REVIEW

Glucocorticoids, antenatal corticosteroid therapy and fetal heart maturation

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Abstract

Glucocorticoids are essential in mammals to mature fetal organs and tissues in order to survive after birth. Hence, antenatal glucocorticoid treatment (termed antenatal corticosteroid therapy) can be life-saving in preterm babies and is commonly used in women at risk of preterm birth. While the effects of glucocorticoids on lung maturation have been well described, the effects on the fetal heart remain less clear. Experiments in mice have shown that endogenous glucocorticoid action is required to mature the fetal heart. However, whether the potent synthetic glucocorticoids used in antenatal corticosteroid therapy have similar maturational effects on the fetal heart is less clear. Moreover, antenatal corticosteroid therapy may increase the risk of cardiovascular disease in adulthood. Here, we present a narrative review of the evidence relating to the effects of antenatal glucocorticoid action on the fetal heart and discuss the implications for antenatal corticosteroid therapy.

Introduction

Through most of gestation, the mammalian fetus is maintained in a low glucocorticoid environment, with fetal glucocorticoid concentrations typically five- to ten-fold lower than maternal. As birth approaches, fetal plasma glucocorticoid concentration rises dramatically. This is essential for survival once born. Without glucocorticoid action, the lungs, heart and other organs and tissues are immature at birth, resulting in neonatal death (Fowden et al. 1998). Preterm birth (prior to 37 weeks completed gestation) occurs before this physiological rise in endogenous glucocorticoids. Hence, antenatal corticosteroid therapy (ACT) – in which a potent synthetic glucocorticoid, betamethasone or dexamethasone, is administered to women at risk of preterm delivery – is widely used to mature the fetal lungs. This aims to reduce neonatal morbidity and improve survival of preterm babies. However, whether ACT faithfully mimics the effects of endogenous glucocorticoids on the maturation of other fetal organs, including the heart, is currently unclear. Crucially, less than half of ACT administered prior to preterm birth is optimally timed, and around 50% of women who receive ACT go on to deliver at or near term (Razaz et al. 2015, Makhija et al. 2016, Grzeskowiak et al. 2017) (reviewed in Kemp et al. 2016). Given evidence associating excessive prenatal glucocorticoid exposure with long-term adverse cardiovascular consequences (Fowden et al. 2016), it is important to establish exactly how fetal glucocorticoids – either endogenous or...
exogenous – ‘programme’ cardiovascular health in later life. This knowledge is essential to allow an informed analysis of the benefits and harms resulting from ACT and to refine the use of ACT during pregnancy.

