *et al.* (2002b) reported that exposure of the ovine fetus to reversible chronic hypoxia lasting a few days suppressed the femoral vasoconstriction but enhanced umbilical vasodilatation during subsequent acute hypoxia through elevated nitric oxide (NO) activity.

Combined, therefore, available data imply that the fetal cardiovascular system may adopt different strategies to spare the fetal brain during acute hypoxia depending on whether this occurs as an isolated event or superimposed on chronic hypoxia or following recovery from chronic hypoxia. While acute-on-chronic hypoxia may sensitise cardiovascular defence mechanisms (Block *et al.* 1984; Gardner *et al.*

2002a), acute-after-chronic hypoxia may switch the fetal defence strategy from a reliance on vasoconstrictor mechanisms to those promoting NO-dependent vasodilatation and increased perfusion and cardiac output (Gardner *et al*. 2002b). Clearly, these broad interpretations must now lay the foundation for future research asking focused and mechanistic questions, which do not shy away from but rather embrace the complexity of studying the cardiovascular function *in vivo* of the chronically hypoxic fetus.

REFERENCES

Abrams ME, Meredith KS, Kinnard P, Clark RH (2007). Hydrops fetalis: a retrospective review of cases reported to a large national database and identification of risk factors associated with death. *Pediatrics* **120(1)**, 84-9.

Adams MB, Simonetta G, McMillen IC (1996). The non-neurogenic catecholamine response of the fetal adrenal to hypoxia is dependent on activation of voltage sensitive Ca2+ channels. *Brain Res Dev Brain Res.* **94(2)**, 182-9.

Alayed N, Kezouh A, Oddy L, Abenhaim HA (2014). Sickle cell disease and pregnancy outcomes: population-based study on 8.8 million births. *J Perinat Med* **42(4)**, 487-92.

Alvarez-Buylla R, de Alvarez-Buylla ER (1988). Carotid sinus receptors participate in glucose homeostasis. *Respir Physiol* **72(3)**, 347-5.

Allen VM, Joseph K, Murphy KE, Magee LA, Ohlsson A (2004). The effect of hypertensive disorders in pregnancy on small for gestational age and stillbirth: a population based study. *BMC Pregnancy Childbirth* **4(1)**, 17.

Anderson PA, Glick KL, Killam AP, Mainwaring RD (1986). The effect of heart rate on in utero left ventricular output in the fetal sheep. *Journal of Physiology* **372**, 557-573.

Barcroft J (1935). The Croonian lecture: foetal respiration. Proc R Soc Lond B **118**, 242-263.

Barker DJP (1998). Mothers, Babies, and Disease in Later Life (Churchill Livingstone, Edinburgh, UK).

Bartelds B, van Bel F, Teitel DF & Rudolph AM. (1993). Carotid, not aortic, chemoreceptors mediate the fetal cardiovascular response to acute hypoxemia in lambs. Pediatric Research 34, 51-55.

Baschat AA (2004). Fetal responses to placental insufficiency: an update. Br J Obstet Gynaecol **111**, 1031-1041.

Bekedam DJ, Visser GH (1985). Effects of hypoxemic events on breathing, body movements, and heart rate variation: a study in growth-retarded human fetuses. *Am J Obstet Gynecol* **153(1)**, 52-6.

Block BS, Llanos AJ, Creasy RK (1984). Responses of the growth-retarded fetus to acute hypoxemia. *Am J Obstet Gynecol* **148(7)**, 878-85.

Blood AB, Hunter CJ, Power GG (2002). The role of adenosine in regulation of cerebral blood flow during hypoxia in the near-term fetal sheep. *J Physiol* **543(Pt 3)**, 1015-23.

Boddy K, Dawes GS, Fisher R, Pinter S, Robinson JS (1974). Foetal respiratory movements, electrocortical and cardiovascular responses to hypoxaemia and hypercapnia in sheep. *J Physiol* **243(3)**, 599-618.

Boudoulas H, Rittgers SE, Lewis RP, Leier CV, Weissler AM (1979). Changes in diastolic time with various pharmacologic agents: implication for myocardial perfusion. *Circulation* **60**, 164-169.

Boyle DW, Hirst K, Zerbe GO, Meschia G, Wilkening RB (1990). Fetal hind limb oxygen consumption and blood flow during acute graded hypoxia. Pediatr Res. **28(2)**, 94-100.

Booth LC, Malpas SC, Barrett CJ, Guild SJ, Gunn AJ, Bennet L (2012). Renal sympathetic nerve activity during asphyxia in fetal sheep. *Am J Physiol Regul Integr Comp Physiol* **303(1)**, R30-8.

Boshier DP, Gavin CB, Holloway H (1989). Morphometric analyses of adrenal gland growth in fetal and neonatal sheep. II. The adrenal medulla, with some observations on its ultrastructure. *J Anat*. **167**, 15-30.

Brace RA (1997). Physiology of amniotic fluid volume regulation. *Clin Obstet Gynecol* **40(2)**, 280-9.

Brenner B, Kupferminc MJ (2003). Inherited thrombophilia and poor pregnancy outcome. *Best Pract Res Clin Obstet Gynaecol* **17(3)**, 427-39.

Broughton-Pipkin PF, Lumbers ER & Mott JC (1974). Factors influencing plasma renin and angiotensin II in the conscious pregnant ewe and its foetus. *J Physiol* **243**, 619-636.

Burrage DM, Braddick L, Cleal JK, Costello P, Noakes DE, Hanson MA, Green LR (2009). The late gestation fetal cardiovascular response to hypoglycaemia is modified by prior peri-implantation undernutrition in sheep. J Physiol. **587(Pt 3)**, 611-24.

Burrage D, Green LR, Moss TJ, Sloboda DM, Nitsos I, Newnham JP, Hanson MA (2008). The carotid bodies influence growth responses to moderate maternal undernutrition in late-gestation fetal sheep. *Brit Journal Obstet Gynaecol* **115(2)**, 261-8.

Cheung CY (1990). Fetal adrenal medulla catecholamine response to hypoxia-direct and neural components. *Am J Physiol*. **258(6 Pt 2)**, R1340-6.

Cleal JK, Bagby S, Hanson MA1, Gardiner HM, Green LR (2010). The effect of late gestation foetal hypoglycaemia on cardiovascular and endocrine function in sheep. *J Dev Orig Health Dis*. **1(1)**, 42-9.

Cohn HE, Sacks EJ, Heymann MA & Rudolph AM (1974). Cardiovascular responses to hypoxemia and acidemia in fetal lambs. *Am J Obstet Gynecol* **120**, 817-824.

Comline RS, Silver M (1961). The release of adrenaline and noradrenaline from the adrenal glands of the foetal sheep. *J Physiol*. **156**, 424-44.

Court DJ & Parer JT (1984). Experimental studies of fetal asphyxia and fetal heart rate interpretation. In: *Research in Perinatal Medicine*. Eds. Nathanielsz PW and Parer JT. Perinatology Press. Chapter 5, pp. 113-169.

Davis L, Thornburg KL, Giraud GD (2005). The effects of anaemia as a programming agent in the fetal heart. *J Physiol* **565(Pt 1)**, 35-41.

Dawes GS, Lewis BV, Milligan JE, Roach MR, Talner NS (1968). Vasomotor responses in the hind limbs of foetal and new-born lambs to asphyxia and aortic chemoreceptor stimulation. *J Physiol*. **195(1)**, 55-81.

Dawes GS, Duncan SL, Lewis BV, Merlet CL, Owen-Thomas JB, Reeves JT (1969). Hypoxaemia and aortic chemoreceptor function in foetal lambs. J Physiol. **201(1)**, 105-16.

Derks JB, Oudijk MA, Torrance HL, Rademaker CM, Benders MJ, Rosen KG, Cindrova-Davies T, Thakor AS, Visser GH, Burton GJ, van Bel F, Giussani DA (2010). Allopurinol reduces oxidative stress in the ovine fetal cardiovascular system after repeated episodes of ischemia-reperfusion. *Pediatr Res* **68(5)**, 374-80.

Dorey ES, Pantaleon M, Weir KA, Moritz KM. Adverse prenatal environment and kidney development: implications for programing of adult disease. *Reproduction* **147(6)**, R189-98.

Edelstone DI (1980). Regulation of blood flow through the ductus venosus. *J Dev Physiol* **2**, 219-238.

Edelstone DI, Holzman IR (1982). Fetal intestinal oxygen consumption at various levels of oxygenation. *Am J Physiol*. **242(1)**, H50-4.

