DOI: 10.1113/JP274949

The fetus at the tipping point: modifying the outcome of fetal asphyxia

Simerdeep K. Dhillon,[1] Christopher A. Lear,[1] Robert Galinsky,[1,2] Guido Wassink,[1] Joanne O. Davidson,[1] Sandra Juul,[3] Nicola J. Robertson,[4] Alistair J. Gunn,[1] Laura Bennet.[1]

[1] The Department of Physiology, University of Auckland, Auckland, New Zealand

[2] The Ritchie Centre, Hudson Institute of Medical Research, Melbourne, Victoria, Australia

[3] Department of Pediatrics, University of Washington, Seattle, Washington, USA

[4] Institute for Women's Health, University College London, London, UK.

Running title: Modulating fetal asphyxia

TOC: Reproduction and developmental

Figure Count: 1 + graphical abstract

Corresponding Author:

Professor Laura Bennet

Department of Physiology,

Faculty of Medical and Health Sciences,

The University of Auckland

85 Park Road, Grafton

Auckland 1142

New Zealand

Email: l.bennet@auckland.ac.nz

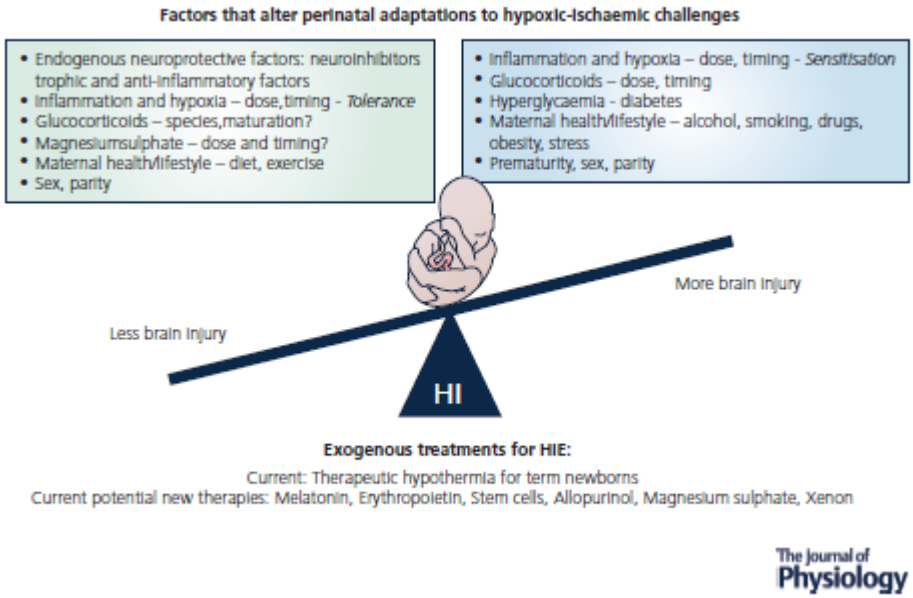
Phone: (649) 373 7599

This is an Accepted Article that has been peer-reviewed and approved for publication in the The Journal of Physiology, but has yet to undergo copy-editing and proof correction. Please cite this article as an 'Accepted Article'; doi: 10.1113/JP274949

Abstract

Brain injury around birth is associated with nearly half of all cases of cerebral palsy. Although brain injury is multifactorial, particularly after preterm birth, acute hypoxia-ischaemia is a major contributor to injury. It is now well established that the severity of injury after HI is determined by a dynamic balance between injurious and protective processes. In addition, mothers who are at risk of premature delivery have high rates of diabetes and antepartum infection/inflammation and are almost universally given treatments such as antenatal glucocorticoids and magnesium sulphate to reduce the risk of death and complications after preterm birth. We review evidence that these common factors affect responses to fetal asphyxia, often in unexpected ways. For example, glucocorticoid exposure dramatically increases delayed cell loss after acute hypoxia-ischaemia, largely through secondary hyperglycaemia. This critical new information is important to understand the effects of clinical treatments of women whose fetuses are at risk of perinatal asphyxia.

Graph Abstract:



The global burden of hypoxic-ischaemic brain injury

Hypoxic-ischaemic (HI) events at birth and during the first 28 days of life represent the single greatest contribution to overall disability worldwide. Overall, they account for one-tenth of all disability adjusted life years (DALY) (GBD 2016 Disease and Injury Incidence and Prevalence Collaborators, 2016), and preterm birth and neonatal encephalopathy are in the top 10 leading causes of DALY. Moreover, intrapartum-related death is the leading cause of neonatal mortality and the third leading cause of death in children under five (Liu *et al.*, 2015). In practice, these statistics likely considerably under-estimate the impact of HI given that many countries do not have robust maternal and perinatal mortality and morbidity databases (Blencowe *et al.*, 2016).

Further, there is a need to improve precision around terms such as 'birth asphyxia', which lack specificity, to improve our understanding of the causal pathologies (Ariff *et al.*, 2016). Imprecise terminology narrows our focus on *when* we consider injurious or life threatening HI may occur. The words birth and asphyxia, for example, tend to be become synonymous, leading us to focus on birth as the only time significant HI insults may happen. Yet, as we will discuss in this review, adverse events can affect the entire perinatal period, whether in isolation, acutely, chronically or in combination. For example, antenatal HI and other insults contribute to the antenatal origins of at least some cases of cerebral palsy (CP) (Tan, 2014; Shepherd *et al.*, 2017) (*see also Grigsby et al., in this issue*), and impaired maturation of oligodendrocytes in the preterm brain (Back, 2015). While postnatal cardiorespiratory and metabolic dysfunction (Laptook, 2013) (s*ee also the review by Bennet et al, in this issue*), and intermittent or sustained systemic infection and inflammation are all significantly associated with later-life disability (Dammann & Leviton, 2014; Hagberg *et al.*, 2015; Bennet *et al.*, 2018).

To reduce neonatal mortality and morbidity, as well as life-long disability, we need to address the significant global challenge of ensuring timely and equitable access to obstetric and neonatal care by trained staff, particularly in developing nations, although equity remains a problem even in many resource-rich nations (Tagin *et al.*, 2015; Ariff *et al.*, 2016). Further, creating maternal and perinatal mortality and morbidity databases will help determine risk factors and the success of interventions (Blencowe *et al.*, 2016). Importantly, however, it is the advances we must make in our scientific understanding about the adaptation of the fetus and the neonate to adverse events (both injurious and endogenously protective) that will allow us to unravel the complexity of the pathological causes underpinning perinatal mortality and morbidity. Such advances are vital if we are to truly improve our detection of the at risk baby and for the development of treatments which will prevent death and prevent, reduce or repair injury.

The purpose of this review is to highlight the scientific challenges we face in understanding the nature and timing of adverse perinatal insults such as HI and inflammation, their interaction with each other from fetal to newborn life, and how other factors such as clinical treatments and maternal health act to modify these interactions and thus outcomes. Our primary focus for the review is on the role of HI in neonatal encephalopathy and impaired neurodevelopmental disabilities.

Hypoxic-ischaemic challenges

Hypoxia-ischaemia at birth

While the causes of brain injury and impaired brain development are complex and multifactorial (Galinsky *et al.*, 2017b), HI contributes to injury and impaired development in both preterm and term babies (Laptook, 2016; Gale *et al.*, 2017; Huang *et al.*, 2017) and represents around 50-80% of cases of neonatal encephalopathy (NE) (Ahearne *et al.*, 2016). The majority of cases of NE occur in low-middle income countries (Lee *et al.*, 2013; Tagin *et al.*, 2015), and in developed countries, the prevalence of fetal asphyxia at delivery is around 25/1000 live births (Low, 2004), of which ~1-3/1000 live births at term will develop early onset HI encephalopathy (HIE) (Lee *et al.*, 2013; Gale *et al.*, 2017).

Few studies have evaluated HI events during *preterm* birth, but it has been suggested that the prevalence of asphyxia is around 73 per 1000 live births, of whom 50% are moderate or severe (Low *et al.*, 2003). In relatively small, retrospective studies, the rates of HIE vary between 1.4/1000 (Chalak *et al.*, 2012), 5/1000 (Schmidt & Walsh, 2010), and 9/1000 (Salhab & Perlman, 2005). However, more recently, a large cohort of 115,502 deliveries in the USA between 2008 and 2011 reported that HI in preterm birth maybe significantly higher with 37.3/1000 babies born before 37 weeks of gestation reported as having moderate to severe HIE (Manuck *et al.*, 2016). Importantly, this study demonstrated that mortality and morbidity rates rose significantly with falling gestational age at birth, such that infants born before 28 weeks of gestation had an overall rate of HIE of 120/1000, underscoring the need to define age ranges when comparing studies.

Differences between studies may relate to the size of the cohorts studied, how HI was defined, and a lack of standardised data collection (Laptook, 2016). Further, while determining if an injurious HI event has occurred can be difficult in term births (Ahearne *et al.*, 2016; Ariff *et al.*, 2016; Laptook, 2016), it is much more difficult in preterm babies, particularly in infants <30 weeks of gestation (Logitharajah *et al.*, 2009; Laptook, 2016). Thus, it is likely that HI at birth is underappreciated in very young preterm infants (Laptook, 2016). An example of how we may be under-reporting HIE in preterm infants is given by Logitharajah and colleagues who observed that around 30% of babies with HIE had a cord blood pH of >7.0 (Logitharajah *et al.*, 2009), suggesting that studies such as that of Salhab *et al*, which included only babies with a pH of <7.0 may underestimate the number of babies affected by HI. Finally, postnatal cardio-respiratory compromise further complicates the diagnosis of HI at birth (Laptook, 2016).

Antenatal hypoxia-ischaemia

The studies discussed above have evaluated the occurrence of an HI occurring around the time of birth. However, HI insults may occur both before birth (*e.g*. in association with intrauterine growth retardation (IUGR)/small for gestational age (SGA) or with discrete HI), as well as after birth (*e.g.* apnoea), particularly in preterm infants (Streimish *et al.*, 2012). IUGR/SGA is defined as birth weight below the 10th percentile (Ehrenkranz, 2007), and is seen in around 3-9% of all births in high income nations, and is more than six-fold higher in low-middle income countries (Lee *et al.*, 2013; Miller *et al.*, 2016). While many factors contribute to IUGR/SGA, including malnutrition and fetal chromosomal abnormalities, many cases relate to placental insufficiency leading to hypoxia as well

as reduced nutrition. IUGR/SGA is associated with an increased risk of death and, in survivors, with impaired neurodevelopment, CP, and increased risk for cardio-metabolic diseases (Ehrenkranz, 2007; Streimish *et al.*, 2012; Miller *et al.*, 2016). The prevalence of CP increases, for example, from 1.33 to 59.2/1000 live births in moderate-late preterm infants who weigh less than 1500g compared to 2500g (Hirvonen *et al.*, 2014).

Acute on chronic hypoxia-ischaemia

Clinically, IUGR/SGA is associated with both chronic antenatal hypoxia, with basal hypercarbia and elevated lactate levels consistent with significant chronic placental impairment, and a higher risk of death and abnormal neurodevelopmental outcomes (Nicolaides *et al.*, 1989; Arcangeli *et al.*, 2012). The adverse outcomes are at least in part associated with increased vulnerability to HI at birth (Hayes *et al.*, 2013). Consistent with this, in near-term fetal sheep, healthy normoxic fetuses adapt well to brief umbilical cord occlusions repeated every 5 minutes; a rate consistent with early labour, with minimal metabolic acidosis and stable hypertension during occlusion (Westgate *et al.*, 2005). In contrast, fetuses with pre-existing but stable moderate hypoxia develop with severe metabolic acidosis and hypotension (Westgate *et al.*, 2005). In turn, the intermitted hypotension during occlusions was associated with greater EEG suppression, inter-occlusion seizures, and more sustained cytotoxic cerebral oedema, consistent with early onset of neural injury (Wassink *et al.*, 2013). IUGR is associated with reduced stores of cardiac glycogen (Takahashi *et al.*, 1995). Given that the ability of the fetus to survive prolonged asphyxia is highly associated with levels of cardiac glycogen (Shelley, 1961), it is likely that the early onset of hypotension during umbilical cord occlusion in fetuses with pre-existing hypoxia was associated with more rapid depletion of cardiac glycogen. Evolving myocardial injury may also have contributed particularly once hypotension was established during the series of occlusions (Gunn *et al.*, 2000).

Tolerance to acute asphyxia falls towards term

Identifying acute HI insults *before* birth is more difficult, for obvious reasons. However, in the search of the pathological factors which cause injury, it is important to appreciate that immature animals, both term and preterm, show high cardiac and neural tolerance to HI (*for review see* (Bennet, 2017)). An early observation of this phenomenon came from Robert Boyle and colleagues who demonstrated in 1670 that term newborn kittens could tolerate anoxia in a vacuum chamber for far longer than adult animals (Boyle, 1670). Studies in a variety of species, including humans, have since demonstrated that immature animals have greater cardiac glycogen stores that ensure the heart can continue to beat through an HI challenge (Shelley, 1961). It is notable that cardiac glycogen stores peak in fetal life around 0.5-0.6 of gestation (Shelley, 1961), suggesting the intriguing possibility that hypoxic challenges may be particularly common in preterm life. Higher glycogen stores facilitate anaerobic metabolism, and so, healthy preterm fetuses can survive much longer periods of HI induced by umbilical cord occlusion than their term counterparts (Bennet, 2017). Moreover, the preterm fetus can tolerate longer periods of hypoxia, hypoperfusion and hypotension before developing injury to the brain and other organs (Keunen *et al.*, 1997; Quaedackers *et al.*, 2004a; Quaedackers *et al.*, 2004b; Wassink *et al.*, 2007; Bennet, 2017).

However, even in the very preterm fetus, there comes a point when a sufficient duration of severe HI, hypoperfusion and hypotension will cause neural injury. A preterm fetus exposed to severe HI insult *in-utero* can continue to survive with evolving brain injury. Studies examining neural outcomes after severe HI in preterm fetal sheep have shown that by 72 hours there is diffuse white matter loss, subcortical neuronal injury and no cortical neuronal loss (Bennet *et al.*, 2007; Wassink *et al.*, 2017). Diffuse white matter injury evolves over time involving degenerative, proliferative and arrested maturation processes. One week after prolonged cerebral ischaemia or severe HI in preterm fetal sheep, proliferation of oligodendrocyte progenitor cells restored the number of total oligodendrocytes (Riddle *et al.*, 2011; Drury *et al.*, 2014). However, newly formed pre-oligodendrocytes failed to differentiate into mature oligodendrocytes and there was loss of white matter volume (Riddle *et al.*, 2011).

Chronic activation of microglia and astrogliosis persisted three weeks after severe HI in preterm fetal sheep, with the reduced number of mature, myelin-producing oligodendrocytes, altered myelination in the subcortical white matter tracts and reduced cortical thickness (van den Heuij *et al.*, 2017). Furthermore, MRI data from preterm fetal sheep, four weeks after prolonged cerebral ischaemia, showed altered microstructural development of grey matter with reduced dendritic arbour complexity and spine density of cortical projection neurons and medium spiny neurons of caudate nucleus, and functional disturbance in glutamatergic signalling (Dean *et al.*, 2013; McClendon *et al.*, 2014). The patterns of injury seen in preterm fetal sheep after HI are highly consistent with the spectrum of injury seen in contemporary cohorts of preterm infants (Buser *et al.*, 2012; Ball *et al.*, 2015; Thomason *et al.*, 2017).

Further, milder insults, which are less easy to diagnose, can also have long-term adverse effects on the brain. Transient moderate hypoxia in preterm fetal sheep, at 0.65-0.7 gestation (equivalent to 28-30 week human brain maturation), significantly impaired maturation of the fetal sub-plate neuron arborisation and activity (McClendon *et al.*, 2017) with the impact on maturation related to the severity of hypoxia. Similarly, impaired brain development and delayed cerebral injury was seen after mild HI in preterm-equivalent neonatal rats at postnatal day (P) 3 (Sizonenko *et al.*, 2003), and at P7 (Geddes *et al.*, 2001). Thus, it is entirely feasible that HI insults can occur well before birth, which are undetected and which the fetus can survive and then continue to develop until either preterm or term birth. In turn brain injury or impairment sustained before birth may then be added to by HI events during birth. It is notable that fetal heart rate (FHR) monitoring; the gold standard for monitoring fetal well-being during labour (*for review see* (Lear *et al.*, 2016)), assumes that without evidence to the contrary that the fetus being monitored is neurologically intact. Research is now being undertaken to begin to determine the effect of the fetal adaptation to HI insults *in utero* on FHR parameters (*see Yamaguchi et al, this issue*).