**Endogenous glucocorticoids and fetal maturation**

The adrenal gland is the site of synthesis and release of corticosteroid hormones: mineralocorticoids and the glucocorticoids, cortisol and corticosterone (cortisol predominates in most mammals, whereas rats and mice produce only corticosterone). In human embryos, the adrenal cortex is discernible from the eighth week of gestation, though the morphology differs from the adult (Mesiano & Jaffe 1997). Prior to birth, the fetal zone (comprising most of the adrenal cortex) is the major site of steroidogenesis. The definitive zone starts to produce mineralocorticoids in late gestation, whereas the transitional zone produces cortisol, transitioning into the zona fasciculata, the site of glucocorticoid production, from late gestation (Mesiano & Jaffe 1997). De novo synthesis of cortisol from cholesterol initiates in the human fetal adrenal gland around the 28th week of gestation (Mastorakos & Ilias 2003), but plasma glucocorticoid concentrations remain low until the week before birth (Fowden et al. 1998). Similarly, in fetal mice, plasma glucocorticoid concentrations are low during early-to-mid-gestation then increase rapidly following initiation of adrenal steroidogenesis at embryonic day (E) 14.5 (Michelsohn & Anderson 1992), approximately 5 days before birth. The low glucocorticoid environment during gestation is maintained by at least two placental mechanisms: 11β-hydroxysteroid dehydrogenase (11β-HSD)-2, which converts active cortisol and corticosterone into their intrinsically inert 11-keto-metabolites (Chapman et al. 2013) and P-glycoprotein-mediated active retrograde transport of glucocorticoids from fetus to mother, maintaining the feto–maternal glucocorticoid gradient in both endogenous and exogenous glucocorticoids (Varma 1986, Fowden & Forhead 2004, Walker et al. 2017). 11β-HSD2 is also widely expressed in the fetus during early-to-mid-gestation, to ‘mop-up’ any glucocorticoid that reaches sensitive tissues, particularly the brain (Wyrwoll et al. 2011, 2015). In late gestation, placental 11β-HSD-2 activity markedly declines (Brown et al. 1996, Murphy & Clifton 2003) and maternal glucocorticoid concentrations rise, coincident with increased fetal production of glucocorticoids (Mastorakos & Ilias 2003). Together, these generate the dramatic rise in fetal plasma glucocorticoid concentrations close to term. These increased concentrations of endogenous glucocorticoids act, via glucocorticoid receptor (GR), to mature the fetal lungs (Jobe & Ikegami 2000, Bird et al. 2015) and other organs, in order to survive in the extra-uterine environment after birth. Mice with global knockout of GR die shortly after birth with immature and severely impaired lung function (Cole et al. 1995). A proportion of GR knockout mice die earlier, in late gestation, with immature and poorly functional hearts (Rog-Zielinska et al. 2013), suggesting cardiac immaturity contributes to perinatal mortality and morbidity with deficiency in glucocorticoid action.

**ACT: a life-saving therapy**

In high-income countries, and increasingly in low- and middle-income countries, antenatal corticosteroids (24 mg betamethasone or dexamethasone, administered over 48h) are routinely administered to women considered at imminent risk of preterm delivery (birth prior to 37 weeks gestation and before the natural increase in endogenous glucocorticoid concentrations would be expected). Antenatal corticosteroids are widely accepted to be the most effective therapy to reduce neonatal morbidity and mortality in preterm infants born between 24 and 34 weeks gestation: ACT reduces the incidence of neonatal death and the incidence and severity of respiratory distress syndrome, cerebral haemorrhage and necrotising enterocolitis (Roberts et al. 2017). Thus, in high-income settings, antenatal corticosteroids are undoubtedly life-saving in preterm infants delivered at 24–34 weeks of gestation within a 2- to 7-day window following initiation of a single course of ACT. However, the safety and efficacy of ACT in other settings have been called into question: most notably in low- and middle-income settings, following multiple courses of ACT, in late preterm infants (born at 34–37 weeks of gestation) and where birth occurs outside the 2- to 7-day window following steroid administration (reviewed in Kemp et al. 2016). The latter is a particular concern in high-income settings where estimates suggest 50–80% of pregnant women that receive ACT remain undelivered at 7 days after treatment (McPheters et al. 2005, Razaz et al. 2015), and 50% actually go on to deliver their babies at or near term (Razaz et al. 2015) (reviewed in Kemp et al. 2016). This suggests widespread over-treatment with antenatal corticosteroids resulting in unnecessary and inappropriately timed corticosteroid exposure in babies, many of which subsequently deliver at term. Similar concerns surround the use of repeat courses of ACT, administered when birth does not occur
within 7 days of the first course and preterm delivery remains likely (McKinlay et al. 2015b, Kemp et al. 2016). There are potential short-term and long-term adverse effects associated with unnecessary or inappropriately timed corticosteroid treatment. Delivery later than 7 days after a single course of ACT is associated with an increased risk of perinatal death and maternal infection (McLaughlin et al. 2003). Some follow-up studies have demonstrated adverse neurodevelopmental effects in children born preterm or exposed to multiple courses of ACT (Asztalos et al. 2014, Crowther et al. 2016). Whether ACT affects risk of cardiovascular disease in humans remains unclear (Dalziel et al. 2005, de Vries et al. 2008, McKinlay et al. 2015a). However, cardiovascular disease can take decades to manifest, and it may yet be too early to fully assess the risk in adults. Nevertheless, excessive or mistimed exposure to glucocorticoids in utero is widely acknowledged to have detrimental long-term adverse effects in animals, including non-human primates, that increase the risk of cardiovascular and/or metabolic disease in adulthood (Seckl & Holmes 2007, Rog-Zielinska et al. 2014). The mechanisms remain to be fully elucidated, but undoubtedly include long-term adverse effects on the heart and vasculature.