Escobar J, Teramo K, Stefanovic V, Andersson S, Asensi MA, Arduini A, Cubells E, Sastre J, Vento M (2013). Amniotic fluid oxidative and nitrosative stress biomarkers correlate with fetal chronic hypoxia in diabetic pregnancies. *Neonatology***103(3)**, 193-8.

Fisher DJ, Heymann MA, Rudolph AM (1982). Fetal myocardial oxygen and carbohydrate consumption during acutely induced hypoxemia. *American Journal of Physiology* **242**, H657-H661.

Fletcher AJ, Edwards CM, Gardner DS, Fowden AL, Giussani DA (2000). Neuropeptide Y in the sheep fetus: effects of acute hypoxemia and dexamethasone during late gestation. *Endocrinology* **141(11)**, 3976-82.

Fletcher AJ, McGarrigle HH, Edwards CM, Fowden AL, Giussani DA (2002). Effects of low dose dexamethasone treatment on basal cardiovascular and endocrine function in fetal sheep during late gestation. *J Physiol* **545(Pt 2)**, 649-60.

Fletcher AJ, Gardner DS, Edwards CM, Fowden AL, Giussani DA (2003). Cardiovascular and endocrine responses to acute hypoxaemia during and following dexamethasone infusion in the ovine fetus. *J Physiol* **549(Pt 1)**, 271-87

Fletcher AJ, Forhead AJ, Fowden AL, Ford WR, Nathanielsz PW, Giussani DA (2005). Effects of gestational age and cortisol treatment on ovine fetal heart function in a novel biventricular Langendorff preparation. *J Physiol* **562(Pt 2)**, 493-505.

Fletcher AJ, Gardner DS, Edwards CM, Fowden AL, Giussani DA (2006). Development of the ovine fetal cardiovascular defense to hypoxemia towards full term. *Am J Physiol Heart Circ Physiol* **291(6)**, H3023-34.

Fowden AL, Giussani DA, Forhead AJ (2006). Intrauterine programming of physiological systems: causes and consequences. Physiology (Bethesda) **21**, 29-37.

Gao L, Ortega-Sáenz P, García-Fernández M, González-Rodríguez P, Caballero-Eraso C, López-Barneo J (2014). Glucose sensing by carotid body glomus cells: potential implications in disease. *Front Physiol*. **5**, 398.

Gagnon R, Murotsuki J, Challis JR, Fraher L, Richardson BS (1997). Fetal sheep endocrine responses to sustained hypoxemic stress after chronic fetal placental embolization. *Am J Physiol* **272(5 Pt 1)**, E817-23.

Gardner DS, Fletcher AJ, Fowden AL, Giussani DA (2001). A novel method for controlled and reversible long term compression of the umbilical cord in fetal sheep. *J Physiol* **535(Pt 1)**, 217-29.

Gardner DS, Fletcher AJ, Bloomfield MR, Fowden AL & Giussani DA (2002a). Effects of prevailing hypoxaemia, acidaemia or hypoglycaemia upon the cardiovascular, endocrine and metabolic responses to acute hypoxaemia in the ovine fetus. *J Physiol* **540**, 351-366.

Gardner DS, Fowden AL & Giussani DA (2002b). Adverse intrauterine conditions diminish the fetal defense against acute hypoxia by increasing nitric oxide activity. *Circulation* **106**, 2278-2283.

Gardner DS & Giussani DA (2003). Enhanced umbilical blood flow during acute hypoxemia after chronic umbilical cord compression: a role for nitric oxide. *Circulation* **108**, 331-335.

Gardner DS, Giussani DA & Fowden AL (2003). Hindlimb glucose and lactate metabolism during umbilical cord compression and acute hypoxaemia in the late-gestation ovine fetus. *Am J Physiol Regulatory Integreative Comp Physiol* **284**, 954-964.

Gardner DS, Powlson AS & Giussani DA (2001). An in vivo nitric oxide clamp to investigate the influence of nitric oxide on continuous umbilical blood flow during acute hypoxaemia in the sheep fetus. *J Physiol* **537**, 587-596.

Gilbert RD (1980). Control of fetal cardiac output during changes in blood volume. *American Journal of Physiology* **238**, H80-H86.

Giussani DA, Spencer JA, Moore PJ, Bennet L & Hanson MA (1993). Afferent and efferent components of the cardiovascular reflex responses to acute hypoxia in term fetal sheep. *J Physiol* **461**, 431-449.

Giussani DA, Spencer JA & Hanson MA. (1994a). Fetal cardiovascular reflex responses to hypoxia. *Fetal and Maternal Medicine Review* **6**, 17-37.

Giussani DA, McGarrigle HH, Spencer JA, Moore PJ, Bennet L, Hanson MA (1994b). Effect of carotid denervation on plasma vasopressin levels during acute hypoxia in the late-gestation sheep fetus. *J Physiol* **477 (Pt 1)**, 81-7.

Giussani DA, McGarrigle HH, Moore PJ, Bennet L, Spencer JA, Hanson MA (1994c). Carotid sinus nerve section and the increase in plasma cortisol during acute hypoxia in fetal sheep. *J Physiol*. 477 (Pt 1), 75-80.

Giussani DA, Moore PJ, Bennet L, Spencer JA, Hanson MA (1995). Alpha 1- and alpha 2-adrenoreceptor actions of phentolamine and prazosin on breathing movements in fetal sheep in utero. *J Physiol* **486 (Pt 1)**, 249-55.

Giussani DA, Unno N, Jenkins SL, Wentworth RA, Derks JB, Collins JH, Nathanielsz PW (1997). Dynamics of cardiovascular responses to repeated partial umbilical cord compression in late-gestation sheep fetus. *Am J Physiol*. **273(5 Pt 2)**, H2351-60.

Giussani DA, Riquelme RA, Sanhueza EM, Hanson MA, Blanco CE, Llanos AJ (1999). Adrenergic and vasopressinergic contributions to the cardiovascular response to acute hypoxaemia in the llama fetus. J Physiol **515 (Pt 1)**, 233-41.

Giussani DA, Gardner DS, Cox DT, Fletcher AJ (2001). Purinergic contribution to circulatory, metabolic, and adrenergic responses to acute hypoxemia in fetal sheep. *Am J Physiol Regul Integr Comp Physiol* **280(3)**, R678-85.

Giussani DA, Salinas CE, Villena M, Blanco CE (2007). The role of oxygen in prenatal growth: studies in the chick embryo. *J Physiol* **585(Pt 3)**, 911-7.

Giussani DA, Davidge ST (2013).Developmental programming of cardiovascular disease by prenatal hypoxia. J Dev Orig Health Dis **4(5)**, 328-37.

Gouin K, Murphy K, Shah PS; Knowledge Synthesis group on Determinants of Low Birth Weight and Preterm Births (2011). Effects of cocaine use during pregnancy on low birthweight and preterm birth: systematic review and metaanalyses. *Am J Obstet Gynecol.* **204(4)**, 340.e1-12.

Godfrey KM, Haugen G, Kiserud T, Inskip HM, Cooper C, Harvey NC, Crozier SR, Robinson SM, Davies L; Southampton Women's Survey Study Group, Hanson MA (2012). Fetal liver blood flow distribution: role in human developmental strategy to prioritize fat deposition versus brain development. *PLoS One* **7(8)**, e41759.

Green-top Guideline No. 63. London (UK). *Royal College of Obstetrics & Gynaecology*; 20.

Gluckman PD, Hanson MA, Cooper C, Thornburg KL (2008). Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med* **359(1)**, 61-73.

Green LR, Bennet L & Hanson MA (1996). The role of nitric oxide synthesis in cardiovascular responses to acute hypoxia in the late gestation sheep fetus. *J Physiol* **497**, 271–277.

Green LR, Bennet L, Robson S, Hanson MA (1997). The role of carotid chemoreceptors in the effects of hypoxia on renal blood flow in the late gestation sheep fetus. *Exp Physiol*. **82(1)**, 183-92.

Green LR, McGarrigle HH, Bennet L, Hanson MA (1998). Angiotensin II and cardiovascular chemoreflex responses to acute hypoxia in late gestation fetal sheep. *J Physiol.* **507 (Pt 3)**, 857-67.

Gunn AJ, Bennet L (2009). Fetal hypoxia insults and patterns of brain injury: insights from animal models. *Clin Perinatol*. **36(3)**, 579-93.

Hall DM (1989). Birth asphyxia and cerebral palsy. *Brit Med Journal* **299**, 279-282.

Halliday HL (2009). Neonatal management and long-term sequelae. *Best Pract Res Clin Obstet Gynaecol.* **23(6)**, 871-80.