Perinatal events are associated with approximately half of all cases of cerebral palsy (CP) (Reid *et al.*, 2016). Approximately 15 to 20% are related to acute HIE at term (Reid *et al.*, 2016), while a third of cases are related to preterm birth (Committee on Understanding Premature Birth and Assuring Healthy Outcomes). In preterm neonates, the causes of brain injury are very complex, but a recent study has demonstrated perinatal HI-related risk factors such as acidaemia, and Apgar score are strongly associated with the development of periventricular white matter injury (Huang *et al.*, 2017). Recent magnetic resonance imaging cohort studies of children with CP show that only ~13% have

normal imaging, and another 10% have malformations (Reid *et al.*, 2014). Most of the remainder show overt white or grey matter injury or focal vascular insults. Thus, it is plausible that at least some children born at term with no apparent intrapartum risk factors had had undetected pre-partum HI or infection\inflammation. Further, even in infants with known acute HIE at birth, over half also had antepartum risk factors (Badawi *et al.*, 1998).

Further, there is increasing evidence that late stillbirth before the onset of labour is presumptively related to impaired placental perfusion causing fetal HI. Rates of stillbirth have been reduced by targeting fetuses who show acute reductions in fetal movements (Stacey *et al.*, 2011). Moreover, the New Zealand multicentre stillbirth case-control study recently showed that when mother went to sleep in the supine position the risk of stillbirth was increased (adjusted OR 3.7), independent from other common risk factors, and thus may be a modifiable risk factor (McCowan *et al.*, 2017). The mechanism is likely reduced uterine perfusion. Consistent with this, in a study of healthy women in late pregnancy, the semi-recumbent and supine positions were associated with fetal sleep state switching to quiet sleep, in which fetal oxygen consuming activity is reduced with correspondingly reduced fetal heart rate variation (Stone *et al.*, 2017).

Postnatal hypoxia-ischaemia

Apnoea of prematurity and periodic breathing cause repeated, mild hypoxic insults that are associated with neurodevelopmental and motor impairments (Schmidt *et al.*, 2017) (*also see Bennet et al, in this issue*). Further, potential dysregulation of cerebral autoregulation in sick infants may lead to reduced cerebral perfusion and oxygenation (Vesoulis & Mathur, 2017). In preterm babies, perfusion and thus oxygenation may be complicated by their immature lungs and by persistent patent ductus arteriosus (Di Fiore *et al.*, 2013). Periodic breathing and apnoea are more common in preterm babies, but breathing is also often irregular in term babies with HIE and may require ventilation; stable management of these infants remains a key challenge (Martinello *et al.*, 2017). Cerebral desaturation during apnoeic periods may be sufficiently challenging to cerebral metabolism that there is EEG suppression (Low *et al.*, 2012). The preterm brain may also be exposed to spontaneous periods of desaturation (Baerts *et al.*, 2011), but correction with oxygen supplementation can lead to intermittent cerebral hyperoxia, which may itself be injurious (Baerts *et al.*, 2011).

Worryingly, many preterm babies continue to have periodic breathing (Decima *et al.*, 2015) and persistent apnoea (Horne *et al.*, 2017) after being discharged and these events are associated with cerebral desaturation, and thus may contribute to later life neurocognitive impairment (Decima *et al.*, 2015; Horne *et al.*, 2017). Such events are more prevalent around 2-3 and 5-6 months of life than during the first few weeks of life (Horne *et al.*, 2017). Further, individuals born preterm are 3-4 times more likely as children and adults to experience sleep disordered breathing such as snoring and obstructive sleep apnea (Rosen *et al.*, 2004). These pathological conditions can also causes intermittent hypoxia and are associated with learning and behavioural difficulties (Rosen *et al.*, 2004).

Preclinical data support these findings. Mild, intermittent hypoxia given to rats P2 and P12, with follow-up to P18 and P22, was associated with evidence systemic and brain inflammation, impaired

white matter integrity, and metabolic changes consistent with hypoxia (Darnall *et al.*, 2017). Repetitive apnoea in anaesthetised newborn piglets was associated with progressive cortical oxygenation (Schears *et al.*, 2005) and evidence of cortical and subcortical injury (Mendoza-Paredes *et al.*, 2008). Further, in a study where LPS was given to P2 rats who were then followed-up to P10, showed that inflammation may cause more episodes of periodic breathing, potentially by altering carotid body structure and function (Master *et al.*, 2016).

Recovery from hypoxic-ischaemic insults

Contributing to the difficulty in determining whether an injurious insult has occurred is that injury evolves over time (Bennet *et al.*, 2006; Iwata *et al.*, 2008). As discussed below, external factors such as inflammation and clinical treatments can modulate how injury evolves, and affect the measurements used for diagnosis and prognosis. For example, therapeutic hypothermia for HIE significantly alters the temporal expression of seizures (Davidson *et al.*, 2015a; Lynch *et al.*, 2015). It is now well established in term infants and animals that there can be considerable cell survival after severe HI, followed by progressive evolution of bulk cell death over hours to days (Wyatt *et al.*, 1989; Lorek *et al.*, 1994). There are limited data on the post-HI evolution of injury in the preterm brain, however, preclinical data suggest that temporal changes in blood flow, cerebral oxygenation and seizures occur in a similar temporal pattern (Bennet *et al.*, 2006; Bennet *et al.*, 2010; Bennet *et al.*, 2012a).

Latent phase

Following reperfusion there is recovery of depleted high energy phosphates and at least partial resolution of cellular oedema in a so called 'latent' phase of recovery. The extent of recovery during this phase correlates with severity of injury (Iwata *et al.*, 2008). The latent phase is further characterised by suppression of electroencephalographic activity (EEG), which is mediated by neuroinhibitors such as neurosteroids (Nguyen *et al.*, 2004; Yawno *et al.*, 2007), and upregulation of the sympathetic nervous system (Quaedackers *et al.*, 2004a; Dean *et al.*, 2006). Inhibition of these neuromodulators markedly increased cerebral injury, strongly suggesting that these endogenous responses are beneficial (Dean *et al.*, 2006; Yawno *et al.*, 2007). In addition to neuronal inhibition, multiple neuroendocrine responses also help protect the brain (Robertson *et al.*, 2012). For example, in newborn piglets, P7 rats and fetal sheep there is release of melatonin early in the latent phase, and delayed upregulation of multiple anti-apoptotic growth factors such as erythropoietin (Epo) and insulin like growth factor 1 (IGF-1) in the secondary and tertiary phases after HI (Guan *et al.*, 2003; Miller *et al.*, 2005; Robertson *et al.*, 2013; Ohls *et al.*, 2015).

Studies of the preterm fetal brain have shown, however, that EEG suppression is not complete. Epileptiform transient activity (e.g. sharp waves) is observed throughout the latent phase, peaking around 2-3 hours post-HI (Bennet *et al.*, 2010). The maximum frequency of these events after HI is associated with cerebral deoxygenation and with the severity of neural injury (Bennet *et al.*, 2006). These data suggest that transients may stress injured cells and propagate injury in a similar manner to spreading depolarisations (Hartings *et al.*, 2017). Consistent with this, studies in sheep fetuses and multiple adult species (Davidson *et al.*, 2012; Hartings *et al.*, 2017; Kim *et al.*, 2017) show that astrocytic and microglial responses contribute to spreading injury from the most severely affected

regions to previously undamaged areas of the brain, in part by opening of cell membrane channels such as connexin 43 hemichannels, leading to release of excitatory small molecules such as ATP and glutamate (Davidson *et al.*, 2013; Hartings *et al.*, 2017).

EEG suppression during the latent phase is coupled with cerebral hypoperfusion, with data suggesting that this is coupled to reduced cerebral metabolism in immature (Jensen *et al.*, 2006) and adult animals (Michenfelder & Milde, 1990). Hypoperfusion is also seen in peripheral organ beds, mediated by increased vascular resistance not hypotension (*for review see* (Bennet *et al.*, 2012a). This is an important observation, as low blood pressure and hypoperfusion is a frequently seen in preterm newborns during the first few days after birth (Dempsey, 2017), and there is debate about the contribution of this apparent 'cardiovascular instability' to evolving injury and how to best to clinically manage haemodynamic changes (Dempsey, 2017). In part this relates to the variability in what is defined as normal blood pressure, but it is also clear that there is a poor relationship between blood pressure and blood flow (Dempsey, 2017), and low blood flow often does not change in response to increasing blood pressure and some cases treating hypotension is associated with adverse outcomes (Fanaroff *et al.*, 2006; Dempsey, 2017). For some infants, low blood flow may in fact be a post-HI adaptation, as seen experimentally (Bennet *et al.*, 2012a).

Secondary and tertiary phases

The latent phase is followed by a secondary deterioration in cerebral oxidative metabolism starting 6-15 hours after birth (Azzopardi *et al.*, 1989; Lorek *et al.*, 1994; Gunn *et al.*, 1997; Penrice *et al.*, 1997), due to failure of mitochondrial function (Leaw *et al.*, 2017). This phase is associated with the onset of seizures in both preterm and term fetuses, and in the term fetus, where there is cortical neuronal maturation, there is secondary cortical cytotoxic oedema (Lorek *et al.*, 1994; Gunn *et al.*, 1997; Penrice *et al.*, 1997). The timing of energy failure after HI is tightly coupled with the appearance of histologic brain damage (Blumberg *et al.*, 1997; Roth *et al.*, 1997; Vannucci *et al.*, 2004), suggesting that it is primarily a function of evolving cell death (Figure 1). Neuroprotection treatments such as therapeutic hypothermia that are effective when started in the latent phase, progressively lose effectiveness when started during the secondary phase with efficacy rapidly lost thereafter (Gunn *et al.*, 1997; Wassink *et al.*, 2014). It is unclear why mitochondria become dysfunctional when the supply of oxygen is normal, but it does provide a target for treatment (Leaw *et al.*, 2017).

Blood flow changes in the secondary phase can be variable. Preterm fetal sheep studies show that central and peripheral hypoperfusion may resolve or partly resolve after asphyxia (Bennet *et al.*, 2012a), and CBF in human preterm babies naturally rises during the first three days of life (Meek *et al.*, 1998). Preterm babies may be at risk of loss of cerebral autoregulation leading to impaired cerebral perfusion with low blood pressure (Vesoulis & Mathur, 2017), and blood flow can fluctuate during events like seizures with hypoperfusion seen in peripheral organs like the gut in preterm fetal sheep mediated by sympathetic activity (Bennet *et al.*, 2012a). In preterm infants, increased systemic perfusion and cerebral blood flow is associated with increased risk for GMH-IVH in younger preterm babies (Noori *et al.*, 2014). In term HIE infants and term fetal sheep, cerebral hyperaemia (oedema and increased cerebral blood flow) is observed (Meek *et al.*, 1999; Greisen, 2014), and, perhaps counter-intuitively, increased CBF correlates with adverse outcomes (Meek *et al.*, 1999).

The secondary phase resolves over 3-4 days post-HI into a tertiary phase of ongoing injury, involving repair and reorganisation which may last weeks to months and even years (Hagberg *et al.*, 2015; Bennet *et al.*, 2018), but there is also chronic inflammation and epigenetic changes lasting for weeks to months after injury that may prevent optimal neurorepair (Fleiss & Gressens, 2012; Galinsky *et al.*, 2017b; Bennet *et al.*, 2018). Key neuroprotection strategies in this phase include treating chronic inflammation and stimulation of endogenous factors which support proliferation, migration, and maturation of glia and neurons (Hagberg *et al.*, 2015; Bennet *et al.*, 2018). Treatments such as stem cell therapy have utility in this phase, given their multimodal effects in reducing inflammation and promoting release of trophic factors (Fleiss *et al.*, 2014; van den Heuij *et al.*, 2017; Bennet *et al.*, 2018). Further, there is a clear role for postnatal neurorehabilitation for optimising development of the neural network (Pitcher *et al.*, 2009; Maitre, 2015) (*see also Bennet et al, in this issue*). Importantly, as part of the challenges we face, it is clear that early and accurate diagnosis of conditions such as CP make a significant difference to providing the right neurorehabilitation treatment in a timely manner (Novak *et al.*, 2017).

Modification of neural outcome by multiple insults

A recent MRI study in preterm infants demonstrated that a synergy between prenatal and postnatal insults, such as intrauterine growth restriction and prolonged mechanical ventilation had a cumulative effect on white matter injury, as shown by lower white matter fractional anisotropy at term equivalent age, and impaired neurodevelopmental outcomes at 20 months corrected age (Barnett *et al.*, 2018). In the section below we discuss how exposure to inflammation, antenatal treatments (e.g. glucocorticoids and magnesium sulphate), and maternal diabetes, obesity and other lifestyle factors may modulate fetal response to HI insults and resultant neural injury.

Inflammation

It is now recognised that fetal inflammation is associated with adverse life-long outcomes such as impaired neurodevelopment, particularly after preterm birth (Dammann & Leviton, 2014; Back, 2015; Hagberg *et al.*, 2015; Bennet *et al.*, 2018). Inflammation of chorionic and amniotic membranes (chorioamnionitis), for example, is reported in nearly 95% of preterm births at 21 – 24 weeks of gestation, and in about 10% of deliveries at 33 – 36 weeks (Kim *et al.*, 2015). While infection of the fetus occurs in approximately 20-30% of confirmed intrauterine infections (Cordeiro *et al.*, 2015). Fetal inflammation (funisitis) and early neonatal bacteraemia have been shown to be independent risk factors for encephalopathy (Tann *et al.*, 2017), and late onset bacteraemia (due to factors such as long-term indwelling catheters) in preterm infants during postnatal weeks 2-4 is associated with a greater risk of neurocognitive limitations at age 10 years (Bright *et al.*, 2017). Further, there is some evidence from a cohort study of 8299 women that the combination of cord blood acidosis and maternal pyrexia independently greatly increased the risk of neonatal encephalopathy (Impey *et al.*, 2008).

Currently, the mechanisms mediating the association between infection and inflammation and neonatal encephalopathy are unclear (Hagberg *et al.*, 2015). However NE is strongly associated with elevations of pro-inflammatory mediators such as tumour necrosis factor (TNF)-α, interleukin (IL)-6, IL-8 and IL-1β and toll-like receptors (TLRs) in plasma and in the brain (Dammann & Leviton, 2014;

Hagberg *et al.*, 2015; Bennet *et al.*, 2018). Recent data from the Extremely Low Gestational Age Newborns (ELGANs) study measured pro-inflammatory cytokine patterns in whole blood of preterm infants <28 weeks gestation in the first month of life and demonstrated that elevated systemic levels of pro-inflammatory cytokines are associated with adverse neurological outcomes up to the age of 10 years (Kuban *et al.*, 2017), and that both antenatal and postnatal inflammation play a role (Yanni *et al.*, 2017). Further, data suggest that in children with CP associated with white matter loss, early exposure to inflammation is associated with chronic inflammation and increased sensitivity to inflammatory mediators later in life (Lin *et al.*, 2010).

Perinatal inflammation can function as a second hit in preterm infants with SGA, acting to increase the risk of impaired neurodevelopmental outcomes (Leviton *et al.*, 2013). Exposure to both sepsis and HI during the perinatal period increases the risk of cerebral palsy in very premature infants (Wang *et al.*, 2014). Similarly, the combination of fetal growth restriction, denoting prenatal hypoxia and postnatal inflammation markedly increases the risk of impaired neurodevelopmental scores at 2 years of age compared to either alone (Leviton *et al.*, 2013). That two injurious insults are additive is not surprising, however, considerable data suggest that inflammation can modify the responses to an HI insult in both positive (tolerance) and negative (sensitisation) ways depending on the order, intensity and time of the insults (Hagberg *et al.*, 2015; Bennet *et al.*, 2018).