Maturation of the heart, before and after birth

In the time shortly before and continuing after birth, the heart undergoes extensive growth and remodelling, driven by cardiomyocyte hyperplasia and associated with structural, functional and biochemical maturation. These remarkable changes are driven by mechanical and hormonal factors and are crucial for survival following birth. They also set the foundation for later life and, importantly, influence the risk of developing heart disease in adulthood. Increased cardiac load stimulates fetal myocyte proliferation (Sedmera et al. 2003, Drenckhahn 2009). The fetal heart needs to pump against the resistance of the placental vascular bed with the resulting haemodynamic forces being important drivers of cardiac maturation. The complexity of the fetal part of the placental vasculature increases through branching morphogenesis, though exactly how this influences fetal haemodynamics is currently unclear. In sheep, fetal hypertension promotes cardiomyocyte proliferation prior to terminal differentiation. Conversely, reduced systolic load reduces cardiomyocyte proliferation and, thus, fetal heart weight (reviewed in Thornburg et al. 2011). However, placental insufficiency, with high placental impedance against pulsatile flow, also impairs myocardial maturation (Thornburg et al. 2010); the mechanism remains to be fully elucidated.

Fetal systolic and diastolic functions improve before and shortly after birth, with increasing maturation of the contractile and relaxation properties of cardiomyocytes (Harada et al. 1997, Corrigan et al. 2010). It is proliferation of differentiated mononuclear diploid cardiomyocytes that increases heart mass in late gestation and early after birth in mice and sheep (Soonpaa et al. 1996, Porrello et al. 2011, Alkass et al. 2015, Jonker et al. 2015). The very limited evidence available is consistent with a similar capacity for cardiomyocyte proliferation in neonatal human hearts (Molova et al. 2013). Beyond the first postnatal week in mice, cardiomyocyte proliferation is negligible, and increases in heart mass occur through cardiomyocyte hypertrophy following binucleation and terminal differentiation of cardiomyocytes. Any subsequent proliferation that occurs is restricted to rare mononucleated diploid cardiomyocytes (Patterson et al. 2017). Structural changes that occur in cardiomyocyte maturation during the neonatal period include increased myofibril density and organisation, maturation of mechanical and electrical coupling between cardiomyocytes and the appearance of binucleated cells. This latter is associated with a wave of DNA synthesis that occurs in mouse cardiomyocytes between postnatal day P4 and P7 (Soonpaa et al. 1996). Additionally, as perinatal cardiomyocytes mature, some nuclei become polyploid. In mice, increases in ploidy account for all cell-cycle activity following P13 (Brodsky et al. 1980, Alkass et al. 2015). The majority of murine cardiomyocyte nuclei are diploid, with only 10% being polyploid (Adler et al. 1996). Similarly, in humans, most cardiomyocyte nuclei are diploid up to 7 years of age (though higher levels of ploidy are observed following childhood cardiac disease) (Brodsky et al. 1994). Glucocorticoids play a vital role in the normal maturational changes that occur in cardiomyocytes before birth. The extent to which endogenous glucocorticoids directly affect cardiac maturation in the neonate remains unclear. Even more unclear is how ACT affects these maturational processes in heart structure and function, both before and after birth.