Harlev A, Levy A, Zaulan Y, Koifman A, Mazor M, Wiznitzer A, Faizayev E, Sheiner E (2008). Idiopathic bleeding during the second half of pregnancy as a risk factor for adverse perinatal outcome. *J Matern Fetal Neonatal Med*. **21(5)**, 331-5.

Hashimoto K, Clapp JF (2003). The effect of nuchal cord on amniotic fluid and cord blood erythropoietin at delivery. *J Soc Gynecol Investig* **10(7)**, 406-11.

Hawkins J, Van Hare GF, Schmidt KG, Rudolph AM (1989). Effects of increasing afterload on left ventricular output in fetal lambs. *Circulation Research* **65**, 127-134.

Hayes EK, Lechowicz A, Petrik JJ, Storozhuk Y, Paez-Parent S, Dai Q, Samjoo IA, Mansell M, Gruslin A, Holloway AC, Raha S (2012). Adverse fetal and neonatal outcomes associated with a life-long high fat diet: role of altered development of the placental vasculature. PLoS One. **7(3)**, e33370.

Herrera EA, Kane AD, Hansell JA, Thakor AS, Allison BJ, Niu Y, Giussani DA (2012). A role for xanthine oxidase in the control of fetal cardiovascular function in late gestation sheep. *J Physiol* **590(Pt 8)**, 1825-37.

Howarth C, Gazis A, James D (2007). Associations of Type 1 diabetes mellitus, maternal vascular disease and complications of pregnancy. Diabet Med. **24(11)**, 1229-34.

Huch A, Huch R, Schneider H & Rooth G (1977). Continuous transcutaneous monitoring of fetal oxygen tension during labour. *Br J Obstet Gynaecol* **84** Suppl 1, 1-39.

Hunter CJ, Blood AB, White CR, Pearce WJ, Power GG (2003). Role of nitric oxide in hypoxic cerebral vasodilatation in the ovine fetus. J Physiol **549(Pt 2)**, 625-33.

Itskovitz J, LaGamma EF, Bristow J, Rudolph AM (1991). Cardiovascular responses to hypoxemia in sinoaortic-denervated fetal sheep. Pediatr Res **30(4)**, 381-5.

Iwamoto HS, Rudolph AM, Mirkin BL, Keil LC (1983). Circulatory and humoral responses of sympathectomized fetal sheep to hypoxemia. *Am J Physiol* **245(5 Pt 1)**, H767-72.

Itskovitz J, LaGamma EF, Rudolph AM (1987). Effects of cord compression on fetal blood flow distribution and O2 delivery. *Am J Physiol* **252(1 Pt 2)**, H100-9.

Iwamoto HS, Kaufman T, Keil LC & Rudolph AM (1989). Responses to acute hypoxemia in fetal sheep at 0.6-0.7 gestation. *Am J Physiol* **256**, H613-H620.

Jansen AH, Belik J, Ioffe S, Chernick V (1989). Control of organ blood flow in fetal sheep during normoxia and hypoxia. *Am J Physiol*. **257(4 Pt2)**, H1132-H1139.

Jellyman JK, Gardner DS, Edwards CM, Fowden AL, Giussani DA (2005). Fetal cardiovascular, metabolic and endocrine responses to acute hypoxaemia during and following maternal treatment with dexamethasone in sheep. J Physiol **567(Pt 2)**, 673-88.

Jellyman JK, Gardner DS, McGarrigle HH, Fowden AL, Giussani DA (2009). Antenatal glucocorticoid therapy increases glucose delivery to cerebral circulations during acute hypoxemia in fetal sheep during late gestation. *Am J Obstet Gynecol* **201(1)**, 82.e1-8.

Jensen A, Hanson MA (1995). Circulatory responses to acute asphyxia in intact and chemodenervated fetal sheep near term. *Reprod Fertil Dev*. **7(5)**, 1351-9.

Jones CT, Robinson RO (1975). Plasma catecholamines in foetal and adult sheep. J Physiol **248(1)**, 15-33.

Jones CT, Ritchie JW (1983). The effects of adrenergic blockade on fetal response to hypoxia. *J Dev Physiol* **5(4)**, 211-22.

Jones CT, Roebuck MM, Walker DW, Johnston BM (1988). The role of the adrenal medulla and peripheral sympathetic nerves in the physiological responses of the fetal sheep to hypoxia. *J Dev Physiol*. **10(1)**, 17-36.

Kamitomo M, Alonso JG, Okai T, Longo LD, Gilbert RD (1993). Effects of long-term, high-altitude hypoxemia on ovine fetal cardiac output and blood flow distribution. Am J Obstet Gynecol. **169(3)**, 701-7.

Kane AD, Herrera EA, Hansell JA, Giussani DA (2012). Statin treatment depresses the fetal defence to acute hypoxia via increasing nitric oxide bioavailability. *J Physiol* **590(Pt 2)**, 323-34.

Kane AD, Hansell JA, Herrera EA, Allison BJ, Niu Y, Brain KL, Kaandorp JJ, Derks JB, Giussani DA (2014). Xanthine oxidase and the fetal cardiovascular defence to hypoxia in late gestation ovine pregnancy. *J Physiol* **592(Pt 3)**:475-89.

Kaplan-Sturk R, Åkerud H, Volgsten H, Hellström-Westas L, Wiberg-Itzel E (2013). Outcome of deliveries in healthy but obese women: obesity and delivery outcome. *BMC Res Notes* **6**, 50.

Katz O, Sheiner E (2008). Asthma and pregnancy: a review of two decades. *Expert Rev Respir Med* **2(1)**, 97-107.

Kemp PJ, Telezhkin V (2014). Oxygen sensing by the carotid body: is it all just rotten eggs? *Antioxid Redox Signal*. **20(5)**, 794-804.

Kennare R, Heard A, Chan A (2005). Substance use during pregnancy: risk factors and obstetric and perinatal outcomes in South Australia. *Aust N Z J Obstet Gynaecol* **45(3)**, 220-5.

Kingdom JC, Kaufmann P (1997). Oxygen and placental villous development: origins of fetal hypoxia. *Placenta* **18(8)**, 613-21.

Kirkpatrick SE, Pitlick PT, Naliboff J, Friedman WF (1976). Frank-Starling relationship as an important determinant of fetal cardiac output. *American Journal of Physiology* **231**, 495-500.

Kjellmer I, Andiné P, Hagberg H, Thiringer K (1989). Extracellular increase of hypoxanthine and xanthine in the cortex and basal ganglia of fetal lambs during hypoxia-ischemia*. Brain Res* **478(2)**, 241-7.

Koos BJ (2011). Adenosine A_2a receptors and O_2 sensing in development. *Am J Physiol Regul Integr Comp Physiol.* **301(3)**, R601-22.

Kumar P, Prabhakar NR (2012). Peripheral chemoreceptors: function and plasticity of the carotid body*. Compr Physiol.* **2(1)**, 141-219.

Leung TY, Stuart O, Sahota DS, Suen SS, Lau TK, Lao TT (2011). Head-to-body delivery interval and risk of fetal acidosis and hypoxic ischaemic encephalopathy in shoulder dystocia: a retrospective review. *Brit J Obstet Gynaecol* **118(4)**, 474-9.

Lewis AB, Donovan M & Platzker AC (1980). Cardiovascular responses to autonomic blockade in hypoxemic fetal lambs. *Biol Neonate* **37**, 233-242.

Li HP, Chen X, Li MQ (2013). Gestational diabetes induces chronic hypoxia stress and excessive inflammatory response in murine placenta. *Int J Clin Exp Pathol* **6(4)**, 650-9.

Limesand SW, Jensen J, Hutton JC, Hay WW Jr (2005). Diminished beta-cell replication contributes to reduced beta-cell mass in fetal sheep with intrauterine growth restriction. *Am J Physiol Regul Integr Comp Physiol* **288(5)**, R1297-305.

Llanos AJ, Court DJ, Block BS, Germain AM, Parer JT (1986). Fetal cardiorespiratory changes during spontaneous prelabor uterine contractions in sheep. *Am J Obstet Gynecol*. **155(4)**, 893-7.

Longo LD (1976). Carbon monoxide: effects on oxygenation of the fetus in utero. *Science* **194(4264)**, 523-5.

López-Barneo J, López-López JR, Ureña J, González C (1988). Chemotransduction in the carotid body: K+ current modulated by PO2 in type I chemoreceptor cells. *Science* **241(4865)**, 580-2.

Low JA, Galbraith RS, Muir DW, Killen HL, Pater EA & Karchmar EJ (1985). The relationship between perinatal hypoxia and newborn encephalopathy. *Am J Obstet Gynecol* **152**, 256-260.