Sensitisation

Clinical data suggest that prior inflammatory stimulus can enhance metabolic decompensation during subsequent HI. For example, using NIRS in preterm infants Stark *et al*., showed that intrauterine inflammation was associated with an increase in cerebral oxygen consumption after birth (Stark *et al.*, 2016). In preclinical studies, systemic inflammation induced with injection of TLR-2 agonist Pam3CSK4, given 14 hours before an HI insult in P8 mice, increased loss of brain tissue and demyelination, potentially through suppression of ADP induced oxidative phosphorylation in mitochondria (Mottahedin *et al.*, 2017). These data suggest that inflammation may play a role in the loss of mitochondrial function post-HI. Similarly, inflammation induced by the viral protein mimetic polyinosinic-polycytidylic acid (poly(I:C)) 14 hours before HI in P8 mice also increased injury and this was associated with increased pro-inflammatory cytokines and apoptotic proteins in the brain (Stridh *et al.*, 2013).

Exposure of P2 rat pups to lipopolysaccharide (LPS), a component of cell wall of gram negative bacteria, given 2 hours before HI augmented microglia activation, cerebral pro-inflammatory cytokines, blood brain barrier damage and white matter damage compared to HI alone (Wang *et al.*, 2010), with similar effects observed if LPS was given at 4 hours to P7 rats (Eklind *et al.*, 2001) or 2 and 72 hours pre-HI (Eklind *et al.*, 2005). Similar results were observed when LPS was given 14 hours before HI, with evidence that injury involved TLR-4 and the recruitment of the MyD88 adaptor protein (Wang *et al.*, 2009). Recently, fetal embryonic day 18 rat model of LPS and HI exposure demonstrated that the patterns of brain injury and motor function assessed one month after birth, were different between HI and HI+LPS, and HI with or without LPS produced patterns similar to those seen in infants with neural injury the clinical ELGANS study (Jantzie *et al.*, 2014). All pups experienced gait abnormalities, with function worse in the HI group alone. LPS alone caused inflammation, but significantly less inflammation and injury than HI and HI+LPS, with greater acute

glial activation and inflammation seen after HI+LPS. HI alone, however, was associated with greater chronic white matter and axonal injury.

Notably, in this study, the loss of myelin basic protein (a marker for myelination) was observed up to P15 in both HI and HI+LPS groups, but worsened beyond this time only in the HI alone group (Jantzie *et al.*, 2014). These data further demonstrate the need to study the evolution of injury over time to understand the relative contributions of insults. Further, the data support the concept that HI insults alone can be associated with dysmaturation of oligodendrocytes and thus the development of subsequent myelination (Back, 2015), and may contribute to more severe or chronic white matter injury patterns. LPS appears to positively moderate the severity of HI injury, and this may occur through progressive restoration of Epo receptor and ligand expression observed in this study in the HI+LPS but not HI alone group (Jantzie *et al.*, 2014).

Tolerance

In contrast to the studies demonstrating sensitisation by inflammation to greater HI mediated injury, several studies demonstrate that depending on the insult severity, the time interval between insults and maturational stage of the brain, the interaction between inflammation and HI insults can be protective. We have demonstrated in preterm fetal sheep that inflammation induced by chronic low-dose infusion of LPS (100-250ng) for five days with superimposed 1μg boluses was associated with white matter inflammation and loss of mature oligodendrocytes (Mathai *et al.*, 2013; van den Heuij *et al.*, 2014). However, exposure to inflammation was associated with a significant reduction in HI injury when an HI insult was given four hours after the last bolus of LPS (van den Heuij *et al.*, 2014). Upregulated plasma concentrations of the anti-inflammatory IL-10 and cortisol may have contributed to neuroprotection (van den Heuij *et al.*, 2014). Further, pre-treatment of preterm fetal sheep with a bolus dose of LPS (50–100 ng/kg) led to differentially regulated TLR mRNA expression and increased protein expression of interferon-beta when exposed to HI at 24 hours after LPS treatment, whereas no effect was seen with the time interval of 4 hours (Dhillon *et al.*, 2015). In this study, LPS preconditioned fetuses had reduced loss of oligodendrocytes, with reduced microgliosis and astrogliosis, at 5 days after HI (Dhillon *et al.*, 2015).

Consistent with this, in P7 rats, exposure to LPS 24 hours before HI conferred protection, in contrast to increased injury seen when it was given 2, 4, 14 or 72 hours before HI (Eklind *et al.*, 2005). This timing likely reflects, at least in part, the time needed for upregulation of type I interferon and interferon regulatory factors (Marsh *et al.*, 2009). However, the dose of the inflammatory agent and the age of exposure are also important factors. For example, tolerance to HI is observed when LPS is given at P7, P9 or P14 (Eklind *et al.*, 2005; Hickey *et al.*, 2011), and in response to polyI:C at P5 (Hickey *et al.*, 2011; Shi *et al.*, 2013), but not when LPS is given P3 and P5 rat pups, or Poly I:C given at P7 (Hickey *et al.*, 2011; Shi *et al.*, 2013). The differences may be due to the developmental differences in TLR induction (Shi *et al.*, 2013). Consistent with this, preterm neonates (<30 weeks) have attenuated innate immune responses to TLR agonists in the first 28 days of age (Marchant *et al.*, 2015). Consideration should also be given to the dose of LPS. In P7 rats, a 0.3mg/kg bolus dose of LPS given 24 hours before an HI insult increased injury, whereas a 0.05mg/kg bolus dose reduced injury and improved neurological outcomes, and this was associated with reduced microglial activation and pro-inflammatory cytokine production (Lin *et al.*, 2009).

Exposure to hypoxia can also confer tolerance to subsequent insults. The neuroinflammatory response after HI was attenuated in P7 rats preconditioned with transient asphyxia *in utero* or mild hypoxia postnatally, and the interaction between the insults was found to be neuroprotective (Park *et al.*, 2011; Vlassaks *et al.*, 2013). A 3 hour period of 8% hypoxia alone 24 hours before an HI insult in P6-7 rats suppressed glial and pro-inflammatory cytokine production (Chen *et al.*, 2015; Parmar & Jones, 2015). Intermittent periods of mild hypoxia have also been shown to cause preconditioning against later injurious HI in P7 rats (Ota *et al.*, 1998). However, prolonged spontaneous mild hypoxemia for at least 5 days before carotid artery ischaemia in near-term fetal sheep did not alter brain injury (Davidson *et al.*, 2015b). This negative finding suggests that gene induction by pre-conditioning is transient and therefore resolves during chronic hypoxia.

Modification of neural outcome by antenatal treatment

Antenatal glucocorticoids and hyperglycaemia

When considering factors which modulate the perinatal responses to HI, we should remember that the fetus and newborn are not naïve to clinical drugs treatments ranging from routine antenatal glucocorticoids and magnesium sulphate ($MgSO_4$) and to postnatal steroids, pain and anti-seizure medications, sedatives and anaesthetics, and treatments such as glucose supplementation. The catch-22 is, of course, that many of the conditions being treated (e.g. seizures, pain and hypoglycaemia) themselves modulate outcomes. However, this does not mean the treatment *per se* is without effect.

Glucocorticoids are routinely given to women at risk of preterm delivery to reduce mortality and morbidity associated with complications of being born prematurely. To date, there is no clear clinical information on the interaction between antenatal glucocorticoids and HIE in preterm infants, as such cases were excluded from many randomised controlled trials (Roberts *et al.*, 2017). However, it is striking how diverse data from preclinical studies are on the effect of glucocorticoids on the adaptation to HI insults. Maternal administration of dexamethasone given 48 hours pre-HI had no effect on injury (Elitt *et al.*, 2003). However, when given 15 minutes after HI in preterm fetal sheep dexamethasone increased injury (Koome *et al.*, 2013), and this was associated with increased EEG activity and seizure activity, and evidence of uncoupling of CBF and cerebral metabolism as well as exacerbated hyperglycaemia (Lear *et al.*, 2014). In contrast, when dexamethasone was given 4 hours pre-HI, there was a significant increase in neural injury, including induction of cystic lesions, despite evidence of reduced cytotoxic oedema during HI (Lear *et al.*, 2018). Interestingly, glucocorticoids given to normal healthy preterm fetal sheep, who have not had HI, also causes dysregulation of EEG activity and can induce seizures (Davidson *et al.*, 2011).

Similarly, some studies in neonatal rats have also reported exacerbation of HI induced neural injury with prior dexamethasone exposure (Chang *et al.*, 2013; Yeh *et al.*, 2016). For example, administering a tapering course of dexamethasone 0.5, 0.3 and 0.1 mg/Kg on postnatal days 1-3 in neonatal rats, and subsequently subjecting them to HI on P7, caused greater loss of oligodendrocytes, reduced myelin thickness, and worse functional outcome in the long-term as compared to animals subjected to HI alone (Yeh *et al.*, 2016). A role for increased excitotoxicity is

postulated as the effect of dexamethasone on HI mediated injury correlated with decreased glutamate transporter-1 (GLT-1)-mediated glutamate reuptake observed after HI (Chang *et al.*).

In contrast, many studies in postnatal rats have shown neuroprotection with glucocorticoids given 4-48 hours before HI (Barks *et al.*, 1991; Chumas *et al.*, 1993; Ekert *et al.*, 1997; Dardzinski *et al.*, 2000; Felszeghy *et al.*, 2004; Ikeda *et al.*, 2005; Feng *et al.*, 2011), but no effect if given within 3 hours of an HI insult. Post-treatment is associated with no effect when given immediately, 24 or 48 hours post HI (Barks *et al.*, 1991), or protection when given 2 hours post-HI (Harding *et al.*, 2016). Differences in dose, timing of glucocorticoid administration and route of administration; e.g. intracerebroventricular injection (Harding *et al.*, 2016) and intraperitoneal injection (Barks *et al.*, 1991) remain to be explored. It is noted, however, that clinically, antenatal steroid exposure is associated with risk of increasing adverse neurodevelopmental outcomes (Qin *et al.*, 2017). Finally, Ikeda and colleagues have demonstrated in P6 rats, that the protection conferred by exposure to LPS 24 hours before HI was prevented by co-administration of the glucocorticoid receptor blocker RU486 (Ikeda *et al.*, 2005).

One significant effect of glucocorticoids is to increase glucose, and the differences between the perinatal rat and sheep data of the effects of HI mediated injury may be explained by differences in glucose handling in the newborn period between species, which is discussed in the next section. Developmental changes in glucose handling may also explain why pre-treatment with glucocorticoids in adult rats is usually associated with increased HI mediated brain injury, as reviewed (Bennet *et al.*, 2012b).

Glycaemia

Term infants with HIE (Nadeem *et al.*, 2011) and preterm infants (McKinlay *et al.*, 2017; Sharma *et al.*, 2017), show highly variable blood glucose levels during the early period after birth, and a current clinical challenge is to understand what constitutes euglycaemia and how fluctuating glucose may contribute to NE and thus how best to manage changes in glucose (Ogilvy-Stuart & Beardsall, 2010). Clinical data from term infants with HIE shows adverse neurodevelopmental outcomes associated with both hyperglycaemia and hypoglycaemia during the first day after birth (Chouthai *et al.*, 2015; Basu *et al.*, 2016). In preterm infants, hypoglycaemia is often followed by hyperglycaemia, mediated by insulin resistance and insulin deficiency (Ogilvy-Stuart & Beardsall, 2010). In preterm infants hyperglycaemia is associated with increased mortality (Alexandrou *et al.*, 2010; van der Lugt *et al.*, 2010), adverse neurodevelopmental outcomes (van der Lugt *et al.*, 2010), and injury to white matter (Alexandrou *et al.*, 2010). Currently, the role of glycaemia in modulating HI in preterm births is not known.

As with glucocorticoids, the data on the HI modulating effects of glucose are variable. In preterm fetal sheep increasing glucose to similar levels seen after dexamethasone produced the same severe cystic injury patterns as seen with dexamethasone (Lear *et al.*, 2014; Lear *et al.*, 2018). Given that post-HI dexamethasone was associated with more modest injury (Koome *et al.*, 2013), this suggests that increased glucose compromises cellular function during the HI insult. *In vitro* evidence supports this concept and further suggests that increased opening of connexin hemichannels may be a key factor in the detrimental effects of hyperglycaemia during HI (Orellana *et al.*, 2010). Similarly,

hyperglycaemia during HI exacerbates neural injury in newborn piglets (LeBlanc *et al.*, 1993), term-equivalent fetal sheep (Petersson *et al.*, 2004), and adult rats (Lin *et al.*, 1998).

In marked contrast, hyperglycaemia is independently protective and, at least in part, mediates the protective effects of dexamethasone in P7 rats after HI (Vannucci & Mujsce, 1992; Tuor *et al.*, 1997). The most likely explanation for the age-related difference in rats, and the difference with other species is the much lower uptake of glucose into the neonatal rat brain (Vannucci, 1994; Vannucci *et al.*, 1996). Therefore, the reassuring neuroprotective effects of dexamethasone for HI induced neural injury observed in neonatal rat studies might not translate into human infants. Consistent with this, a recent meta-analysis reported lack of evidence for antenatal glucocorticoid treatment to have a preventive effect on CP (Shepherd *et al.*, 2017), and that both hypoglycaemia and hyperglycaemia were associated with adverse outcomes in term infants with HIE (Basu *et al.*, 2016). Intriguingly, hyperglycaemia infants with HIE birth actually showed significantly greater improvement with therapeutic hypothermia compared to normothermia (Basu *et al.*, 2017). This suggests either that infants with hyperglycaemia may have been in an early, more treatable phase of injury or that the injurious effects of hyperglycaemia on the brain may be treatable. Thus, there is an urgent need to better understand the impact of glucose management in preterm and sick babies.

Magnesium sulphate

Evidence from meta-analyses and systemic reviews show that $MgSO_4$ administered to women at risk of preterm labour is associated with small, but significant reduction in the risk of CP at 18 months to 2 years of age (Doyle *et al.*, 2009). However, the long-term follow-up studies show that $MgSO_4$ treatment is not associated with significant improvement in neurodevelopmental outcomes at school age, although these were small studies (Chollat *et al.*, 2014; Doyle *et al.*, 2014). Preclinical studies in term equivalent animals of effects of $MgSO_4$ for HIE have reported highly inconsistent outcomes, ranging from neuroprotection, to no effect or increased neuronal loss; it is highly likely that apparent neuroprotection was mediated by drug induced hypothermia (Galinsky *et al.*, 2014).

Magnesium's primary neural effect is to inhibit glutamatergic signalling through binding its specific site on the N-methyl-D-aspartate receptor (Zeevalk & Nicklas, 1992). Consistent with this, reduced basal brain activity was reported in preterm infants treated with $MgSO_4$ (Stark *et al.*, 2015), and in preterm fetal sheep (Galinsky *et al.*, 2016). There is some evidence for anti-oxidative and anti-inflammatory effects for $MgSO_4$ (Maulik *et al.*, 1999; Sugimoto *et al.*, 2012). In preterm fetal sheep, $MgSO_4$ for 24 hours before and after asphyxia was associated with a significant reduction in basal EEG activity and seizure burden after asphyxia (*see also Bennet et al., in this issue*), but no effect on microglial activation, macrophage infiltration, astrogliosis or neuronal loss. Indeed, it was associated with *increased* loss of oligodendrocytes 72 hours after injury (Galinsky *et al.*, 2017a). A recent study in P7 rats suggests that the interaction between $MgSO_4$ and HI is time dependent, with neuroprotection seen when it was administered between 6 days and 12 hours before HI, but not at 3 hours or 30 minutes before HI (Koning *et al.*, 2017). This effect was likely mediated by improved mitochondrial resistance to HI. Overall, these studies suggest the impact of $MgSO_4$ on HIE is complex and possibly time dependant. Thus, further careful investigation into the effects of $MgSO_4$ in preterm and term-equivalent translation animal models is essential before undertaking large randomised clinical trials for HIE.