Glucocorticoid action in the fetal heart

Glucocorticoids bind to intracellular receptors: the glucocorticoid receptor (GR or type II receptor) and the high-affinity mineralocorticoid receptor (MR or type I receptor). In mineralocorticoid target tissues, MR is
Glucocorticoids and fetal heart

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and suppression of the hypothalamic–pituitary–thyroid
instability that is common in preterm infants and which
contributes to morbidity and mortality (Ng et al. 2004,
Ibrahim et al. 2011). The non-cardiac sites for these
important hemodynamic effects are currently unclear,
though the fetal and placental vasculature are candidates.
Antenatal dexamethasone treatment in rats dramatically
reduces the extent and complexity of the vasculature
within the labyrinth zone (the site of feto–maternal
exchange), causing intrauterine growth restriction (IUGR)
(Hewitt et al. 2006). In pregnant mice, corticosterone or
dexamethasone treatment (at doses sufficient to cause
IUGR) reduce placental vascularisation if administered
in mid-gestation (E11–16) but not if administered from
E14–E19, the time when glucocorticoid concentrations
naturally rise in mice (Vaughan et al. 2012, 2013). Excessive
glucocorticoid concentrations may also impact feto–
placental vasculature in humans. In pregnant women,
repeat courses of ACT starting at gestational ages ranging
from 27 to 32 weeks impaired the normal gestational
increase in villous capillarisation (Elfayom & Almasry
2014). Similarly, glucocorticoid usage in pregnant women
with asthma was associated with reduced fetal capillary
length and volume (Mayhew et al. 2008). Given the crucial
role of the placental vasculature in cardiac development
(Thornburg et al. 2010), glucocorticoids very likely
contribute to feto cardiac maturation via effects on feto–
placental vasculature. Support for this idea comes from a
recent study in Hsd11b2−/− mice, a model of antenatal GC
excess and IUGR. Hsd11b2−/− mice show abnormal cardiac
maturation, with impaired heart function in late gestation
(Wyrwoll et al. 2016). This is associated with restricted
growth of fetal vessels in the placenta and reduced
Restoration of the normal placental vasculature and blood
flow restored normal heart function in Hsd11b2−/− mice
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The mechanisms by which glucocorticoids mature
fetal cardiomyocytes have been investigated in vitro.

co-expressed with 11β-HSD2, conferring mineralocorticoid
specificity upon MR (Chapman et al. 2013). However,
in certain MR-expressing tissues in which 11β-HSD2
is not co-expressed, most notably the heart and the
hippocampus, MR is activated by cortisol/corticosterone.
Moreover, because of its high affinity, cardiac MR is
likely to be occupied even at the glucocorticoid nadir.
Importantly, while betamethasone and dexamethasone
(the synthetic glucocorticoids commonly used in ACT)
potently activate GR, they are mineralocorticoid sparing,
showing little activation of MR (we return to this below).
Ligand-bound GR and MR translocate to the nucleus
where they are active as transcription factors, triggering
cascades of gene expression (Coutinho & Chapman 2011).

Glucocorticoids, through GR, promote structural,
functional and metabolic remodelling in mouse fetal
cardiomyocytes (Rog-Zielinska et al. 2013, 2015). Mice
with global GR knockout have small and immature hearts
that function poorly. The E/A wave ratio, a marker of
cardiac maturity, is reduced in GR knockout mice and early
systolic and diastolic function are impaired (Rog-Zielinska
et al. 2013), similar to the preterm heart (reviewed in
Rog-Zielinska et al. 2014). This functional immaturity
goes hand-in-hand with structural immaturity (short and
disorganised myofibrils, a lack of cardiomyocyte alignment
in the outer ventricle wall) and biochemical immaturity
(calcium handling, energy metabolism). Endogenous
glucocorticoids directly impact cardiomyocytes. SMGRKO
mice, with GR deficiency restricted to cardiomyocytes and
vascular smooth muscle cells (VSMCs), replicate much of
the cardiac phenotype of global GR knockout mice (Rog-
Zielinska et al. 2013). SMGRKO mice have immature
systolic function in late gestation, associated with short and
disorganised myofibril structure (Rog-Zielinska et al.
2013). This is underpinned by reduced expression of genes
encoding structural proteins myosin heavy chain (MHCα)
and proteins vital for calcium handling (SERCA2, RYR2)
and energy metabolism in late gestation (Rog-Zielinska
et al. 2013).