Mc Millen IC, Adams MB, Ross JT, Coulter CL, Simonetta G, Owens JA, Robinson J, Edwards LJ (2001) Fetal growth restriction: adaptations and consequences. *Reproduction* **122**, 195–204.

Maberry MC, Ramin SM, Gilstrap LC 3rd, Leveno KJ, Dax JS (1990). Intrapartum asphyxia in pregnancies complicated by intra-amniotic infection. *Obstet Gynecol* **76(3 Pt 1)**, 351-4.

Makowski EL, Battaglia FC, Meschia G, Behrman RE, Schruefer J, Seeds AE, Bruns PD (1968). Effect of maternal exposure to high altitude upon fetal oxygenation. *Am J Obstet Gynecol.* **100(6)**, 852-61.

Mandel D, Oron T, Mimouni GS, Littner Y, Dollberg S, Mimouni FB (2005). The effect of prolonged rupture of membranes on circulating neonatal nucleated red blood cells. *J Perinatol* **25(11)**, 690-3.

Marshall JM (1999). The Joan Mott Prize Lecture. The integrated response to hypoxia: from circulation to cells. *Exp Physiol* **84(3)**, 449-70.

Maurer HS, Behrman RE, Honig GR (1970). Dependence of the oxygen affinity of blood on the presence of foetal or adult haemoglobin. *Nature* **227(5256)**, 388-90.

McMillen IC, Simonetta G, Roberts ML, Adams MB (1997). Catecholamines, enkephalins and the response of the fetal adrenal medulla to hypoxaemia. *Equine Vet J Suppl*. **24**, 68-73.

McMillen IC, Adams MB, Ross JT, Coulter CL, Simonetta G, Owens JA, Robinson JS, Edwards LJ (2001). Fetal growth restriction: adaptations and consequences. *Reproduction* **122(2)**, 195-204.

Moodley S1, Sanatani S, Potts JE, Sandor GG (2013). Postnatal outcome in patients with fetal tachycardia. *Pediatr Cardiol* **34(1)**, 81-7.

Moore PJ, Hanson MA (1991). The role of peripheral chemoreceptors in the rapid response of the pulmonary vasculature of the late gestation sheep fetus to changes in PaO_2. *J Dev Physiol* **16(3)**, 133-8.

Morrison S, Gardner DS, Fletcher AJ, Bloomfield MR, Giussani DA (2003). Enhanced nitric oxide activity offsets peripheral vasoconstriction during acute hypoxaemia via chemoreflex and adrenomedullary actions in the sheep fetus. *J Physiol* **547(Pt 1)**, 283-91.

Morrison JL (2008). Sheep models of intrauterine growth restriction: fetal adaptations and consequences. *Clin Exp Pharmacol Physiol* **35(7)**, 730-43.

Mulder AL, van Golde JC, Prinzen FW, Blanco CE (1998). Cardiac output distribution in response to hypoxia in the chick embryo in the second half of the incubation time. *J Physiol* **508 (Pt 1)**, 281-7.

Nicolaides KH, Economides DL & Soothill PW (1989). Blood gases, pH, and lactate in appropriate- and small-for-gestational-age fetuses. *Am J Obstet Gynecol* **161**, 996-1001.

Nishida N, Blood AB, Hunter CJ, Bragg S, Williams J, Pearce WJ, Power GG (2006). Role of prostanoids in the regulation of cerebral blood flow during normoxia and hypoxia in the fetal sheep. *Pediatr Res* **60(5)**, 524-9.

Pardal R, López-Barneo J (2002). Low glucose-sensing cells in the carotid body. *Nat Neurosci*. **5(3)**, 197-8.

Pardi G, Cetin I, Marconi AM, Lanfranchi A, Bozzetti P, Ferrazzi E, Buscaglia M, Battaglia FC (1993). Diagnostic value of blood sampling in fetuses with growth retardation. *N Engl J Med* **328(10)**, 692-6.

Parer JT (1984). The effect of atropine on heart rate and oxygen consumption of the hypoxic fetus. *Am J Obstet Gynecol* **148(8)**, 1118-22.

Peeters LL, Sheldon RE, Jones MD, Jr., Makowski EL & Meschia G (1979). Blood flow to fetal organs as a function of arterial oxygen content. *Am J Obstet Gynecol* **135**, 637-646.

Pearce WJ (1995). Mechanisms of hypoxic cerebral vasodilatation. *Pharmacol Ther*. 65(1):75-91.

Peebles DM (2012). Radical change for the fetus. *J Physiol* 590(Pt 8):1773

Peebles DM, Spencer JA, Edwards AD, Wyatt JS, Reynolds EO, Cope M, Delpy DT (1994). Relation between frequency of uterine contractions and human fetal cerebral oxygen saturation studied during labour by near infrared spectroscopy. *Br J Obstet Gynaecol* **101(1)**, 44-8.

Peréz R, Espinoza M, Riquelme R, Parer JT, Llanos AJ (1989). Arginine vasopressin mediates cardiovascular responses to hypoxemia in fetal sheep. *Am J Physiol*. **256(5 Pt 2)**, R1011-8.

Poudel R, McMillen IC, Dunn SL, Zhang S, Morrison JL (2015). Impact of chronic hypoxemia on blood flow to the brain, heart, and adrenal gland in the late-gestation IUGR sheep fetus. *Am J Physiol Regul Integr Comp Physio* **308(3)**, R151-62.

Prabhakar NR, Semenza GL (2012). Gaseous messengers in oxygen sensing. *J Mol Med (Berl)*. **90(3)**, 265-72.

Ratcliffe FM, Evans JM (1993). Neonatal wellbeing after elective caesarean delivery with general, spinal, and epidural anaesthesia. *Eur J Anaesthesiol* **10**, 175–181.

Reller MD, Morton MJ, Reid DL, Thornburg KL (1987). Fetal lamb ventricles respond differently to filling and arterial pressures and to in utero ventilation. *Pediatric Research* **22**, 621-626.

Reuss ML, Parer JT, Harris JL & Krueger TR (1982). Hemodynamic effects of alpha-adrenergic blockade during hypoxia in fetal sheep. *Am J Obstet Gynecol* **142**, 410-415.

Roberts CL, Algert CS, Nippita TA, Bowen JR, Shand AW (2015). Association of prelabor cesarean delivery with reduced mortality in twins born near term. *Obstet Gynecol* **125(1)**, 103-10.

Robillard JE, Nakamura KT, DiBona GF (1986). Effects of renal denervation on renal responses to hypoxemia in fetal lambs. *Am J Physiol* **250(2 Pt 2)**, F294-301.

Roux JC, Brismar H, Aperia A, Lagercrantz H (2005). Developmental changes in HIF transcription factor in carotid body: relevance for O2 sensing by chemoreceptors. *Pediatr Res.* **58(1)**, 53-7.

Rowell LB, Blackmon JR (1987). Human cardiovascular adjustments to acute hypoxaemia. *Clin Physiol* **7(5)**, 349-76.

Rudolph AM, Heymann MA (1968). The fetal circulation. *Annu Rev Med* **19**, 195-206.

Rudolph AM (1984). The fetal circulation and its response to stress. *J Dev Physiol* **6(1)**, 11-9.

Rurak DW (1978). Plasma vasopressin levels during hypoxaemia and the cardiovascular effects of exogenous vasopressin in foetal and adult sheep. *J Physiol* **277**, 341-357.

Rurak DW, Gruber NC (1983). Increased oxygen consumption associated with breathing activity in fetal lambs. *J Appl Physiol Respir Environ Exerc Physiol* **54(3)**, 701-7.

Richardson BS, Carmichael L, Homan J, Patrick JE (1993). Cerebral oxidative metabolism in fetal sheep with prolonged and graded hypoxemia. *J Dev Physiol* **19(2)**, 77-83.

Richardson BS, Bocking AD (1998). Metabolic and circulatory adaptations to chronic hypoxia in the fetus. *Comp Biochem Physiol A Mol Integr Physiol* **119(3)**, 717-23.

Robinson JS, Jones CT, Kingston EJ (1983). Studies on experimental growth retardation in sheep. The effects of maternal hypoxaemia. *J Dev Physio* **5(2)**, 89-100.

Robson SC, Crawford RA, Spencer JA, Lee A (1992). Intrapartum amniotic fluid index and its relationship to fetal distress. *Am J Obstet Gynecol* **166(1 Pt 1)**, 78-82.

Salinas CE, Blanco CE, Villena M, Camm EJ, Tuckett JD, Weerakkody RA, Kane AD, Shelley AM, Wooding FB, Quy M, Giussani DA (2010). Cardiac and vascular disease prior to hatching in chick embryos incubated at high altitude. *J Dev Orig Health Dis.* **1(1)**, 60-6.