Maternal health and lifestyle associated risks

A variety of maternal health and lifestyle factors can affect normal fetal development. Decades of research have confirmed the considerable potential for harm to the fetus associated with maternal alcohol intake and smoking, including impaired fetal neurodevelopment (Polanska *et al.*, 2015). Despite public health warnings, however, it remains a challenge to improve rates of cessation. It is striking, for example, how many women in both developed and developing nations binge drink before and during pregnancy (Lange *et al.*, 2017). Added to these perennial health problems, maternal obesity and diabetes are increasing (Langer, 2018).

The previous section detailed experimental research which suggests that hyperglycaemia can increase the risk of perinatal brain injury after HI, suggesting that the clinical association between hyperglycaemia and adverse perinatal outcomes is at least partly causal. This is of particular concern given that there is a world-wide "epidemic" of obesity, such that in the United States and Germany, for example, at least half of all women are overweight or obese before and during pregnancy (Dudenhausen *et al.*, 2015). Obesity is associated with increased risks of miscarriage, premature birth, stillbirth, and gestational diabetes (Kalliala *et al.*, 2017), and both clinical and preclinical data show that maternal obesity is strongly associated with later life risk for cardiometabolic disease in offspring, highlighting the transgenerational risk of maternal obesity (Mehta *et al.*, 2014; Nicholas *et al.*, 2016).

Maternal obesity before and during pregnancy is associated with impaired neurodevelopmental and behavioural and psychiatric outcomes in term and preterm offspring (Mehta *et al.*, 2014; Reynolds *et al.*, 2014; Edlow, 2017). The greater the maternal weight, the greater risk of adverse perinatal outcomes (Smid *et al.*, 2016). Maternal obesity is associated with impaired white matter development in term infants assessed 2 weeks after normal delivery (Ou *et al.*, 2015), and obesity and chorioamnionitis were independently correlated with periventricular white matter injury in preterm babies (Herzog *et al.*, 2015). Obesity is associated with greater complications leading to an

increased risk for mortality, and adverse outcomes including seizures (Yao *et al.*, 2017), this includes an increased risk of severe HI (Persson *et al.*, 2014). Maternal obesity is often accompanied by an increased risk for gestational diabetes and both are also associated with poor placental perfusion and conditions such as pre-eclampsia that are associated with fetal inflammation, hypoxia and IUGR (Spradley, 2017). The factors that mediate the impact of obesity during development and which may interact with HI insults are multifactorial and include chronic neuroinflammation, oxidative stress, as well functional changes in maternal and perinatal insulin, glucose and leptin signalling (Edlow, 2017).

Collectively this overview of some of the additive physiological and clinical factors the fetus and newborn are exposed to during and after an HI insult highlights the magnitude of the challenges that we face in dissecting mechanisms of action. While the challenge is substantial, this knowledge gives us many targets to base therapeutic strategies on. Some of them are commitments to lifestyle changes such as diet and exercise, which reduce obesity and can prevent gestational diabetes leading to improved pregnancy outcomes (Brown *et al.*, 2017). Others require clinical interventions. In the final section below, we address potential therapeutic targets.

Improving outcomes by augmenting endogenous protective responses

As previously discussed, HI triggers multiple endogenous protective responses. Here we review two promising examples of how we can augment these responses to protect the perinatal brain.

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is released from the pineal gland and helps entrain circadian rhythms (McMillen *et al.*, 1995). A role for melatonin in modulating HI injury has been demonstrated in both adult and neonatal animals through anti-oxidant, anti-inflammatory and oxygen free radical scavenging effects (Hassell *et al.*, 2015). In addition, melatonin also mediates systemic effects on vascular reactivity and immune system that may provide indirect neuroprotective effects (Colella *et al.*, 2016).

Fetuses receive melatonin through the placenta from mother and therefore they have a circadian melatonin rhythm (McMillen *et al.*, 1995; Seron-Ferre *et al.*, 2012). This is lost at birth, and neonates have low, arrhythmic levels of melatonin in plasma, until the pineal gland begins production (Kennaway *et al.*, 1992). This absence of circadian release of melatonin for the first few weeks of life may help facilitate adaptation of the newborn to the physiological demands of the post-natal environment, including the need to eat regularly both day and night (Mirmiran *et al.*, 2003).

Endogenous melatonin production is increased after traumatic brain injury in human adults and children (Marseglia *et al.*, 2017), raising the possibility that endogenous melatonin may help protect the brain. Supporting this hypothesis, HI injury is significantly increased after pinealectomy in adult rats (Kilic *et al.*, 1999), and exogenous administration of melatonin after HI reduced neural injury in a range of preclinical paradigms, as reviewed (Robertson *et al.*, 2012). Similarly, an acute increase in endogenous melatonin levels was seen in newborn piglets after HI (Robertson *et al.*, 2013). Interestingly, an injurious stimulus can also induce extra-pineal melatonin production in different organ systems, but it is not known if this would lead to an increase in circulating levels (Acuna-Castroviejo *et al.*, 2014).

There is now consistent evidence in neonatal animals that exogenous melatonin can reduce HI brain injury (Robertson *et al.*, 2012). In preterm fetal sheep, infusion of low-dose melatonin (0.1mg/kg bolus followed by 0.1 mg/kg/h for 6 hours) to the mother starting 15 minutes before severe global asphyxia induced by umbilical cord occlusion reduced microglia activation and improved survival of mature oligodendrocytes in the periventricular white matter at seven days after asphyxia (Drury et al., 2014). Critically, exogenous melatonin can also be protective *after* HI. For example, high-dose melatonin (20mg/kg) starting 10 minutes after asphyxia and continued for 6 hours was associated with slower recovery of fetal blood pressure but reduced numbers of activated microglia and cell death (Welin *et al.*, 2007). Further, melatonin given to preterm fetuses from 2 to 6 hours after asphyxia reduced apoptosis, inflammation and oxidative metabolism (Yawno et al., 2017). Supporting this, a recent study in newborn lambs demonstrated that melatonin given either by IV injection or transdermal patch starting 30 minutes after acute asphyxia at birth reduced neuroinflammation, oxidative stress in white matter, and improved survival of mature oligodendrocytes and myelin density by ten days after HI (Aridas *et al.*, 2018). Finally, in term piglets, exogenous infusion of high-dose melatonin starting 10 minutes after HI significantly augmented hypothermic neuroprotection (Robertson *et al.*, 2013). Brain protection was dependent on the timing and dose of intravenous melatonin in the piglet, such that administration 2 hours after HI was less protective than when it was given at 10 minutes after HI.

Clinically, a small randomised control trial has assessed the feasibility of using melatonin in combination with therapeutic hypothermia after HIE at term (Aly *et al.*, 2015). Melatonin was given as five daily enteral doses (10mg/kg). The study found that melatonin during hypothermia was associated with a reduction in seizures, white matter abnormalities, and appeared to improve survival without neurological or developmental abnormalities at 6 months (Aly *et al.*, 2015). These encouraging, preliminary data suggest that melatonin can improve neural outcomes, and its effects are not altered by hypothermia, which is seen with some potential treatments (Gunn & Groenendaal, 2016).

However, despite the promising neuroprotective effects of melatonin caution is needed for its use to treat neonates with HIE. First, the reader should appreciate that preclinical studies examining neuroprotection with melatonin typically dissolved melatonin in ethanol. In one study in preterm fetal sheep, even a very small amount of ethanol had regional specific effects to improve neuronal survival in the caudate nucleus, but increased neuronal loss in regions of the hippocampus (Drury *et al.*, 2014). Further, in P7 rats, an alternative solvent for melatonin, dimethyl sulfoxide, also affected cerebral energy metabolism and neurotransmitter concentrations as measured by MR spectroscopy

both in sham controls after HI (Berger *et al.*, 2017). This illustrates the complexity of developing therapies for translation and highlights the urgent need for safe formulations of melatonin.

Moreover, there is limited data on the pharmacokinetics of melatonin in preterm infants, the threshold dose of melatonin and optimal route for melatonin administration required for neuroprotection in neonates (Colella *et al.*, 2016). Recent studies have demonstrated a different pharmacokinetic profile of melatonin in preterm infants compared with adults and children, therefore data from adult studies should be used with caution to guide neonatal administration (Merchant *et al.*, 2013). Furthermore, melatonin metabolites might have unintended side effects like sedation (Colella *et al.*, 2016). Different doses and treatment regimens of melatonin also need to be tested, to establish if substitutive or supra-physiological doses are required for an optimal neuroprotective effect. A recent study in newborn lambs showed that during the early postnatal period when endogenous melatonin levels are low, high-dose exogenous melatonin treatment interfered with the postnatal adaptation of adrenocortical function and heart development (Seron-Ferre *et al.*, 2017). Melatonin injections (0.25 mg/Kg) on postnatal days 1-5 altered clock-time related changes in levels of hormones and metabolic markers, affected the expression of clock genes and functional genes in adrenals and heart, and deceased heart/body weight ratio (Seron-Ferre *et al.*, 2017). Although the long-term consequences of these changes are not known, these data suggest the need for more preclinical and clinical research on the systemic effects of melatonin treatment during the early postnatal period.

Erythropoietin (Epo)

By contrast with the very rapid release of melatonin, the endogenous growth factor Epo shows slower upregulation that is seen mainly during the secondary and tertiary phases after HI. Epo and Epo receptor protein expression were increased in the injured hemisphere of P7 rats at 24 hours and one week after HI (Sun *et al.*, 2004). Similarly, Epo receptors were upregulated in the P2 rat brain after exposure to transient HI *in-utero* at embryonic day 18 (Mazur *et al.*, 2010). In contrast, brain-specific gene deletion (EpoR / Epo) renders neurons more susceptible to glutamate and hypoxia, and impairs cell survival after ischaemia (Chen *et al.*, 2007). Moreover, there is evidence in adult mice that pre-conditioning with hypoxia before stroke was mediated by induction of endogenous Epo (Prass *et al.*, 2003).

Recently, elevated serum Epo concentrations were reported in full term infants exposed to perinatal asphyxia on days one and two after birth, and were associated with severity of HIE on MRI (Sweetman *et al.*, 2017). Similarly, baseline endogenous Epo levels (pre-Epo infusion and therapeutic hypothermia) in term infants with moderate to severe injury undergoing hypothermia were positively correlated with injury in the basal ganglia and brainstem (Massaro *et al.*, 2018). Endogenous upregulation of Epo after HI is delayed but prolonged, suggesting that it likely has a role both in limiting injury in the secondary phase and promoting neurorepair in the long-term. However, it should be noted that milder HI brain injury in non-human primates did not stimulate Epo production, despite causing injury, and this is one rationale for exogenous treatment strategies (Traudt *et al.*, 2013). Thus, exogenous Epo treatment has potential to further improve outcomes.

Studies of exogenous treatment support this hypothesis. Delayed treatment with 5000 U/Kg human recombinant Epo (rEpo) at 24, 48 and 72 hours after HI in P7 rats was associated with decreased neuroinflammation and improved neural outcome (Sun *et al.*, 2005). Furthermore, delayed treatment with rEpo starting 48 hours after HI in P7 rats did not reduce tissue volume loss, and yet increased oligodendrogenesis at five days after HI, with improved oligodendrocyte maturation, reduced white matter injury and increased neurogenesis at 14 days after injury (Iwai *et al.*, 2010). In preterm fetal sheep, a prolonged infusion of rEpo from 30 minutes to 72 hours after severe HI improved electrophysiological and cerebrovascular recovery in association with reduced apoptosis and inflammation, three days after HI (Wassink *et al.*, 2017).

Clinically, postnatal treatment with Epo as monotherapy or in combination with therapeutic hypothermia improved neurodevelopmental outcomes in several trials of term neonates with hypoxic-ischaemic brain injury (Zhu *et al.*, 2009; Rogers *et al.*, 2014; Wu *et al.*, 2016; Malla *et al.*, 2017). A recent meta-analysis of 1133 very preterm infants ($\leq$ 32 weeks gestation) randomised to early Epo for neuroprotection found reduced incidence of severely impaired neurodevelopmental scores at 18-24 months post menstrual age, odds ratio 0.51 ($P < 0.005$), with a number needed to treat of 14 (Fischer *et al.*, 2017).

Conclusions

Preclinical studies have provided significant evidence for interaction between multiple insults modifying neural outcome after asphyxia and have demonstrated that time and dose dependent interactions could act in synergy to exacerbate or attenuate the damage induced by HI. However, there is only limited clinical data examining the effect of multiple interactions on neurodevelopmental outcome. In addition, there is a significant gap in knowledge of mechanisms underlying the interactions between various factors. There are additional factors that contribute to the modulation of HI outcomes not discussed in this review, such as the role of the peripheral immune system, fetal parity, and importantly fetal sex. Nevertheless, the evidence presented above highlights the importance of assessing the effect of multiple hits on neural outcomes in infants with HIE. Potentially, identification of high-risk groups can inform the development of future treatments. Furthermore, there is a need for more preclinical studies examining the efficacy of neuroprotective treatments for injury induced with multiple insults to examine the realistic clinical scenario. Identification of endogenous neuroprotective mechanisms has provided a rationale for exogenous treatment with these factor agents to further augment neuroprotective effects. It remains to be determined if multiple treatments given in a similar temporal profile to their endogenous upregulation will have an optimal neuroprotective effect.

AUTHOR CONTRIBUTION STATEMENT

Laura Bennet, Simerdeep Dhillon and Alistair J. Gunn, conceptualised this topical review. Christopher Lear, Robert Galinsky, Guido Wassink, Joanne Davidson, Sandra Juul and Nicola Robertson provided important intellectual input and preparation of figures. All authors reviewed and edited this manuscript, and have approved the final version as submitted to the Journal of Physiology.

ACKNOWLEDGEMENTS

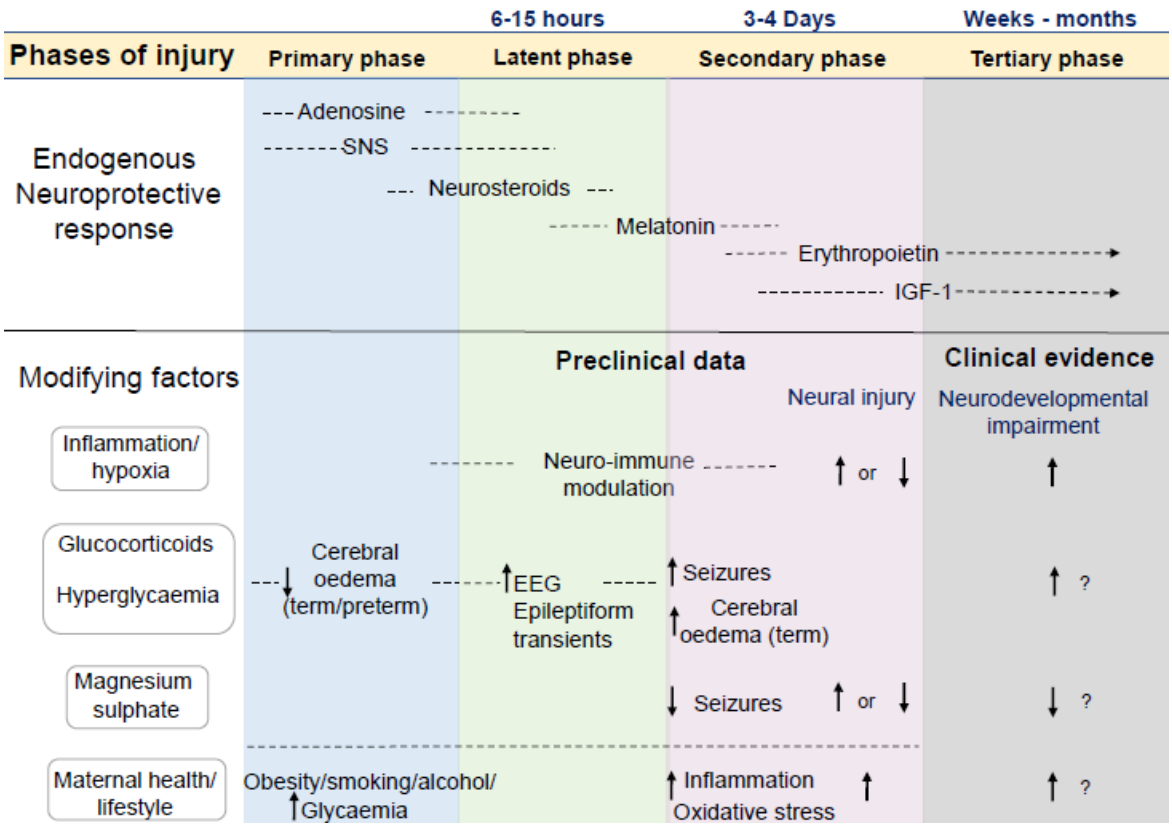
The author's studies discussed in this review received support from the Health Research Council of New Zealand (HRC ID# 12/613, 14/216, 17/601) and Neurological Foundation of New Zealand (NF1715-PG). Dr Davidson holds a Sir Charles Hercus Fellowship from the Health Research Council of New Zealand.