Not all the actions of endogenous glucocorticoids
upon the fetal heart are direct. The use of a cardiomyocyte-
specific approach avoids the perturbation of blood pressure
and suppression of the hypothalamic–pituitary–thyroid
(HPA) axis associated with exogenous glucocorticoid
administration. While SMGRKO mice reproduce much
of the cardiac phenotype of global GR-knockout mice,
some aspects differ. In contrast to global GR knockout
mice, SMGRKO mice have normal-sized hearts, a normal
E/A-wave ratio and normal cardiac expression of Nppa
(encoding atrial natriuretic peptide, ANP) (Rog-Zielinska
et al. 2013). This suggests that these deficits in the global
knockout mice are attributable to lack of GR elsewhere
than in cardiomyocytes and VSMC. Hemodynamic
forces may account for at least some of these differences.
Fetal ANP responds to volume stimuli and regulates
blood pressure (Cameron & Ellmers 2003). The normal
cardiac expression of ANP in SMGRKO fetuses suggests
blood pressure is normal (as it is in adult SMGRKO
mice (Richardson et al. 2017)). Glucocorticoids exert
powerful pressor effects, including in the fetus (Unno
et al. 1999, Forhead & Fowden 2004). Indeed, adrenal
insufficiency is likely to be a factor in the haemodynamic
instability that is common in preterm infants and which
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The mechanisms by which glucocorticoids mature
fetal cardiomyocytes have been investigated in vitro.
Treatment of mouse fetal cardiomyocytes with either dexamethasone or corticosterone for 24 h promotes their structural, functional and biochemical maturation (Rog-Zielinska et al. 2015). GR targets in these cells include a number of transcription factors (Rog-Zielinska et al. 2015), supporting a cascade effect of GR activation. In *vivo* and *in vitro* dexamethasone rapidly induces PGC-1α, a critical regulator of cardiac mitochondrial capacity and vital for the functional and metabolic maturation of the fetal heart (Lai et al. 2008). Knockdown of PGC-1α in fetal cardiomyocytes abolished the glucocorticoid effects on mitochondrial O$_2$ consumption and myofibril structure, suggesting it is a key mediator of glucocorticoid-induced maturation of cardiomyocyte structure and mitochondrial capacity (Rog-Zielinska et al. 2015). Whether PGC-1α mediates other aspects of glucocorticoid-induced cardiomyocyte maturation is unknown. However, PGC-1α knockdown in human embryonic stem cell-derived cardiomyocytes decreased mitochondrial content and activity (as expected) and also reduced concentrations of reactive oxygen species (ROS) and heightened vulnerability to metabolic stress (Birket et al. 2013). This suggests that glucocorticoids, via PGC-1α might increase ROS production in maturing cardiomyocytes. Other primary targets of GR in fetal cardiomyocytes include Ppara, Klf15 and Lipin1 (Rog-Zielinska et al. 2015). This points to a direct role for GR in promoting the capacity of cardiomyocytes for fatty acid oxidation as the preferred substrate for cardiac mitochondrial ATP generation, consistent with the increase in cardiac ATP concentration and ATP delivery to myofibrils induced by dexamethasone administration in late gestation rats (Tsuzuki et al. 2009, Mizuno et al. 2010). Other rapidly induced targets of GR in primary fetal mouse cardiomyocytes include Dio2, encoding deiodinase (D2) the enzyme that converts thyroxine (T$_4$) into the more biologically active thyroid hormone, T$_3$ (Rog-Zielinska et al. 2015). Thus, amplification of intracellular thyroid hormone action is part of the cascade of events initiated by GR activation and is consistent with evidence that at least some of the effects of the prepartum increase in glucocorticoids are mediated via T$_3$ (Forhead & Fowden 2014). Indeed, cortisol increases plasma availability of T$_3$ in the fetus by inducing hepatic expression of D1 (responsible for producing most of the circulating T$_3$), and downregulates placental clearance of T$_3$ by the D3 enzyme (Forhead & Fowden 2014, Jonker & Louey 2016). Many of the actions of glucocorticoid and thyroid hormones during fetal and neonatal heart maturation overlap. Both accelerate the switch from MHC-β to MHC-α and both increase ANP (van Tuyl et al. 2004, Chattergoon et al. 2012a) (these are indirect effects in the case of glucocorticoids (Rog-Zielinska et al. 2015)). In *vivo*, haemodynamic forces are changed by both glucocorticoids and thyroid hormones. Like synthetic glucocorticoids (Rog-Zielinska et al. 2014), *in vitro* and possibly *in vivo*, T$_3$ is anti-proliferative in cardiomyocytes, promoting the switch to hypertrophic growth and increasing the population of terminally differentiated binucleated myocytes (Chattergoon et al. 2007, 2012a,b). If GR is precociously activated before the HPA axis has started to produce circulating fetal thyroid hormones in any substantial amount, this could limit maturation of fetal organs by glucocorticoids. This may be more important in rodents (where fetal thyroid hormone synthesis only initiates in late gestation) than in humans or in sheep models, where thyroid hormone synthesis occurs by mid-gestation (Forhead & Fowden 2014). Interestingly, while dexamethasone alone is ineffective in human induced pluripotent cell (iPSC)-derived cardiomyocytes, it acts in concert with T$_3$ to improve the electrophysiology, bioenergetics and contractile force generation of the cells (Birket et al. 2015). Both T$_3$ and dexamethasone are also required for T-tubule development in human iPSC-derived cardiomyocytes (Parikh et al. 2017). This inter-dependent relationship of thyroid hormone and glucocorticoids in cardiac maturation is an important consideration for ACT, where only glucocorticoid is administered.