Shinozuka N, Yen A, Nathanielsz PW (1999). Alteration of fetal oxygenation and responses to acute hypoxemia by increased myometrial contracture frequency produced by pulse administration of oxytocin to the pregnant ewe from 96 to 131 days' gestation. *Am J Obstet Gynecol* **180(5)**, 1202-8.

Smith GC, Fleming KM, White IR (2007). Birth order of twins and risk of perinatal death related to delivery in England, Northern Ireland, and Wales, 1994-2003: retrospective cohort study. *Brit Med Journal* **334(7593)**, 576.

Soleymanlou N, Jurisica I, Nevo O, Ietta F, Zhang X, Zamudio S, Post M & Caniggia I (2005). Molecular evidence of placental hypoxia in preeclampsia. *J Clin Endocrinol Metab* **90**, 4299-4308.

Soothill PW, Nicolaides KH, Rodeck CH, Clewell WH, Lindridge J (1987). Relationship of fetal hemoglobin and oxygen content to lactate concentration in Rh isoimmunized pregnancies. *Obstet Gynecol* **69(2)**, 268-71.

Soria R, Julian CG, Vargas E, Moore LG, Giussani DA (2013). Graduated effects of high-altitude hypoxia and highland ancestry on birth size. *Pediatr Res* **74(6)**, 633-8.

Snoeck A, Remacle C, Reusens B, Hoet JJ (1990). Effect of a low protein diet during pregnancy on the fetal rat endocrine pancreas. *Biol Neonate* **57(2)**, 107-18.

Skilton MR, Evans N, Griffiths KA, Harmer JA, Celermajer DS (2005). Aortic wall thickness in newborns with intrauterine growth restriction. *Lancet* **365**, 1484–1486.

Spinillo A, Cesari S, Bariselli S, Tzialla C, Gardella B, Silini EM (2015). Placental lesions associated with oligohydramnios in fetal growth restricted (FGR) pregnancies. *Placenta* **36(5)**, 538-44.

Thakor AS, Bloomfield MR, Patterson M, Giussani DA (2005). Calcitonin gene-related peptide antagonism attenuates the haemodynamic and glycaemic responses to acute hypoxaemia in the late gestation sheep fetus. *J Physiol* **566(Pt 2)**, 587-97.

Thakor AS, Giussani DA (2009). Effects of acute acidemia on the fetal cardiovascular defense to acute hypoxemia. *Am J Physiol Regul Integr Comp Physiol* **296(1)**, R90-9.

Thakor AS, Richter HG, Kane AD, Dunster C, Kelly FJ, Poston L, Giussani DA (2010b). Redox modulation of the fetal cardiovascular defence to hypoxaemia. *J Physiol* **588(Pt 21)**, 4235-47.

Thakor AS, Herrera EA, Serón-Ferré M, Giussani DA (2010a). Melatonin and vitamin C increase umbilical blood flow via nitric oxide-dependent mechanisms. *J Pineal Res.* **49(4)**, 399-406.

Thakor AS, Allison BJ, Niu Y, Botting KJ, Serón-Ferré M, Herrera EA, Giussani DA (2015). Melatonin modulates the fetal cardiovascular defense response to acute hypoxia. *J Pineal Res*. Apr 22. doi: 10.1111/jpi.12242. [Epub ahead of print].

Thornburg KL, Morton MJ (1986). Filling and arterial pressures as determinants of left ventricular stroke volume in fetal lambs. *American Journal of Physiology* **251**, H961-H968.

Tissot van Patot MC, Ebensperger G, Gassmann M, Llanos AJ (2012). The hypoxic placenta. High Alt Med Biol. 2012 Sep;13(3):176-84.

Umbers AJ, Aitken EH, Rogerson SJ (2011). Malaria in pregnancy: small babies, big problem. Trends Parasitol **27(4)**, 168-75.

Valsky DV, Eixarch E, Martinez JM, Crispi F, Gratacós E (2010). Selective intrauterine growth restriction in monochorionic twins: pathophysiology, diagnostic approach and management dilemmas. *Semin Fetal Neonatal Med*. **15(6)**, 342-8.

van Bel F, Sola A, Roman C & Rudolph AM (1995). Role of nitric oxide in the regulation of the cerebral circulation in the lamb fetus during normoxemia and hypoxemia. *Biol Neonate* **68**, 200-210.

Veille JC, Hanson R, Sivakoff M, Hoen H, Ben-Ami M (1993). Fetal cardiac size in normal, intrauterine growth retarded, and diabetic pregnancies. *Am J Perinatol* **10**, 275–279.

Vilares Conde SV, Peers C (2013). Carotid body chemotransduction gets the human touch. *J Physiol*. **591(Pt 24)**, 6131-2.

Vorherr H (1975). Placental insufficiency in relation to postterm pregnancy and fetal postmaturity. Evaluation of fetoplacental function; management of the postterm gravida. *Am J Obstet Gynecol* **123(1)**, 67-103.

Whittemore R, Hobbins JC, Engle MA (1982). Pregnancy and its outcome in women with and without surgical treatment of congenital heart disease. *Am J Cardiol* **50(3)**, 641-51.

Yaffe H, Parer JT, Block BS & Llanos AJ (1987). Cardiorespiratory responses to graded reductions of uterine blood flow in the sheep fetus*. J Dev Physiol* **9**, 325-336.

Yamada T, Yamada T, Morikawa M, Minakami H (2012). Clinical features of abruptio placentae as a prominent cause of cerebral palsy. *Early Hum Dev* **88(11)**, 861-4

Yasuda M, Takakuwa K, Tokunaga A, Tanaka K (1995). Prospective studies of the association between anticardiolipin antibody and outcome of pregnancy. *Obstet Gynecol* **86(4 Pt 1)**, 555-9.

FIGURE LEGENDS

Figure 1. Fetal cardiovascular responses to acute hypoxia. The data show the mean±SEM for the change in fetal carotid blood flow (a), fetal femoral blood flow (b) and fetal heart rate (c) in intact (○, n=14) and carotid body denervated (●, n=12) chronically-instrumented sheep fetuses at 0.8 of gestation during a 1 hour episode of acute hypoxia (PaO_2 reduced from *ca*. 23 to 13 mmHg, box). Calculation of the ratio between simultaneous measurements of carotid and femoral blood yields the fetal brain sparing index (d). Carotid body denervation prevents the fetal bradycardia and diminishes the fall in fetal femoral blood flow and the increase in the fetal brain sparing index during acute hypoxia. However, carotid body denervation does not affect the increment in carotid femoral blood flow during acute hypoxia. *P<0.05, intact *vs.* denervated. Redrawn from Giussani *et al*. 1993, with permission.

Figure 2. Physiology of fetal brain sparing during hypoxia. The fetal brain sparing response to acute hypoxia is triggered by a carotid chemoreflex which leads to bradycardia and an increase in peripheral vasoconstriction. The bradycardia is mediated by a dominant vagal influence on the fetal heart. The neurally triggered peripheral vasoconstriction is maintained by the release of constrictor hormones into the fetal circulation as well as a local vascular oxidant tone, determined by the interaction between nitric oxide (•NO) and ROS, such as the superoxide anion ($\bullet O_2^-$). Numbers represent some of the evidence available in the literature.

1. Kjellmer I, Andiné P, Hagberg H, Thiringer K (1989). *Brain Res* **478(2)**, 241-7.

2. van Bel F, Sola A, Roman C & Rudolph AM (1995). *Biol Neonate* **68**, 200-210.

3. Pearce WJ (1995). *Pharmacol Ther*. **65(1)**, 75-91.

4. Green LR, Bennet L & Hanson MA (1996). *J Physiol* **497**, 271–277..

5. Blood AB, Hunter CJ, Power GG (2002). *J Physiol* **543(Pt 3)**, 1015-23.

6. Hunter CJ, Blood AB, White CR, Pearce WJ, Power GG (2003). *J Physiol* **549(Pt 2)**, 625-33.

7. Nishida N, Blood AB, Hunter CJ, Bragg S, Williams J, Pearce WJ, Power GG (2006). *Pediatr Res* **60(5)**, 524-9.

8. Giussani DA, Spencer JA, Moore PJ, Bennet L & Hanson MA (1993). *J Physiol* **461**, 431-449.

9. Bartelds B, van Bel F, Teitel DF & Rudolph AM. (1993). *Pediatric Research* **34**, 51-55.

10. Giussani DA, Gardner DS, Cox DT, Fletcher AJ (2001). *Am J Physiol Regul Integr Comp Physiol* **280(3)**, R678-85.

11. Parer JT (1984). *Am J Obstet Gynecol* **148(8)**, 1118-22.

12. Court DJ & Parer JT (1984). In: *Research in Perinatal Medicine*. Eds. Nathanielsz PW and Parer JT. Perinatology Press. Chapter 5, pp. 113-169.