DISCLOSURE / CONFLICT OF INTEREST

The authors declare no potential conflict of interest in this article.

Figure Legend

Figure 1. Schematic diagram illustrating the phases of evolving hypoxic-ischaemic (HI) brain injury. Examples of when endogenous neuroprotective factors are released are shown at the top. Examples of factors which modify the perinatal adaptation to HI are shown below. Factors that can increase neural injury or risk of neurodevelopmental impairment are denoted by up arrows, while factors associated with evidence for decreased injury and impairment are denoted by down arrows. SNS (sympathetic nervous system), insulin-like growth factor 1 (IGF-1), electroencephalographic activity (EEG).



References

Acuna-Castroviejo D, Escames G, Venegas C, Diaz-Casado ME, Lima-Cabello E, Lopez LC, Rosales-Corral S, Tan DX & Reiter RJ (2014). Extrapineal melatonin: sources, regulation, and potential functions. *Cell Mol Life Sci* **71,** 2997-3025.

Ahearne CE, Boylan GB & Murray DM (2016). Short and long term prognosis in perinatal asphyxia: An update. *World J Clin Pediatr* **5,** 67-74.

Alexandrou G, Skiold B, Karlen J, Tessma MK, Norman M, Aden U & Vanpee M (2010). Early hyperglycemia is a risk factor for death and white matter reduction in preterm infants. *Pediatrics* **125,** e584-591.

Aly H, Elmahdy H, El-Dib M, Rowisha M, Awny M, El-Gohary T, Elbatch M, Hamisa M & El-Mashad AR (2015). Melatonin use for neuroprotection in perinatal asphyxia: a randomized controlled pilot study. *J Perinatol* **35,** 186-191.

Arcangeli T, Thilaganathan B, Hooper R, Khan KS & Bhide A (2012). Neurodevelopmental delay in small babies at term: a systematic review. *Ultrasound in Obstetrics & Gynecology* **40,** 267-275.

Aridas JDS, Yawno T, Sutherland AE, Nitsos I, Ditchfield M, Wong FY, Hunt RW, Fahey MC, Malhotra A, Wallace EM, Jenkin G & Miller SL (2018). Systemic and transdermal melatonin administration prevents neuropathology in response to perinatal asphyxia in newborn lambs. *J Pineal Res***,** e12479.

Ariff S, Lee AC, Lawn J & Bhutta ZA (2016). Global burden, epidemiologic trends, and prevention of intrapartum-related deaths in low-resource settings. *Clin Perinatol* **43,** 593-608.

Azzopardi D, Wyatt JS, Cady EB, Delpy DT, Baudin J, Stewart AL, Hope PL, Hamilton PA & Reynolds EO (1989). Prognosis of newborn infants with hypoxic-ischemic brain injury assessed by phosphorus magnetic resonance spectroscopy. *Pediatr Res* **25,** 445-451.

Back SA (2015). Brain injury in the preterm infant: New horizons for pathogenesis and prevention. *Pediatr Neurol* **53,** 185-192.

Badawi N, Kurinczuk JJ, Keogh JM, Alessandri LM, O'Sullivan F, Burton PR, Pemberton PJ & Stanley FJ (1998). Antepartum risk factors for newborn encephalopathy: the Western Australian case-control study. *BMJ* **317,** 1549-1553.

Baerts W, Lemmers PM & van Bel F (2011). Cerebral oxygenation and oxygen extraction in the preterm infant during desaturation: effects of increasing FiO(2) to assist recovery. *Neonatology* **99,** 65-72.

Ball G, Pazderova L, Chew A, Tusor N, Merchant N, Arichi T, Allsop JM, Cowan FM, Edwards AD & Counsell SJ (2015). Thalamocortical connectivity predicts cognition in children born preterm. *Cereb Cortex* **25,** 4310-4318.

Barks JD, Post M & Tuor UI (1991). Dexamethasone prevents hypoxic-ischemic brain damage in the neonatal rat. *Pediatr Res* **29,** 558-563.

Barnett ML, Tusor N, Ball G, Chew A, Falconer S, Aljabar P, Kimpton JA, Kennea N, Rutherford M, David Edwards A & Counsell SJ (2018). Exploring the multiple-hit hypothesis of preterm white matter damage using diffusion MRI. *NeuroImage Clinical* **17,** 596-606.

Basu SK, Kaiser JR, Guffey D, Minard CG, Guillet R & Gunn AJ (2016). Hypoglycaemia and hyperglycaemia are associated with unfavourable outcome in infants with hypoxic ischaemic encephalopathy: a post hoc analysis of the CoolCap Study. *Arch Dis Child Fetal Neonatal Ed* **101,** F149-155.

Basu SK, Salemi JL, Gunn AJ, Kaiser JR & on behalf of the CoolCap Study Group (2017). Hyperglycaemia in infants with hypoxic ischaemic encephalopathy is associated with improved outcomes after therapeutic hypothermia: a post-hoc analysis of the CoolCap Study. *Arch Dis Child Fetal Neonatal Ed* **102,** F299-F306.

Bennet L (2017). Sex, drugs and rock and roll: tales from preterm fetal life. *J Physiol* **595,** 1865-1881.

Bennet L, Booth L & Gunn AJ (2010). Potential biomarkers for hypoxic-ischemic encephalopathy. *Semin Fetal Neonatal Med* **15,** 253-260.

Bennet L, Booth LC, Drury PP, Quaedackers JS & Gunn AJ (2012a). Preterm neonatal cardiovascular instability: does understanding the fetus help evaluate the newborn? *Clin Exp Pharmacol Physiol* **39,** 965-972.

Bennet L, Davidson JO, Koome M & Gunn AJ (2012b). Glucocorticoids and preterm hypoxic-ischemic brain injury: the good and the bad. *J Pregnancy* **2012,** 751694.

Bennet L, Dhillon S, Lear CA, van den Heuij L, King V, Dean JM, Wassink G, Davidson JO & Gunn AJ (2018). Chronic inflammation and impaired development of the preterm brain. *J Reprod Immunol* **125,** 45-55.

Bennet L, Roelfsema V, George S, Dean JM, Emerald BS & Gunn AJ (2007). The effect of cerebral hypothermia on white and grey matter injury induced by severe hypoxia in preterm fetal sheep. *J Physiol* **578,** 491-506.

Bennet L, Roelfsema V, Pathipati P, Quaedackers J & Gunn AJ (2006). Relationship between evolving epileptiform activity and delayed loss of mitochondrial activity after asphyxia measured by near-infrared spectroscopy in preterm fetal sheep. *J Physiol* **572,** 141-154.

Berger HR, Nyman AKG, Morken TS, Vettukattil R, Brubakk AM & Wideroe M (2017). Early metabolite changes after melatonin treatment in neonatal rats with hypoxic-ischemic brain injury studied by in-vivo1H MR spectroscopy. *PloS one* **12,** e0185202.

Blencowe H, Calvert Ph DC, Lawn JE, Cousens S & Campbell OM (2016). Measuring maternal, foetal and neonatal mortality: Challenges and solutions. *Best Pract Res Clin Obstet Gynaecol* **36,** 14-29.

Blumberg RM, Cady EB, Wigglesworth JS, McKenzie JE & Edwards AD (1997). Relation between delayed impairment of cerebral energy metabolism and infarction following transient focal hypoxia-ischaemia in the developing brain. *Exp Brain Res* **113,** 130-137.

Boyle R (1670). New pneumatical experiments about respiration. *Philos Trans R Soc Lond* **62,** 2011-2031.

Bright HR, Babata K, Allred EN, Erdei C, Kuban KCK, Joseph RM, O'Shea TM, Leviton A & Dammann O (2017). Neurocognitive outcomes at 10 years of age in extremely preterm newborns with late-onset bacteremia. *J Pediatr* **187,** 43-49.e41.

Brown J, Alwan NA, West J, Brown S, McKinlay CJ, Farrar D & Crowther CA (2017). Lifestyle interventions for the treatment of women with gestational diabetes. *Cochrane Database Syst Rev* **5,** CD011970.

Buser JR, Maire J, Riddle A, Gong X, Nguyen T, Nelson K, Luo NL, Ren J, Struve J, Sherman LS, Miller SP, Chau V, Hendson G, Ballabh P, Grafe MR & Back SA (2012). Arrested preoligodendrocyte maturation contributes to myelination failure in premature infants. *Ann Neurol* **71,** 93-109.

Chalak LF, Rollins N, Morriss MC, Brion LP, Heyne R & Sanchez PJ (2012). Perinatal acidosis and hypoxic-ischemic encephalopathy in preterm infants of 33 to 35 weeks' gestation. *J Pediatr* **160,** 388-394.

Chang KH, Yeh CM, Yeh CY, Huang CC & Hsu KS (2013). Neonatal dexamethasone treatment exacerbates hypoxic-ischemic brain injury. *Mol Brain* **6,** 18.

Chen CY, Sun WZ, Kang KH, Chou HC, Tsao PN, Hsieh WS & Fu WM (2015). Hypoxic Preconditioning Suppresses Glial Activation and Neuroinflammation in Neonatal Brain Insults. *Mediators Inflamm* **2015,** 632592.

Chen ZY, Asavaritikrai P, Prchal JT & Noguchi CT (2007). Endogenous erythropoietin signaling is required for normal neural progenitor cell proliferation. *J Biol Chem* **282,** 25875-25883.

Chollat C, Enser M, Houivet E, Provost D, Benichou J, Marpeau L & Marret S (2014). School-age outcomes following a randomized controlled trial of magnesium sulfate for neuroprotection of preterm infants. *J Pediatr* **165,** 398-400.e393.

Chouthai NS, Sobczak H, Khan R, Subramanian D, Raman S & Rao R (2015). Hyperglycemia is associated with poor outcome in newborn infants undergoing therapeutic hypothermia for hypoxic ischemic encephalopathy. *J Neonatal Perinatal Med* **8,** 125-131.

Chumas PD, Del Bigio MR, Drake JM & Tuor UI (1993). A comparison of the protective effect of dexamethasone to other potential prophylactic agents in a neonatal rat model of cerebral hypoxia-ischemia. *J Neurosurg* **79,** 414-420.

Colella M, Biran V & Baud O (2016). Melatonin and the newborn brain. *Early Hum Dev* **102,** 1-3.

Committee on Understanding Premature Birth and Assuring Healthy Outcomes. (2007). Preterm Birth: Causes, Consequences, and Prevention, ed. Behrman RE & Butler AS. Institute of Medicine of the National Academies, Washington DC, USA.

Cordeiro CN, Tsimis M & Burd I (2015). Infections and brain development. *Obstet Gynecol Surv* **70,** 644-655.

Dammann O & Leviton A (2014). Intermittent or sustained systemic inflammation and the preterm brain. *Pediatr Res* **75,** 376-380.

Dardzinski BJ, Smith SL, Towfighi J, Williams GD, Vannucci RC & Smith MB (2000). Increased plasma beta-hydroxybutyrate, preserved cerebral energy metabolism, and amelioration of brain damage during neonatal hypoxia ischemia with dexamethasone pretreatment. *Pediatr Res* **48,** 248-255.

Darnall RA, Chen X, Nemani KV, Sirieix CM, Gimi B, Knoblach S, McEntire BL & Hunt CE (2017). Early postnatal exposure to intermittent hypoxia in rodents is proinflammatory, impairs white matter integrity, and alters brain metabolism. *Pediatr Res* **82,** 164-172.

Davidson JO, Green CR, Bennet L, Nicholson LF, Danesh-Meyer H, Carroll SJ & Gunn AJ (2013). A key role for connexin hemichannels in spreading ischemic brain injury. *Current Drug Targets* **14,** 36-46.

Davidson JO, Green CR, Nicholson LF, O'Carroll SJ, Fraser M, Bennet L & Gunn AJ (2012). Connexin hemichannel blockade improves outcomes in a model of fetal ischemia. *Ann Neurol* **71,** 121-132.

Davidson JO, Quaedackers JS, George SA, Gunn AJ & Bennet L (2011). Maternal dexamethasone and EEG hyperactivity in preterm fetal sheep. *J Physiol* **589,** 3823–3835.

Davidson JO, Wassink G, Yuill CA, Zhang FG, Bennet L & Gunn AJ (2015a). How long is too long for cerebral cooling after ischemia in fetal sheep? *J Cereb Blood Flow Metab* **35,** 751-758.

Davidson JO, Yuill CA, Wassink G, Bennet L & Gunn AJ (2015b). Spontaneous pre-existing hypoxia does not affect brain damage after global cerebral ischaemia in late-gestation fetal sheep. *Dev Neurosci* **37,** 56-65.

Dean JM, Gunn AJ, Wassink G, George S & Bennet L (2006). Endogenous alpha(2)-adrenergic receptor-mediated neuroprotection after severe hypoxia in preterm fetal sheep. *Neuroscience* **142,** 615-628.

Dean JM, McClendon E, Hansen K, Azimi-Zonooz A, Chen K, Riddle A, Gong X, Sharifnia E, Hagen M, Ahmad T, Leigland LA, Hohimer AR, Kroenke CD & Back SA (2013). Prenatal cerebral ischemia disrupts MRI-defined cortical microstructure through disturbances in neuronal arborization. *Sci Transl Med* **5,** 168ra167.

Decima PF, Fyfe KL, Odoi A, Wong FY & Horne RS (2015). The longitudinal effects of persistent periodic breathing on cerebral oxygenation in preterm infants. *Sleep Med* **16,** 729-735.

Dempsey EM (2017). What Should We Do about Low Blood Pressure in Preterm Infants. *Neonatology* **111,** 402-407.

Dhillon SK, Gunn AJ, Jung Y, Mathai S, Bennet L & Fraser M (2015). Lipopolysaccharide-induced preconditioning attenuates apoptosis and differentially regulates TLR4 and TLR7 gene expression after ischemia in the preterm ovine fetal brain. *Dev Neurosci* **37,** 497-514.

Di Fiore JM, Martin RJ & Gauda EB (2013). Apnea of prematurity--perfect storm. *Respir Physiol Neurobiol* **189,** 213-222.

Doyle LW, Anderson PJ, Haslam R, Lee KJ & Crowther C (2014). School-age outcomes of very preterm infants after antenatal treatment with magnesium sulfate vs placebo. *JAMA* **312,** 1105-1113.

Doyle LW, Crowther CA, Middleton P & Marret S (2009). Antenatal magnesium sulfate and neurologic outcome in preterm infants: a systematic review. *Obstet Gynecol* **113,** 1327-1333.

Drury PP, Davidson JO, Bennet L, Booth LC, Tan S, Fraser M, van Den Heuij LG & Gunn AJ (2014). Partial neural protection with prophylactic low-dose melatonin after asphyxia in preterm fetal sheep. *J Cereb Blood Flow Metab* **34,** 126-135.

Dudenhausen JW, Grunebaum A & Kirschner W (2015). Prepregnancy body weight and gestational weight gain-recommendations and reality in the USA and in Germany. *Am J Obstet Gynecol* **213,** 591-592.

Edlow AG (2017). Maternal obesity and neurodevelopmental and psychiatric disorders in offspring. *Prenat Diagn* **37,** 95-110.