The transition to neonatal life: a role for glucocorticoids in cardiac adaption?

The transition to extra-uterine life is accompanied by loss of the placenta, a low-resistance vascular bed. In term birth, the associated circulatory changes rapidly increase peripheral resistance in the systemic circulation, with greater cardiac afterload and systemic arterial pressure. Blood and tissue oxygenation rapidly increase (partial pressure of oxygen in arterial blood increases over two-fold), as do plasma glucose and free fatty acid concentrations, to fuel the increased energy needs (Jonker & Louey 2016). Endogenous glucocorticoids are likely to be critical in facilitating these adaptions, although apart from metabolic effects, their role has been little explored. Interestingly, expression profiling and *in silico* transcription factor analysis in sheep heart across the perinatal period suggests glucocorticoids transiently suppress immune responses at birth and that they support metabolic changes during the transition from fetal to neonatal life (Richards et al. 2015).
In mice, cell-cycle arrest in cardiomyocytes occurs shortly after birth and is triggered by increased mitochondrial ROS production and oxidative DNA damage following birth (Puente et al. 2014). Transient hyperoxia in neonatal mice exacerbates oxidative DNA damage and accelerates cell-cycle arrest, whereas hypoxia has the opposite effect. Only hypoxia alters (increases) the heart-to-body weight ratio (Puente et al. 2014). Reduced cell number with hyperoxia is compensated by cardiomyocyte hypertrophy. Transient hyperoxia in neonatal rats causes left ventricular hypertrophy in adulthood and increases vulnerability to pressure overload, with severe heart failure in those exposed to transient high O₂ at birth (Bertagnolli et al. 2014). The mechanism was not explored, but reduced cardiomyocyte endowment as a result of premature cell-cycle exit, is associated with pathological cardiac hypertrophy in adult rats (Porrello et al. 2009). Glucocorticoids may promote this process: in neonatal rats, administration of dexamethasone from P1 to P3 decreased cardiomyocyte proliferation and increased binucleation at P4, with a reduction in cardiomyocyte number and an increase in heart/body weight ratio (presumably as a result of cardiomyocyte hypertrophy) apparent by 2 weeks of age (Gay et al. 2015). Dexamethasone induces oxidative stress in neonatal rat hearts (Adler et al. 2010). Whether this is mediated through PGC-1α will be interesting to establish, given the role of PGC-1α in mitochondrial ROS production in human embryonic stem cell-derived cardiomyocytes (Birket et al. 2013). Deletion of GR in cardiomyocytes/VSMC increases cardiomyocyte proliferation in neonatal mice and increases heart weight, without associated cardiomyocyte hypertrophy (Richardson et al. 2017). This is consistent with GR activation normally restraining cardiomyocyte number in neonatal mice. Glucocorticoid activation of cardiac MR may have the opposite effect. At subpressor doses, cortisol stimulates cardiomyocyte proliferation in fetal sheep without affecting binucleation (Giraud et al. 2006). This hyperplastic effect can be blocked by intra-pericardial antagonism of MR (Feng et al. 2013). Similarly, spironolactone antagonism of MR from birth reduced adult heart weight in mice, independently of cardiomyocyte GR (Richardson et al. 2017). This is consistent with endogenous glucocorticoid action via MR being pro-proliferative in perinatal cardiomyocytes.