13. Lewis AB, Donovan M & Platzker AC (1980). *Biol Neonate* **37**, 233-242.

14. Reuss ML, Parer JT, Harris JL & Krueger TR (1982). *Am J Obstet Gynecol* **142**, 410-415.

15. Iwamoto HS, Rudolph AM, Mirkin BL, Keil LC (1983). *Am J Physiol* **245(5 Pt 1)**, H767-72.

16. Robillard JE, Nakamura KT, DiBona GF (1986). *Am J Physiol* **250(2 Pt 2)**, F294-301.

17. Thakor AS, Bloomfield MR, Patterson M, Giussani DA (2005). *J Physiol* **566(Pt 2)**, 587-97.

18. Booth LC, Malpas SC, Barrett CJ, Guild SJ, Gunn AJ, Bennet L (2012). *Am J Physiol Regul Integr Comp Physiol* **303(1)**, R30-8.

19. Broughton-Pipkin PF, Lumbers ER & Mott JC (1974). *J Physiol* **243**, 619-636.

20. Jones CT, Robinson RO (1975). *J Physiol* **248(1)**, 15-33.

21. Rurak DW (1978). *J Physiol* **277**, 341-357.

22. Peréz R, Espinoza M, Riquelme R, Parer JT, Llanos AJ (1989). *Am J Physiol*. **256(5 Pt 2)**, R1011-8.

23. Giussani DA, McGarrigle HH, Spencer JA, Moore PJ, Bennet L, Hanson MA (1994b). *J Physiol* **477 (Pt 1)**, 81-7.

24. Giussani DA, McGarrigle HH, Moore PJ, Bennet L, Spencer JA, Hanson MA (1994c). *J Physiol* **477 (Pt 1)**, 75-80.

25. Fletcher AJ, Edwards CM, Gardner DS, Fowden AL, Giussani DA (2000). *Endocrinology* **141(11)**, 3976-82.

26. Fletcher AJ, Gardner DS, Edwards CM, Fowden AL, Giussani DA (2006). *Am J Physiol Heart Circ Physiol* **291(6)**, H3023-34.

27. Morrison S, Gardner DS, Fletcher AJ, Bloomfield MR, Giussani DA (2003). *J Physiol* **547(Pt 1)**, 283-91.

28. Thakor AS, Richter HG, Kane AD, Dunster C, Kelly FJ, Poston L, Giussani DA (2010b). *J Physiol* **588(Pt 21)**, 4235-47.

29. Kane AD, Herrera EA, Hansell JA, Giussani DA (2012). *J Physiol* **590(Pt 2)**, 323-34.

30. Kane AD, Hansell JA, Herrera EA, Allison BJ, Niu Y, Brain KL, Kaandorp JJ, Derks JB, Giussani DA (2014). *J Physiol* **592(Pt 3)**:475-89.

31. Thakor AS, Allison BJ, Niu Y, Botting KJ, Serón-Ferré M, Herrera EA, Giussani DA (2015). *J Pineal Res*. Apr 22. doi: 10.1111/jpi.12242.

Figure 3. Ontogeny of the fetal cardiovascular responses to acute hypoxia. The data show mean±SEM for the fetal heart rate, fetal arterial blood pressure, fetal femoral blood flow and fetal femoral vascular resistance during a 1 hour episode of acute hypoxia (box) in 13 fetuses between 125-130 days of gestation, 6 fetuses between 135-140 days of gestation and 6 fetuses >140 days (term is *ca.* 145 days). Basal fetal heart rate and basal fetal femoral blood flow decrease with advancing gestation. In addition, during acute hypoxia, the bradycardia becomes enhanced and persistent and the femoral vasoconstriction is more intense as the fetus approaches term. Redrawn from Fletcher *et al.* (2006), with permission.

Figure 4. Anteanatal glucocorticoid therapy and maturation of the fetal cardiovascular defence to acute hypoxia. The data show mean±SEM for the fetal heart rate, fetal arterial blood pressure, fetal femoral blood flow and fetal femoral vascular resistance during a 1-hour episode of acute hypoxia (box) in 14 fetuses at 127±1 day of gestation (term is *ca.* 145 days) following 2 days of continuous fetal i.v. infusion with saline or with dexamethasone treatment. Fetal treatment with dexamethasone switches the pattern and the magnitude of the fetal heart rate and the femoral vascular resistance responses to acute hypoxia towards those seen in fetuses close to term (see Figure 3). This indicates accelerated maturation of the fetal cardiovascular defence to acute hypoxia by antenatal glucocorticoid treatment. Redrawn from Fletcher *et al.* (2003), with permission.

Abstract Figure. The fetal brain sparing response to hypoxia. The fetal brain sparing response to acute hypoxia is triggered by a carotid chemoreflex which leads to bradycardia and an increase in peripheral vasoconstriction. The bradycardia is mediated by a dominant vagal influence on the fetal heart. The neutrally-triggered peripheral vasoconstriction is maintained by the release of constrictor hormones

into the fetal circulation as well as a local vascular oxidant tone, determined by the interaction between NO and ROS, such as the superoxide anion ($\cdot O_2^-$).

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CONFLICTS OF INTEREST DISCLOSURES

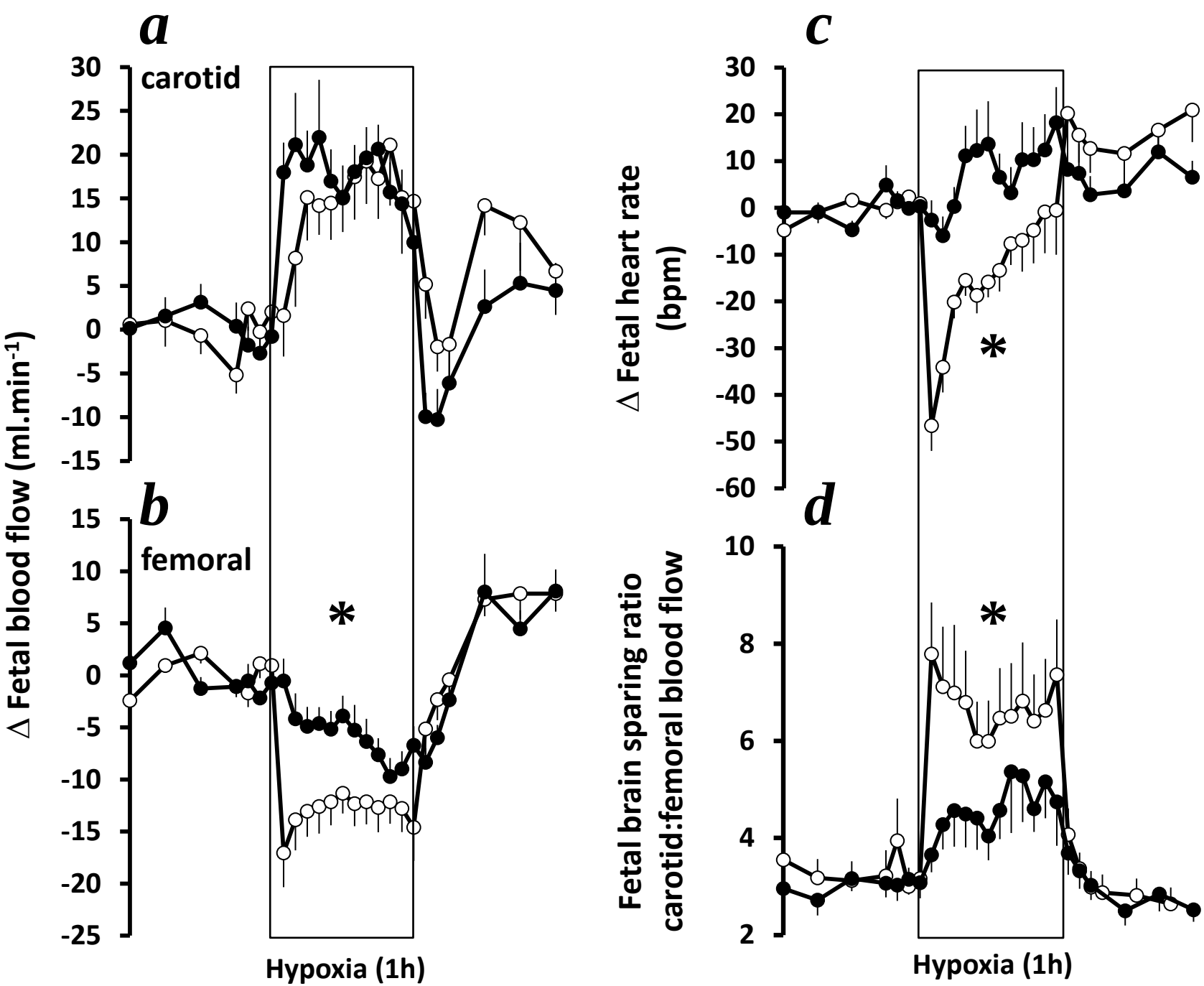
The authors declare no conflicts of interest.