Ehrenkranz RA (2007). Estimated fetal weights versus birth weights: should the reference intrauterine growth curves based on birth weights be retired? *Arch Dis Child Fetal Neonatal Ed* **92,** F161-162.

Ekert P, MacLusky N, Luo XP, Lehotay DC, Smith B, Post M & Tanswell AK (1997). Dexamethasone prevents apoptosis in a neonatal rat model of hypoxic-ischemic encephalopathy (HIE) by a reactive oxygen species-independent mechanism. *Brain Res* **747,** 9-17.

Eklind S, Mallard C, Arvidsson P & Hagberg H (2005). Lipopolysaccharide induces both a primary and a secondary phase of sensitization in the developing rat brain. *Pediatr Res* **58,** 112-116.

Eklind S, Mallard C, Leverin AL, Gilland E, Blomgren K, Mattsby-Baltzer I & Hagberg H (2001). Bacterial endotoxin sensitizes the immature brain to hypoxic--ischaemic injury. *Eur J Neurosci* **13,** 1101-1106.

Elitt CM, Sadowska GB, Stopa EG, Pinar H, Petersson KH & Stonestreet BS (2003). Effects of antenatal steroids on ischemic brain injury in near-term ovine fetuses. *Early Hum Dev* **73,** 1-15.

Fanaroff JM, Wilson-Costello DE, Newman NS, Montpetite MM & Fanaroff AA (2006). Treated hypotension is associated with neonatal morbidity and hearing loss in extremely low birth weight infants. *Pediatrics* **117,** 1131-1135.

Felszeghy K, Banisadr G, Rostene W, Nyakas C & Haour F (2004). Dexamethasone downregulates chemokine receptor CXCR4 and exerts neuroprotection against hypoxia/ischemia-induced brain injury in neonatal rats. *Neuroimmunomodulation* **11,** 404-413.

Feng Y, Rhodes PG & Bhatt AJ (2011). Dexamethasone pre-treatment protects brain against hypoxic-ischemic injury partially through up-regulation of vascular endothelial growth factor A in neonatal rats. *Neuroscience* **179,** 223-232.

Fischer HS, Reibel NJ, Buhrer C & Dame C (2017). Prophylactic early erythropoietin for neuroprotection in preterm infants: a meta-analysis. *Pediatrics* **139**.

Fleiss B & Gressens P (2012). Tertiary mechanisms of brain damage: a new hope for treatment of cerebral palsy? *Lancet Neurol* **11,** 556-566.

Fleiss B, Guillot PV, Titomanlio L, Baud O, Hagberg H & Gressens P (2014). Stem cell therapy for neonatal brain injury. *Clin Perinatol* **41,** 133-148.

Gale C, Statnikov Y, Jawad S, Uthaya SN & Modi N (2017). Neonatal brain injuries in England: population-based incidence derived from routinely recorded clinical data held in the National Neonatal Research Database. *Arch Dis Child Fetal Neonatal Ed*.

Galinsky R, Bennet L, Groenendaal F, Lear CA, Tan S, van Bel F, Juul SE, Robertson NJ, Mallard C & Gunn AJ (2014). Magnesium is not consistently neuroprotective for perinatal hypoxia-ischemia in term-equivalent models in preclinical studies: A systematic review. *Dev Neurosci* **36,** 73-82.

Galinsky R, Davidson JO, Drury PP, Wassink G, Lear CA, van Den Heuij LG, Gunn AJ & Bennet L (2016). Magnesium sulphate and cardiovascular and cerebrovascular adaptations to asphyxia in preterm fetal sheep. *J Physiol* **594,** 1281-1293.

Galinsky R, Draghi V, Wassink G, Davidson JO, Drury PP, Lear CA, Gunn AJ & Bennet L (2017a). Magnesium sulfate reduces EEG activity but is not neuroprotective after asphyxia in preterm fetal sheep. *J Cereb Blood Flow Metab* **37,** 1362-1373.

Galinsky R, Lear CA, Dean JM, Wassink G, Dhillon SK, Fraser M, Davidson JO, Bennet L & Gunn AJ (2017b). Complex interactions between hypoxia-ischemia and inflammation in preterm brain injury. *Developmental Medicine & Child Neurology* **60,** 126-133.

GBD 2016 Disease and Injury Incidence and Prevalence Collaborators (2016). Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* **388,** 1545-1602.

Geddes R, Vannucci RC & Vannucci SJ (2001). Delayed cerebral atrophy following moderate hypoxia-ischemia in the immature rat. *Dev Neurosci* **23,** 180-185.

Greisen G (2014). Cerebral blood flow and oxygenation in infants after birth asphyxia. Clinically useful information? *Early Hum Dev* **90,** 703-705.

Guan J, Bennet L, Gluckman PD & Gunn AJ (2003). Insulin-like growth factor-1 and post-ischemic brain injury. *Prog Neurobiol* **70,** 443-462.

Gunn AJ & Groenendaal F (2016). Delayed neuroprotection in the era of hypothermia: What can we add? *Journal of Clinical Neonatology* **5,** 3-7.

Gunn AJ, Gunn TR, de Haan HH, Williams CE & Gluckman PD (1997). Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal lambs. *J Clin Invest* **99,** 248-256.

Gunn AJ, Maxwell L, de Haan HH, Bennet L, Williams CE, Gluckman PD & Gunn TR (2000). Delayed hypotension and subendocardial injury after repeated umbilical cord occlusion in near-term fetal lambs. *Am J Obstet Gynecol* **183,** 1564-1572.

Hagberg H, Mallard C, Ferriero DM, Vannucci SJ, Levison SW, Vexler ZS & Gressens P (2015). The role of inflammation in perinatal brain injury. *Nat Rev Neurol* **11,** 192-208.

Harding B, Conception K, Li Y & Zhang L (2016). Glucocorticoids protect neonatal rat brain in model of hypoxic-ischemic encephalopathy (HIE). *Int J Mol Sci* **18,** 17.

Hartings JA, Shuttleworth CW, Kirov SA, Ayata C, Hinzman JM, Foreman B, Andrew RD, Boutelle MG, Brennan KC, Carlson AP, Dahlem MA, Drenckhahn C, Dohmen C, Fabricius M, Farkas E, Feuerstein D, Graf R, Helbok R, Lauritzen M, Major S, Oliveira-Ferreira AI, Richter F, Rosenthal ES, Sakowitz OW, Sanchez-Porras R, Santos E, Scholl M, Strong AJ, Urbach A, Westover MB, Winkler MK, Witte OW, Woitzik J & Dreier JP (2017). The continuum of spreading depolarizations in acute cortical lesion development: Examining Leao's legacy. *J Cereb Blood Flow Metab* **37,** 1571-1594.

Hassell KJ, Ezzati M, Alonso-Alconada D, Hausenloy DJ & Robertson NJ (2015). New horizons for newborn brain protection: enhancing endogenous neuroprotection. *Arch Dis Child Fetal Neonatal Ed* **100,** F541-552.

Hayes BC, McGarvey C, Mulvany S, Kennedy J, Geary MP, Matthews TG & King MD (2013). A case-control study of hypoxic-ischemic encephalopathy in newborn infants at >36 weeks gestation. *Am J Obstet Gynecol* **209,** 29 e21-29 e19.

Herzog M, Cerar LK, Srsen TP, Verdenik I & Lucovnik M (2015). Impact of risk factors other than prematurity on periventricular leukomalacia. A population-based matched case control study. *Eur J Obstet Gynecol Reprod Biol* **187,** 57-59.

Hickey E, Shi H, Van Arsdell G & Askalan R (2011). Lipopolysaccharide-induced preconditioning against ischemic injury is associated with changes in toll-like receptor 4 expression in the rat developing brain. *Pediatr Res* **70,** 10-14.

Hirvonen M, Ojala R, Korhonen P, Haataja P, Eriksson K, Gissler M, Luukkaala T & Tammela O (2014). Cerebral palsy among children born moderately and late preterm. *Pediatrics* **134,** e1584-1593.

Horne RSC, Fung ACH, NcNeil S, Fyfe KL, Odoi A & Wong FY (2017). The Longitudinal Effects of Persistent Apnea on Cerebral Oxygenation in Infants Born Preterm. *J Pediatr* **182,** 79-84.

Huang J, Zhang L, Kang B, Zhu T, Li Y, Zhao F, Qu Y & Mu D (2017). Association between perinatal hypoxic-ischemia and periventricular leukomalacia in preterm infants: A systematic review and meta-analysis. *PloS one* **12,** e0184993.

Ikeda T, Mishima K, Aoo N, Liu AX, Egashira N, Iwasaki K, Fujiwara M & Ikenoue T (2005). Dexamethasone prevents long-lasting learning impairment following a combination of lipopolysaccharide and hypoxia-ischemia in neonatal rats. *Am J Obstet Gynecol* **192,** 719-726.

Impey LW, Greenwood CE, Black RS, Yeh PS, Sheil O & Doyle P (2008). The relationship between intrapartum maternal fever and neonatal acidosis as risk factors for neonatal encephalopathy. *Am J Obstet Gynecol* **198,** 49.e41-46.

Iwai M, Stetler RA, Xing J, Hu X, Gao Y, Zhang W, Chen J & Cao G (2010). Enhanced oligodendrogenesis and recovery of neurological function by erythropoietin after neonatal hypoxic/ischemic brain injury. *Stroke* **41,** 1032-1037.

Iwata O, Iwata S, Bainbridge A, De Vita E, Matsuishi T, Cady EB & Robertson NJ (2008). Supra- and sub-baseline phosphocreatine recovery in developing brain after transient hypoxia-ischaemia: relation to baseline energetics, insult severity and outcome. *Brain* **131,** 2220-2226.

Jantzie LL, Corbett CJ, Berglass J, Firl DJ, Flores J, Mannix R & Robinson S (2014). Complex pattern of interaction between in utero hypoxia-ischemia and intra-amniotic inflammation disrupts brain development and motor function. *J Neuroinflammation* **11,** 131.

Jensen EC, Bennet L, Hunter CJ, Power GG & Gunn AJ (2006). Post-hypoxic hypoperfusion is associated with suppression of cerebral metabolism and increased tissue oxygenation in near-term fetal sheep. *J Physiol* **572,** 131-139.

Kalliala I, Markozannes G, Gunter MJ, Paraskevaidis E, Gabra H, Mitra A, Terzidou V, Bennett P, Martin-Hirsch P, Tsilidis KK & Kyrgiou M (2017). Obesity and gynaecological and obstetric conditions: umbrella review of the literature. *BMJ* **359,** j4511.

Kennaway DJ, Stamp GE & Goble FC (1992). Development of melatonin production in infants and the impact of prematurity. *J Clin Endocrinol Metab* **75,** 367-369.

Keunen H, Blanco CE, van Reempts JL & Hasaart TH (1997). Absence of neuronal damage after umbilical cord occlusion of 10, 15, and 20 minutes in midgestation fetal sheep. *Am J Obstet Gynecol* **176,** 515-520.

Kilic E, Ozdemir YG, Bolay H, Kelestimur H & Dalkara T (1999). Pinealectomy aggravates and melatonin administration attenuates brain damage in focal ischemia. *J Cereb Blood Flow Metab* **19,** 511-516.

Kim CJ, Romero R, Chaemsaithong P, Chaiyasit N, Yoon BH & Kim YM (2015). Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. *Am J Obstet Gynecol* **213,** S29-52.

Kim Y, Griffin JM, Harris PW, Chan SH, Nicholson LF, Brimble MA, O'Carroll SJ & Green CR (2017). Characterizing the mode of action of extracellular Connexin43 channel blocking mimetic peptides in an in vitro ischemia injury model. *Biochim Biophys Acta* **1861,** 68-78.

Koning G, Leverin AL, Nair S, Schwendimann L, Ek J, Carlsson Y, Gressens P, Thornton C, Wang X, Mallard C & Hagberg H (2017). Magnesium induces preconditioning of the neonatal brain via profound mitochondrial protection. *J Cereb Blood Flow Metab***,** 271678X17746132.

Koome ME, Davidson JO, Drury PP, Mathai S, Booth LC, Gunn AJ & Bennet L (2013). Antenatal dexamethasone after asphyxia increases neural injury in preterm fetal sheep. *PLoS ONE* **8,** e77480.

Kuban KC, Joseph RM, O'Shea TM, Heeren T, Fichorova RN, Douglass L, Jara H, Frazier JA, Hirtz D, Rollins JV, Paneth N & Extremely Low Gestational Age Newborns (ELGAN) Study Investigators (2017). Circulating inflammatory-associated proteins in the first month of life and cognitive impairment at age 10 years in children born extremely preterm. *J Pediatr* **180,** 116-123.e111.

Lange S, Probst C, Rehm J & Popova S (2017). Prevalence of binge drinking during pregnancy by country and World Health Organization region: Systematic review and meta-analysis. *Reprod Toxicol* **73,** 214-221.

Langer O (2018). Prevention of obesity and diabetes in pregnancy: Is it an impossible dream? *Am J Obstet Gynecol*.

Laptook AR (2013). Neurologic and metabolic issues in moderately preterm, late preterm, and early term infants. *Clin Perinatol* **40,** 723-738.

Laptook AR (2016). Birth Asphyxia and Hypoxic-Ischemic Brain Injury in the Preterm Infant. *Clin Perinatol* **43,** 529-545.

Lear CA, Davidson JO, Mackay GR, Drury PP, Galinsky R, Quaedackers JS, Gunn AJ & Bennet L (2018). Antenatal dexamethasone before asphyxia promotes cystic neural injury in preterm fetal sheep by inducing hyperglycemia. *J Cereb Blood Flow Metab* **38,** 706–718.

Lear CA, Galinsky R, Wassink G, Yamaguchi K, Davidson JO, Westgate JA, Bennet L & Gunn AJ (2016). The myths and physiology surrounding intrapartum decelerations: the critical role of the peripheral chemoreflex. *J Physiol* **594,** 4711-4725.

Lear CA, Koome MM, Davidson JO, Drury PP, Quaedackers JS, Galinsky R, Gunn AJ & Bennet L (2014). The effects of dexamethasone on post-asphyxial cerebral oxygenation in the preterm fetal sheep. *J Physiol* **592,** 5493-5505.

Leaw B, Nair S, Lim R, Thornton C, Mallard C & Hagberg H (2017). Mitochondria, Bioenergetics and Excitotoxicity: New Therapeutic Targets in Perinatal Brain Injury. *Front Cell Neurosci* **11,** 199.

LeBlanc MH, Huang M, Vig V, Patel D & Smith EE (1993). Glucose affects the severity of hypoxic-ischemic brain injury in newborn pigs. *Stroke* **24,** 1055-1062.

Lee AC, Kozuki N, Blencowe H, Vos T, Bahalim A, Darmstadt GL, Niermeyer S, Ellis M, Robertson NJ, Cousens S & Lawn JE (2013). Intrapartum-related neonatal encephalopathy incidence and impairment at regional and global levels for 2010 with trends from 1990. *Pediatr Res* **74,** 50-72.

Leviton A, Fichorova RN, O'Shea TM, Kuban K, Paneth N, Dammann O & Allred EN (2013). Two-hit model of brain damage in the very preterm newborn: small for gestational age and postnatal systemic inflammation. *Pediatr Res* **73,** 362-370.

Lin B, Ginsberg MD & Busto R (1998). Hyperglycemic exacerbation of neuronal damage following forebrain ischemia: microglial, astrocytic and endothelial alterations. *Acta Neuropathol(Berl)* **96,** 610-620.

Lin CY, Chang YC, Wang ST, Lee TY, Lin CF & Huang CC (2010). Altered inflammatory responses in preterm children with cerebral palsy. *Ann Neurol* **68,** 204-212.

Lin HY, Huang CC & Chang KF (2009). Lipopolysaccharide preconditioning reduces neuroinflammation against hypoxic ischemia and provides long-term outcome of neuroprotection in neonatal rat. *Pediatr Res* **66,** 254-259.

Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, Cousens S, Mathers C & Black RE (2015). Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet* **385,** 430-440.