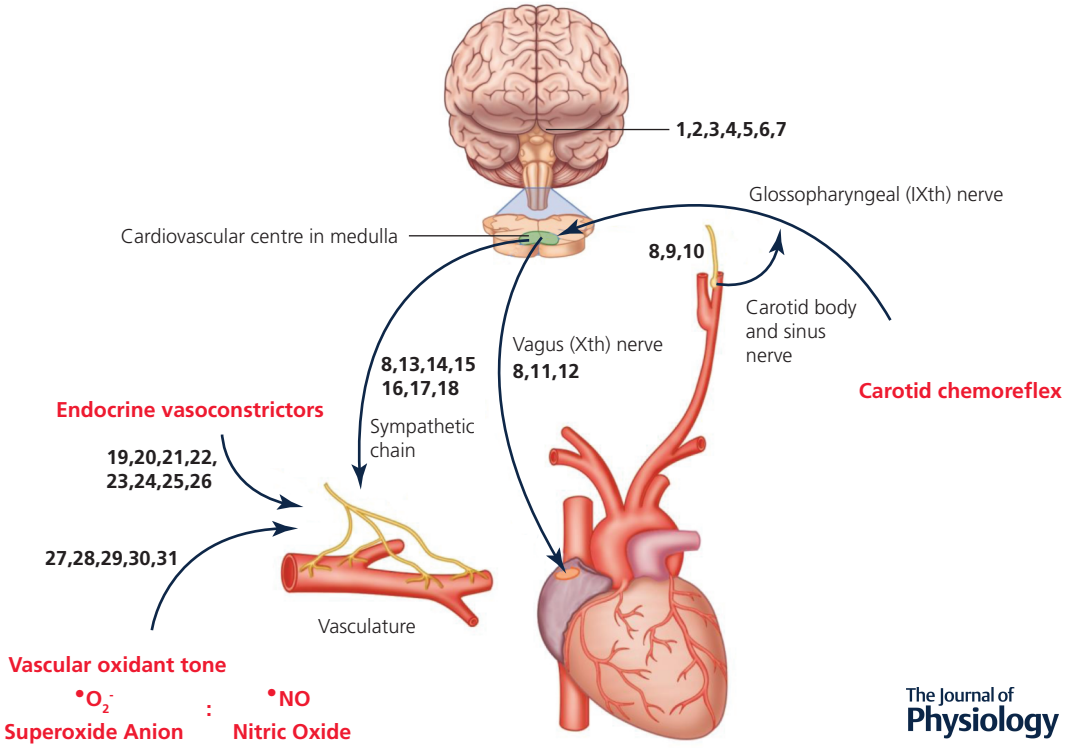
AUTHOR PROFILE

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, a Royal Society Wolfson Research Merit Award Holder and President of the Fetal & Neonatal Physiological Society. Dino's current programmes of research use an integrative approach at the whole animal, isolated organ, cellular and molecular levels to determine the role of fetal oxygenation and reactive oxygen species in cardiovascular development, and in setting an increased risk of cardiovascular disease in later life.

Table 1. Causes and consequences of fetal hypoxia

Acute fetal hypoxia	
Umbilical cord compression	Giussani DA et al. *Am J Physiol* 273(5 Pt 2): H2351-60, 1997
Myometrial contractions during labour	Huch A et al. *Br J Obstet Gynaecol* 84 Suppl 1: 1-39, 1977
Myometrial contractures	Llanos AJ et al. *Am J Obstet Gynecol* 155(4): 893-7, 1986 Shinozuka N, Nathanielsz PW. *Am J Obstet Gynecol* 180: 1202-8, 1999
Short inter-contraction interval	Peebles DM et al. *Br J Obstet Gynaecol* 101(1):44–8, 1994
Placental abruption	Yamada T et al. *Early Hum Dev* 88(11): 861-4, 2012
Major antepartum haemorrhage	Green-top Guideline No. 63. London (UK): RCOG; 20
Abnormal presentation	Leung TY et al. *Brit J Obstet Gynaecol* 118(4): 474-9, 2011
Post-term labour	Vorherr H. Am J Obstet Gynecol 123(1), 67-103, 1975
Multiple pregnancy	Roberts CL. *Obstet Gynecol* 125(1): 103-10, 2015 Smith GC. *BMJ* 17;334:576, 2007
Oligohydramnios	Robson SC et al. *Am J Obstet Gynecol* 166:78–82, 1992
Intrapartum analgesia	Ratcliffe FM, Evans JM. *Eur J Anaesthesiol* 10: 175–181, 1993
Chronic fetal hypoxia	
Uteroplacental dysfunction	Pardi G et al. *N Engl J Med*. 328(10): 692-6, 1993 Baschat AA. *Br J Obstet Gynaecol* 111: 1031-1041, 2004
Pre-eclampsia	Kingdom JC, Kaufmann P. *Placenta* 18(8): 613-21, 1997 Soleymanlou N et al. *J Clin Endocrinol Metab* 90: 4299-4308, 2005
Gestational or Essential Hypertension	Allen VM et al. *BMC Pregnancy Childbirth* 4:17–25, 2004
Chorioamnionitis	Maberry MC et al. *Obstet Gynecol* 76(3 Pt 1): 351-4, 1990
Polyhydramnios	Brace RA. *Clin Obstet Gynecol* 40(2): 280-9, 1997
High altitude pregnancy	Makowski EL et al. *Am J Obstet Gynecol* 100(6): 852-61, 1968 Giussani DA et al. *J Physiol* **585(Pt 3)**: 911-7, 2007
Maternal smoking	Longo LD. *Science* 194(4264): 523-5, 1976
Maternal cyanotic heart disease	Whittemore R et al. *Am J Cardiol* 50(3): 641-51, 1982
Maternal respiratory disease	Katz O, Sheiner E. Expert Rev Respir Med 2(1): 97-107, 2008
Prolonged rupture of membranes	Mandel D et al. *J Perinatol* 25(11); 690-3, 2005
Recurrent antepartum haemorrhage	Harlev A et al. *J Matern Fetal Neonatal Med* 21:331–5, 2008
Nuchal cord	Hashimoto K, Clapp. *J Soc Gynecol Investig* 10(7): 406-11, 2003
Maternal anaemia	Davis L et al. *J Physiol* 565(Pt 1): 35-41, 2005
Immune hydrops (Rhesus disease)	Soothill PW. *Obstet Gynecol* 69(2): 268-71, 1987
Gestational diabetes	Escobar J et al. *Neonatology* 103(3): 193-8, 2013
Maternal obesity	Hayes EK et al. *PLoS One* 7(3): e33370, 2012 Kaplan-Sturk R. *BMC Res Notes* 6: 50, 2013
Multiple Pregnancy	Valsky DV et al. *Semin Fetal Neonatal Med* 15(6):342-8, 2010
Maternal substance abuse	Goiun K et al. *Am J Obstet Gynecol* 204:340:1–12, 2011 Kennare R et al. *ANZJOG* 45:220–5, 2005
Maternal autoimmune disease	Yasuda M et al. *Obstet Gynecol* 86:555–9, 1995
Maternal inherited thrombophilia	Brenner B, Kupferminc MJ. Best Pract Res Clin Obstet Gynaecol 17(3), 427-39, 2003
Diabetes mellitus Type 1	Howarth C et al. *Diabet Med.* 24:1229–34, 2007
Maternal malaria	Umbers AJ et al. *Trends Parasitol* 27(4): 168-75, 2011
Maternal sickle cell disease	Alayed M et al. *J Perinat Med.* 42(4):487-92, 2014
Fetal cardiac structural abnormality or Tachyarrhythmia	Abrams ME et al. *Pediatrics* 120: 84–89, 2007 Moodley S et al. *Pediatr Cardiol*. 34: 81–87, 2013


a
carotid
b
femoral
Δ Fetal blood flow (ml.min-1)
Hypoxia (1h)
c
Δ Fetal heart rate (bpm)
d
Fetal brain sparing ratio carotid:femoral blood flow
Hypoxia (1h)
*