Logitharajah P, Rutherford MA & Cowan FM (2009). Hypoxic-ischemic encephalopathy in preterm infants: antecedent factors, brain imaging, and outcome. *Pediatr Res* **66,** 222-229.

Lorek A, Takei Y, Cady EB, Wyatt JS, Penrice J, Edwards AD, Peebles D, Wylezinska M, Owen-Reece H & Kirkbride V (1994). Delayed ("secondary") cerebral energy failure after acute hypoxia-ischemia in the newborn piglet: continuous 48-hour studies by phosphorus magnetic resonance spectroscopy. *Pediatr Res* **36,** 699-706.

Low E, Dempsey EM, Ryan CA, Rennie JM & Boylan GB (2012). EEG suppression associated with apneic episodes in a neonate. *Case Rep Neurol Med* **2012,** 250801.

Low JA (2004). Determining the contribution of asphyxia to brain damage in the neonate. *J Obstet Gynaecol Res* **30,** 276-286.

Low JA, Killen H & Derrick EJ (2003). Antepartum fetal asphyxia in the preterm pregnancy. *Am J Obstet Gynecol* **188,** 461-465.

Lynch NE, Stevenson NJ, Livingstone V, Mathieson S, Murphy BP, Rennie JM & Boylan GB (2015). The temporal characteristics of seizures in neonatal hypoxic ischemic encephalopathy treated with hypothermia. *Seizure* **33,** 60-65.

Maitre NL (2015). Neurorehabilitation after neonatal intensive care: evidence and challenges. *Arch Dis Child Fetal Neonatal Ed* **100,** F534-540.

Malla RR, Asimi R, Teli MA, Shaheen F & Bhat MA (2017). Erythropoietin monotherapy in perinatal asphyxia with moderate to severe encephalopathy: a randomized placebo-controlled trial. *J Perinatol* **37,** 596-601.

Manuck TA, Rice MM, Bailit JL, Grobman WA, Reddy UM, Wapner RJ, Thorp JM, Caritis SN, Prasad M, Tita AT, Saade GR, Sorokin Y, Rouse DJ, Blackwell SC & Tolosa JE (2016). Preterm neonatal morbidity and mortality by gestational age: a contemporary cohort. *Am J Obstet Gynecol* **215,** 103.e101-e114.

Marchant EA, Kan B, Sharma AA, van Zanten A, Kollmann TR, Brant R & Lavoie PM (2015). Attenuated innate immune defenses in very premature neonates during the neonatal period. *Pediatr Res* **78,** 492-497.

Marseglia L, D'Angelo G, Manti S, Rulli I, Salvo V, Buonocore G, Reiter RJ & Gitto E (2017). Melatonin secretion Is increased in children with severe traumatic brain injury. *Int J Mol Sci* **18**.

Marsh B, Stevens SL, Packard AE, Gopalan B, Hunter B, Leung PY, Harrington CA & Stenzel-Poore MP (2009). Systemic lipopolysaccharide protects the brain from ischemic injury by reprogramming the response of the brain to stroke: a critical role for IRF3. *J Neurosci* **29,** 9839-9849.

Martinello K, Hart AR, Yap S, Mitra S & Robertson NJ (2017). Management and investigation of neonatal encephalopathy: 2017 update. *Arch Dis Child Fetal Neonatal Ed* **102,** F346-f358.

Massaro AN, Wu YW, Bammler TK, Comstock B, Mathur A, McKinstry RC, Chang T, Mayock DE, Mulkey SB, Van Meurs K & Juul S (2018). Plasma biomarkers of brain injury in neonatal hypoxic-ischemic encephalopathy. *J Pediatr* **Epub Jan**.

Master ZR, Porzionato A, Kesavan K, Mason A, Chavez-Valdez R, Shirahata M & Gauda EB (2016). Lipopolysaccharide exposure during the early postnatal period adversely affects the structure and function of the developing rat carotid body. *J Appl Physiol (1985)* **121,** 816-827.

Mathai S, Booth LC, Davidson JO, Drury PP, Fraser M, Jensen EC, George S, Naylor AS, Gunn AJ & Bennet L (2013). Acute on chronic exposure to endotoxin in preterm fetal sheep. *Am J Physiol Regul Integr Comp Physiol* **304,** R189-197.

Maulik D, Zanelli S, Numagami Y, Ohnishi ST, Mishra OP & Delivoria-Papadopoulos M (1999). Oxygen free radical generation during in-utero hypoxia in the fetal guinea pig brain: the effects of maturity and of magnesium sulfate administration. *Brain Res* **817,** 117-122.

Mazur M, Miller RH & Robinson S (2010). Postnatal erythropoietin treatment mitigates neural cell loss after systemic prenatal hypoxic-ischemic injury. *J Neurosurg Pediatr* **6,** 206-221.

McClendon E, Chen K, Gong X, Sharifnia E, Hagen M, Cai V, Shaver DC, Riddle A, Dean JM, Gunn AJ, Mohr C, Kaplan JS, Rossi DJ, Kroenke CD, Hohimer AR & Back SA (2014). Prenatal cerebral ischemia triggers dysmaturation of caudate projection neurons. *Ann Neurol* **75,** 508-524.

McClendon E, Shaver DC, Degener-O'Brien K, Gong X, Nguyen T, Hoerder-Suabedissen A, Molnar Z, Mohr C, Richardson BD, Rossi DJ & Back SA (2017). Transient hypoxemia chronically disrupts maturation of preterm fetal ovine subplate neuron arborization and activity. *J Neurosci* **37,** 11912-11929.

McCowan LME, Thompson JMD, Cronin RS, Li M, Stacey T, Stone PR, Lawton BA, Ekeroma AJ & Mitchell EA (2017). Going to sleep in the supine position is a modifiable risk factor for late

pregnancy stillbirth; Findings from the New Zealand multicentre stillbirth case-control study. *PLoS One* **12,** e0179396.

McKinlay CJD, Chase JG, Dickson J, Harris DL, Alsweiler JM & Harding JE (2017). Continuous glucose monitoring in neonates: a review. *Matern Health Neonatol Perinatol* **3,** 18.

McMillen IC, Houghton DC & Young IR (1995). Melatonin and the development of circadian and seasonal rhythmicity. *Journal of Reproduction & Fertility - Supplement* **49:137-46,** 137-146.

Meek JH, Elwell CE, McCormick DC, Edwards AD, Townsend JP, Stewart AL & Wyatt JS (1999). Abnormal cerebral haemodynamics in perinatally asphyxiated neonates related to outcome. *Arch Dis Child Fetal Neonatal Ed* **81,** F110-F115.

Meek JH, Tyszczuk L, Elwell CE & Wyatt JS (1998). Cerebral blood flow increases over the first three days of life in extremely preterm neonates. *Arch Dis Child Fetal Neonatal Ed* **78,** F33-37.

Mehta SH, Kerver JM, Sokol RJ, Keating DP & Paneth N (2014). The association between maternal obesity and neurodevelopmental outcomes of offspring. *J Pediatr* **165,** 891-896.

Mendoza-Paredes A, Liu H, Schears G, Yu Z, Markowitz SD, Schultz S, Pastuszko P, Greeley WJ, Nadkarni V, Kubin J, Wilson DF & Pastuszko A (2008). Resuscitation with 100%, compared with 21%, oxygen following brief, repeated periods of apnea can protect vulnerable neonatal brain regions from apoptotic injury. *Resuscitation* **76,** 261-270.

Merchant NM, Azzopardi DV, Hawwa AF, McElnay JC, Middleton B, Arendt J, Arichi T, Gressens P & Edwards AD (2013). Pharmacokinetics of melatonin in preterm infants. *Br J Clin Pharmacol* **76,** 725-733.

Michenfelder JD & Milde JH (1990). Postischemic canine cerebral blood flow appears to be determined by cerebral metabolic needs. *J Cereb Blood Flow Metab* **10,** 71-76.

Miller SL, Huppi PS & Mallard C (2016). The consequences of fetal growth restriction on brain structure and neurodevelopmental outcome. *J Physiol* **594,** 807-823.

Miller SL, Yan EB, Castillo-Melendez M, Jenkin G & Walker DW (2005). Melatonin provides neuroprotection in the late-gestation fetal sheep brain in response to umbilical cord occlusion. *Dev Neurosci* **27,** 200-210.

Mirmiran M, Maas YG & Ariagno RL (2003). Development of fetal and neonatal sleep and circadian rhythms. *Sleep Med Rev* **7,** 321-334.

Mottahedin A, Svedin P, Nair S, Mohn CJ, Wang X, Hagberg H, Ek J & Mallard C (2017). Systemic activation of Toll-like receptor 2 suppresses mitochondrial respiration and exacerbates hypoxic-ischemic injury in the developing brain. *J Cereb Blood Flow Metab* **37,** 1192-1198.

Nadeem M, Murray DM, Boylan GB, Dempsey EM & Ryan CA (2011). Early blood glucose profile and neurodevelopmental outcome at two years in neonatal hypoxic-ischaemic encephalopathy. *BMC pediatrics* **11,** 10.

Nguyen P, Yan EB, Castillo-Melendez M, Walker DW & Hirst JJ (2004). Increased allopregnanolone levels in the fetal sheep brain following umbilical cord occlusion. *J Physiol* **560,** 593-602.

Nicholas LM, Morrison JL, Rattanatray L, Zhang S, Ozanne SE & McMillen IC (2016). The early origins of obesity and insulin resistance: timing, programming and mechanisms. *Int J Obes (Lond)* **40,** 229-238.

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.

Noori S, McCoy M, Anderson MP, Ramji F & Seri I (2014). Changes in cardiac function and cerebral blood flow in relation to peri/intraventricular hemorrhage in extremely preterm infants. *J Pediatr* **164,** 264-270 e261-263.

Novak I, Morgan C, Adde L, Blackman J, Boyd RN, Brunstrom-Hernandez J, Cioni G, Damiano D, Darrah J, Eliasson AC, de Vries LS, Einspieler C, Fahey M, Fehlings D, Ferriero DM, Fetters L, Fiori S, Forssberg H, Gordon AM, Greaves S, Guzzetta A, Hadders-Algra M, Harbourne R, Kakooza-Mwesige A, Karlsson P, Krumlinde-Sundholm L, Latal B, Loughran-Fowlds A, Maitre N, McIntyre S, Noritz G, Pennington L, Romeo DM, Shepherd R, Spittle AJ, Thornton M, Valentine J, Walker K, White R & Badawi N (2017). Early, accurate diagnosis and early intervention in cerebral palsy: Advances in diagnosis and treatment. *JAMA Pediatr* **171,** 897-907.

Ogilvy-Stuart AL & Beardsall K (2010). Management of hyperglycaemia in the preterm infant. *Arch Dis Child Fetal Neonatal Ed* **95,** F126-131.

Ohls RK, Christensen RD, Widness JA & Juul SE (2015). Erythropoiesis stimulating agents demonstrate safety and show promise as neuroprotective agents in neonates. *J Pediatr* **167,** 10-12.

Orellana JA, Hernandez DE, Ezan P, Velarde V, Bennett MV, Giaume C & Saez JC (2010). Hypoxia in high glucose followed by reoxygenation in normal glucose reduces the viability of cortical astrocytes through increased permeability of connexin 43 hemichannels. *Glia* **58,** 329-343.

Ota A, Ikeda T, Abe K, Sameshima H, Xia XY, Xia YX & Ikenoue T (1998). Hypoxic-ischemic tolerance phenomenon observed in neonatal rat brain. *Am J Obstet Gynecol* **179,** 1075-1078.

Ou X, Thakali KM, Shankar K, Andres A & Badger TM (2015). Maternal adiposity negatively influences infant brain white matter development. *Obesity (Silver Spring)* **23,** 1047-1054.

Park HK, Seol IJ & Kim KS (2011). Protective effect of hypoxic preconditioning on hypoxic-ischemic injured newborn rats. *J Korean Med Sci* **26,** 1495-1500.

Parmar J & Jones NM (2015). Hypoxic preconditioning can reduce injury-induced inflammatory processes in the neonatal rat brain. *Int J Dev Neurosci* **43,** 35-42.

Penrice J, Lorek A, Cady EB, Amess PN, Wylezinska M, Cooper CE, D'Souza P, Brown GC, Kirkbride V, Edwards AD, Wyatt JS & Reynolds EO (1997). Proton magnetic resonance spectroscopy of the brain during acute hypoxia-ischemia and delayed cerebral energy failure in the newborn piglet. *Pediatr Res* **41,** 795-802.

Persson M, Johansson S, Villamor E & Cnattingius S (2014). Maternal overweight and obesity and risks of severe birth-asphyxia-related complications in term infants: a population-based cohort study in Sweden. *PLoS Med* **11,** e1001648.

Petersson KH, Pinar H, Stopa EG, Sadowska GB, Hanumara RC & Stonestreet BS (2004). Effects of exogenous glucose on brain ischemia in ovine fetuses. *Pediatr Res* **56,** 621-629.

Pitcher JB, Robertson AL, Cockington RA & Moore VM (2009). Prenatal growth and early postnatal influences on adult motor cortical excitability. *Pediatrics* **124,** e128-136.

Polanska K, Jurewicz J & Hanke W (2015). Smoking and alcohol drinking during pregnancy as the risk factors for poor child neurodevelopment - A review of epidemiological studies. *Int J Occup Med Environ Health* **28,** 419-443.

Prass K, Scharff A, Ruscher K, Lowl D, Muselmann C, Victorov I, Kapinya K, Dirnagl U & Meisel A (2003). Hypoxia-induced stroke tolerance in the mouse is mediated by erythropoietin. *Stroke* **34,** 1981-1986.

Qin G, Lo JW, Marlow N, Calvert SA, Greenough A & Peacock JL (2017). Postnatal dexamethasone, respiratory and neurodevelopmental outcomes at two years in babies born extremely preterm. *PLOS ONE* **12,** e0181176.

Quaedackers JS, Roelfsema V, Heineman E, Gunn AJ & Bennet L (2004a). The role of the sympathetic nervous system in post-asphyxial intestinal hypoperfusion in the preterm sheep fetus. *J Physiol* **557,** 1033-1044.

Quaedackers JS, Roelfsema V, Hunter CJ, Heineman E, Gunn AJ & Bennet L (2004b). Polyuria and impaired renal blood flow after asphyxia in preterm fetal sheep. *Am J Physiol Regul Integr Comp Physiol* **286,** R576-R583.

Reid SM, Dagia CD, Ditchfield MR, Carlin JB, Meehan EM & Reddihough DS (2014). An Australian population study of factors associated with MRI patterns in cerebral palsy. *Dev Med Child Neurol* **56,** 178-184.

Reid SM, Meehan E, McIntyre S, Goldsmith S, Badawi N & Reddihough DS (2016). Temporal trends in cerebral palsy by impairment severity and birth gestation. *Dev Med Child Neurol* **58 Suppl 2,** 25-35.

Reynolds LC, Inder TE, Neil JJ, Pineda RG & Rogers CE (2014). Maternal obesity and increased risk for autism and developmental delay among very preterm infants. *J Perinatol* **34,** 688-692.

Riddle A, Dean J, Buser JR, Gong X, Maire J, Chen K, Ahmad T, Cai V, Nguyen T, Kroenke CD, Hohimer AR & Back SA (2011). Histopathological correlates of magnetic resonance imaging-defined chronic perinatal white matter injury. *Ann Neurol* **70,** 493-507.

Roberts D, Brown J, Medley N & Dalziel SR (2017). Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database Syst Rev* **3,** CD004454.

Robertson NJ, Faulkner S, Fleiss B, Bainbridge A, Andorka C, Price D, Powell E, Lecky-Thompson L, Thei L, Chandrasekaran M, Hristova M, Cady EB, Gressens P, Golay X & Raivich G (2013). Melatonin augments hypothermic neuroprotection in a perinatal asphyxia model. *Brain* **136,** 90-105.

Robertson NJ, Tan S, Groenendaal F, van Bel F, Juul SE, Bennet L, Derrick M, Back SA, Valdez RC, Northington F, Gunn AJ & Mallard C (2012). Which neuroprotective agents are ready for bench to bedside translation in the newborn infant? *J Pediatr* **160,** 544-552.e544.

Rogers EE, Bonifacio SL, Glass HC, Juul SE, Chang T, Mayock DE, Durand DJ, Song D, Barkovich AJ, Ballard RA & Wu YW (2014). Erythropoietin and hypothermia for hypoxic-ischemic encephalopathy. *Pediatr Neurol* **51,** 657-662.

Rosen CL, Storfer-Isser A, Taylor HG, Kirchner HL, Emancipator JL & Redline S (2004). Increased behavioral morbidity in school-aged children with sleep-disordered breathing. *Pediatrics* **114,** 1640-1648.

Roth SC, Baudin J, Cady E, Johal K, Townsend JP, Wyatt JS, Reynolds EO & Stewart AL (1997). Relation of deranged neonatal cerebral oxidative metabolism with neurodevelopmental outcome and head circumference at 4 years. *Dev Med Child Neurol* **39,** 718-725.

Salhab WA & Perlman JM (2005). Severe fetal acidemia and subsequent neonatal encephalopathy in the larger premature infant. *Pediatr Neurol* **32,** 25-29.

Schears G, Creed J, Zaitseva T, Schultz S, Wilson DF & Pastuszko A (2005). Cerebral oxygenation during repetitive apnea in newborn piglets. *Adv Exp Med Biol* **566,** 1-7.

Schmidt B, Roberts RS, Anderson PJ, Asztalos EV, Costantini L, Davis PG, Dewey D, D'Ilario J, Doyle LW, Grunau RE, Moddemann D, Nelson H, Ohlsson A, Solimano A & Tin W (2017). Academic Performance, Motor Function, and Behavior 11 Years After Neonatal Caffeine Citrate Therapy for Apnea of Prematurity: An 11-Year Follow-up of the CAP Randomized Clinical Trial. *JAMA Pediatr* **171,** 564-572.

Schmidt JW & Walsh WF (2010). Hypoxic-ischemic encephalopathy in preterm infants. *J Neonatal Perinatal Med* **3,** 277-284.

Seron-Ferre M, Mendez N, Abarzua-Catalan L, Vilches N, Valenzuela FJ, Reynolds HE, Llanos AJ, Rojas A, Valenzuela GJ & Torres-Farfan C (2012). Circadian rhythms in the fetus. *Mol Cell Endocrinol* **349,** 68-75.

Seron-Ferre M, Torres-Farfan C, Valenzuela FJ, Castillo-Galan S, Rojas A, Mendez N, Reynolds H, Valenzuela GJ & Llanos AJ (2017). Deciphering the function of the blunt circadian rhythm of melatonin in the newborn lamb: Impact on adrenal and heart. *Endocrinology* **158,** 2895-2905.

Sharma A, Davis A & Shekhawat PS (2017). Hypoglycemia in the preterm neonate: etiopathogenesis, diagnosis, management and long-term outcomes. *Transl Pediatr* **6,** 335-348.

Shelley HJ (1961). Glycogen reserves and their changes at birth and in anoxia. *Br Med Bull* **17,** 137-143.

Shepherd E, Salam RA, Middleton P, Makrides M, McIntyre S, Badawi N & Crowther CA (2017). Antenatal and intrapartum interventions for preventing cerebral palsy: an overview of Cochrane systematic reviews. *Cochrane Database Syst Rev* **8,** CD012077.

Shi H, Gabarin N, Hickey E & Askalan R (2013). TLR-3 receptor activation protects the very immature brain from ischemic injury. *J Neuroinflammation* **10,** 104.

Sizonenko SV, Sirimanne E, Mayall Y, Gluckman PD, Inder T & Williams C (2003). Selective cortical alteration after hypoxic-ischemic injury in the very immature rat brain. *Pediatr Res* **54,** 263-269.

Smid MC, Vladutiu CJ, Dotters-Katz SK, Manuck TA, Boggess KA & Stamilio DM (2016). Maternal Super Obesity and Neonatal Morbidity after Term Cesarean Delivery. *Am J Perinatol* **33,** 1198-1204.

Spradley FT (2017). Metabolic abnormalities and obesity's impact on the risk for developing preeclampsia. *Am J Physiol Regul Integr Comp Physiol* **312,** R5-r12.

Stacey T, Thompson JM, Mitchell EA, Ekeroma A, Zuccollo J & McCowan LM (2011). Maternal perception of fetal activity and late stillbirth risk: findings from the Auckland Stillbirth Study. *Birth (Berkeley, Calif)* **38,** 311-316.

Stark MJ, Hodyl NA & Andersen CC (2015). Effects of antenatal magnesium sulfate treatment for neonatal neuro-protection on cerebral oxygen kinetics. *Pediatr Res* **78,** 310-314.

Stark MJ, Hodyl NA, Belegar VK & Andersen CC (2016). Intrauterine inflammation, cerebral oxygen consumption and susceptibility to early brain injury in very preterm newborns. *Arch Dis Child Fetal Neonatal Ed* **101,** F137-142.

Stone PR, Burgess W, McIntyre J, Gunn AJ, Lear CA, Bennet L, Mitchell EA, Thompson JM & the Maternal Sleep In Pregnancy Research Group (2017). Effect of maternal position on fetal behavioural state and heart rate variability in healthy late gestation pregnancy. *J Physiol* **595,** 1213-1221.

Streimish IG, Ehrenkranz RA, Allred EN, O'Shea TM, Kuban KC, Paneth N & Leviton A (2012). Birth weight- and fetal weight-growth restriction: impact on neurodevelopment. *Early Hum Dev* **88,** 765-771.

Stridh L, Mottahedin A, Johansson ME, Valdez RC, Northington F, Wang X & Mallard C (2013). Toll-like receptor-3 activation increases the vulnerability of the neonatal brain to hypoxia-ischemia. *J Neurosci* **33,** 12041-12051.

Sugimoto J, Romani AM, Valentin-Torres AM, Luciano AA, Ramirez Kitchen CM, Funderburg N, Mesiano S & Bernstein HB (2012). Magnesium decreases inflammatory cytokine production: a novel innate immunomodulatory mechanism. *J Immunol* **188,** 6338-6346.

Sun Y, Calvert JW & Zhang JH (2005). Neonatal hypoxia/ischemia is associated with decreased inflammatory mediators after erythropoietin administration. *Stroke* **36,** 1672-1678.

Sun Y, Zhou C, Polk P, Nanda A & Zhang JH (2004). Mechanisms of erythropoietin-induced brain protection in neonatal hypoxia-ischemia rat model. *J Cereb Blood Flow Metab* **24,** 259-270.

Sweetman DU, Onwuneme C, Watson WR, Murphy JF & Molloy EJ (2017). Perinatal aand erythropoietin and VEGF: serial serum and cerebrospinal fluid responses. *Neonatology* **111,** 253-259.

Tagin M, Abdel-Hady H, Rahman SU, Azzopardi DV & Gunn AJ (2015). Neuroprotection for perinatal hypoxic ischemic encephalopathy in low- and middle-income countries. *J Pediatr* **167,** 25-28.

Takahashi N, Nishida H, Arai T & Kaneda Y (1995). Abnormal cardiac histology in severe intrauterine growth retardation infants. *Acta Paediatr Jpn* **37,** 341-346.

Tan S (2014). Fault and blame, insults to the perinatal brain may be remote from time of birth. *Clin Perinatol* **41,** 105-117.

Tann CJ, Nakakeeto M, Willey BA, Sewegaba M, Webb EL, Oke I, Mutuuza ED, Peebles D, Musoke M, Harris KA, Sebire NJ, Klein N, Kurinczuk JJ, Elliott AM & Robertson NJ (2017). Perinatal risk factors for neonatal encephalopathy: an unmatched case-control study. *Arch Dis Child Fetal Neonatal Ed*.

Thomason ME, Scheinost D, Manning JH, Grove LE, Hect J, Marshall N, Hernandez-Andrade E, Berman S, Pappas A, Yeo L, Hassan SS, Constable RT, Ment LR & Romero R (2017). Weak functional connectivity in the human fetal brain prior to preterm birth. *Scientific reports* **7,** 39286.

Traudt CM, McPherson RJ, Bauer LA, Richards TL, Burbacher TM, McAdams RM & Juul SE (2013). Concurrent erythropoietin and hypothermia treatment improve outcomes in a term nonhuman primate model of perinatal asphyxia. *Dev Neurosci* **35,** 491-503.

Tuor UI, Yager JY, Bascaramurty S & Del Bigio MR (1997). Dexamethasone prevents hypoxia/ischemia-induced reductions in cerebral glucose utilization and high-energy phosphate metabolites in immature brain. *J Neurochem* **69,** 1954-1963.

van den Heuij LG, Fraser M, Miller SL, Jenkin G, Wallace EM, Davidson JO, Lear CA, Lim R, Wassink G, Gunn AJ & Bennet L (2017). Delayed intranasal infusion of human amnion epithelial cells improves white matter maturation after asphyxia in preterm fetal sheep. *J Cereb Blood Flow Metab* **Epub Sept,** 271678X17729954.

van den Heuij LG, Mathai S, Davidson JO, Lear CA, Booth LC, Fraser M, Gunn AJ & Bennet L (2014). Synergistic white matter protection with acute-on-chronic endotoxin and subsequent asphyxia in preterm fetal sheep. *J Neuroinflammation* **11,** 89.

van der Lugt NM, Smits-Wintjens VE, van Zwieten PH & Walther FJ (2010). Short and long term outcome of neonatal hyperglycemia in very preterm infants: a retrospective follow-up study. *BMC Pediatr* **10,** 52.

Vannucci RC & Mujsce DJ (1992). Effect of glucose on perinatal hypoxic-ischemic brain damage. *Biol Neonate* **62,** 215-224.

Vannucci RC, Towfighi J & Vannucci SJ (2004). Secondary energy failure after cerebral hypoxia-ischemia in the immature rat. *J Cereb Blood Flow Metab* **24,** 1090-1097.

Vannucci SJ (1994). Developmental expression of GLUT1 and GLUT3 glucose transporters in rat brain. *J Neurochem* **62,** 240-246.

Vannucci SJ, Seaman LB & Vannucci RC (1996). Effects of hypoxia-ischemia on GLUT1 and GLUT3 glucose transporters in immature rat brain. *J Cereb Blood Flow Metab* **16,** 77-81.

Vesoulis ZA & Mathur AM (2017). Cerebral Autoregulation, Brain Injury, and the Transitioning Premature Infant. *Front Pediatr* **5,** 64.

Vlassaks E, Strackx E, Vles J, Nikiforou M, Martinez-Martinez P, Kramer BW & Gavilanes AW (2013). Fetal asphyctic preconditioning modulates the acute cytokine response thereby protecting against perinatal asphyxia in neonatal rats. *J Neuroinflammation* **10,** 14.

Wang LW, Chang YC, Lin CY, Hong JS & Huang CC (2010). Low-dose lipopolysaccharide selectively sensitizes hypoxic ischemia-induced white matter injury in the immature brain. *Pediatr Res* **68,** 41-47.

Wang LW, Lin YC, Wang ST, Yeh TF & Huang CC (2014). Hypoxic/ischemic and infectious events have cumulative effects on the risk of cerebral palsy in very-low-birth-weight preterm infants. *Neonatology* **106,** 209-215.

Wang X, Stridh L, Li W, Dean J, Elmgren A, Gan L, Eriksson K, Hagberg H & Mallard C (2009). Lipopolysaccharide sensitizes neonatal hypoxic-ischemic brain injury in a MyD88-dependent manner. *J Immunol* **183,** 7471-7477.

Wassink G, Bennet L, Booth LC, Jensen EC, Wibbens B, Dean JM & Gunn AJ (2007). The ontogeny of hemodynamic responses to prolonged umbilical cord occlusion in fetal sheep. *J Appl Physiol* **103,** 1311-1317.

Wassink G, Bennet L, Davidson JO, Westgate JA & Gunn AJ (2013). Pre-existing hypoxia is associated with greater EEG suppression and early onset of evolving seizure activity during brief repeated asphyxia in near-term fetal sheep. *PLoS ONE* **8,** e73895.

Wassink G, Davidson JO, Dhillon SK, Fraser M, Galinsky R, Bennet L & Gunn AJ (2017). Partial white and grey matter protection with prolonged infusion of recombinant human erythropoietin after asphyxia in preterm fetal sheep. *J Cereb Blood Flow Metab* **37,** 1080-1094.

Wassink G, Gunn ER, Drury PP, Bennet L & Gunn AJ (2014). The mechanisms and treatment of asphyxial encephalopathy. *Front Neurosci* **8,** 40.

Welin AK, Svedin P, Lapatto R, Sultan B, Hagberg H, Gressens P, Kjellmer I & Mallard C (2007). Melatonin reduces inflammation and cell death in white matter in the mid-gestation fetal sheep following umbilical cord occlusion. *Pediatr Res* **61,** 153-158.

Westgate J, Wassink G, Bennet L & Gunn AJ (2005). Spontaneous hypoxia in multiple pregnancy is associated with early fetal decompensation and greater T wave elevation during brief repeated cord occlusion in near-term fetal sheep. *Am J Obstet Gynecol* **193,** 1526-1533.

Wu YW, Mathur AM, Chang T, McKinstry RC, Mulkey SB, Mayock DE, Van Meurs KP, Rogers EE, Gonzalez FF, Comstock BA, Juul SE, Msall ME, Bonifacio SL, Glass HC, Massaro AN, Dong L, Tan KW, Heagerty PJ & Ballard RA (2016). High-dose erythropoietin and hypothermia for hypoxic-ischemic encephalopathy: A phase II trial. *Pediatrics* **137,** e20160191.

Wyatt JS, Edwards AD, Azzopardi D & Reynolds EO (1989). Magnetic resonance and near infrared spectroscopy for investigation of perinatal hypoxic-ischaemic brain injury. *Arch Dis Child* **64,** 953-963.

Yanni D, Korzeniewski S, Allred EN, Fichorova RN, O'Shea TM, Kuban K, Dammann O & Leviton A (2017). Both antenatal and postnatal inflammation contribute information about the risk of brain damage in extremely preterm newborns. *Pediatr Res*.

Yao R, Crimmins SD, Contag SA, Kopelman JN & Goetzinger KR (2017). Adverse perinatal outcomes associated with trial of labor after cesarean section at term in pregnancies complicated by maternal obesity. *J Matern Fetal Neonatal Med***,** 1-6.

Yawno T, Mahen M, Li J, Fahey MC, Jenkin G & Miller SL (2017). The beneficial effects of melatonin administration following hypoxia-ischemia in preterm fetal sheep. *Front Cell Neurosci* **11,** 296.

Yawno T, Yan EB, Walker DW & Hirst JJ (2007). Inhibition of neurosteroid synthesis increases asphyxia-induced brain injury in the late gestation fetal sheep. *Neuroscience* **146,** 1726-1733.

Yeh C, Yeh CM, Yu TH, Chang KH, Huang CC & Hsu KS (2016). Neonatal dexamethasone treatment exacerbates hypoxia/ischemia-induced white matter injury. *Mol Neurobiol*.

Zeevalk GD & Nicklas WJ (1992). Evidence that the loss of the voltage-dependent Mg2+ block at the N-methyl-D-aspartate receptor underlies receptor activation during inhibition of neuronal metabolism. *J Neurochem* **59,** 1211-1220.

Zhu C, Kang W, Xu F, Cheng X, Zhang Z, Jia L, Ji L, Guo X, Xiong H, Simbruner G, Blomgren K & Wang X (2009). Erythropoietin improved neurologic outcomes in newborns with hypoxic-ischemic encephalopathy. *Pediatrics* **124,** e218-226.

Simerdeep Dhillon is a PhD student working with Professor Laura Bennet to understand the role of endogenous growth factors such as erythropoietin in the brain and how best to use them to help improve neural recovery from perinatal hypoxia.

Laura Bennet is a fetal systems physiologist whose foundation studies on preterm brain injury has led to new understanding of how the fetus adapts to key challenges such as oxygen lack, infection, and common maternal therapies. She is building on this work to develop new treatments to protect and potentially rebuild the brain.