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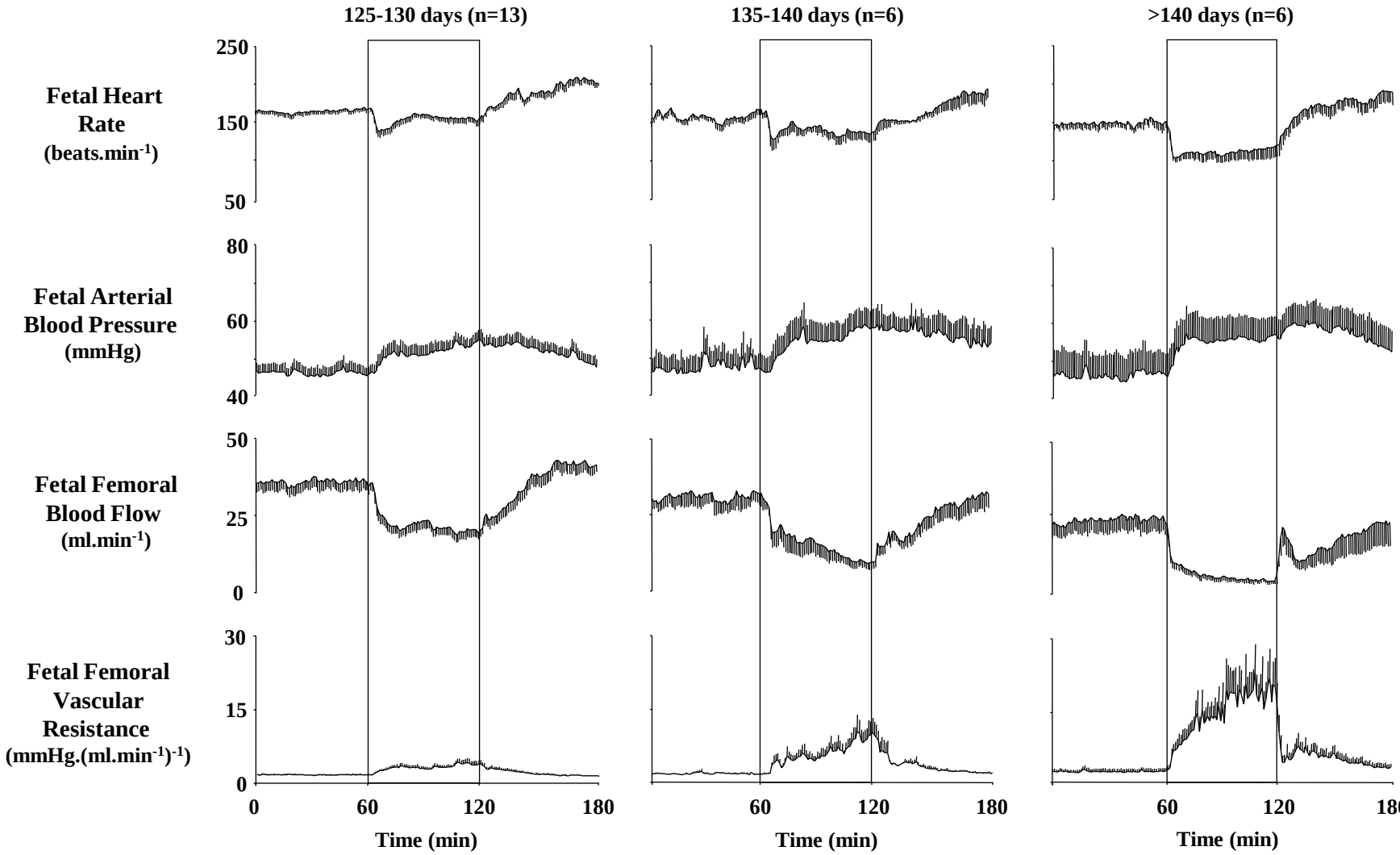
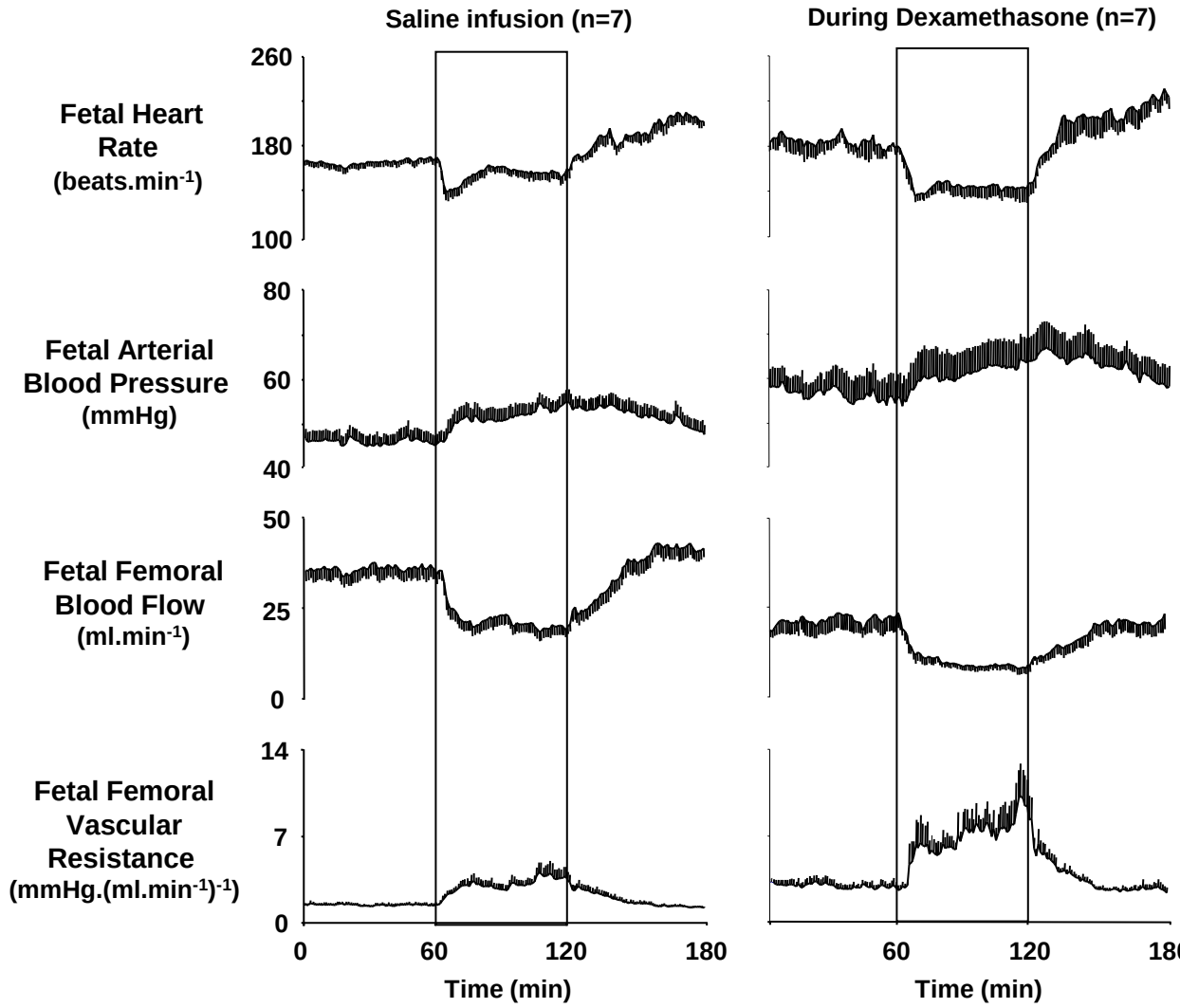

125-130 days (n=13)
135-140 days (n=6)
>140 days (n=6)
Fetal Heart Rate (beats.min-1)
Fetal Arterial Blood Pressure (mmHg)
Fetal Femoral Blood Flow (ml.min-1)
Fetal Femoral Vascular Resistance (mmHg.(ml.min-1)-1)
250
150
50
80
60
40
50
25
0
30
15
0
0
60
120
180
Time (min)


Saline infusion (n=7)
During Dexamethasone (n=7)
Fetal Heart Rate (beats.min-1)
260
180
100
Fetal Arterial Blood Pressure (mmHg)
80
60
40
Fetal Femoral Blood Flow (ml.min-1)
50
25
0
Fetal Femoral Vascular Resistance (mmHg.(ml.min-1)-1)
14
7
0
0
60
120
180
Time (min)
60
120
180
Time (min)