The heart in preterm infants

Preterm birth occurs before the organs have had sufficient time to accrue mass, and when they are structurally and functionally immature. Thus, in preterm infants, heart mass (relative to body weight) is reduced at birth (Aye et al. 2017). Moreover, if driven by cardiac load, oxygenation and arterial pressure, then terminal differentiation of cardiomyocytes will likely occur in the same time scale in the preterm heart as in the term. In an important recent study, hearts from preterm infants who died 1–42 days following birth were compared with appropriately grown stillborn infants (20–40 weeks of gestation). Birth per se reduced markers of cardiomyocyte proliferation compared to age-matched stillborns (Bensley et al. 2018). Therefore, preterm birth prompts the cessation of cardiomyocyte proliferation, plausibly as a result of the oxygen-induced DNA damage discussed above (Puente et al. 2014). This potentially reduces cardiomyocyte endowment in preterm infants compared to the term-born infants, resulting in maladaptive structural remodelling in the neonatal period. Alterations in cardiac geometry and mechanics are already apparent in the preterm-born infant and child (Lee et al. 1992, Aye et al. 2017). The reduced heart mass after preterm birth becomes normalised by disproportionate cardiac hypertrophy and increased left ventricular mass in subsequent ‘catch-up’ growth in the early postnatal period (Kozak-Barany et al. 2001, Aye et al. 2017), with differences in cardiac structure and function persisting in adulthood (reviewed in Bensley et al. 2016, Le et al. 2018). Preterm-born adults show increased left ventricular mass (independent of blood pressure), thickened ventricular walls and displaced apex (Lewandowski et al. 2013). Studies in preterm lambs demonstrate that catch-up growth occurs by cardiomyocyte hypertrophy: cardiomyocyte volume is increased despite no differences in absolute or relative heart weight (Bensley et al. 2010). Similarly, in hypoplastic mouse hearts, myocardial mass is normalised by accelerated cardiomyocyte hypertrophy (Drenckhahn et al. 2015). Thus, myocyte hypertrophy can compensate for fewer cardiomyocytes to maintain mass. However, in the very and extremely preterm infant, the reduction in cardiomyocyte number may be too great for this compensatory mechanism to normalise heart size: left ventricular mass is reduced in adults born extremely preterm (Kowalski et al. 2016) and in children born very preterm (Mohilkert et al. 2018).

Ontogenetically determined cardiomyocyte number (or endowment) is an important factor in vulnerability to cardiac disease (Levkau et al. 2008). In a large study of over 2.6 million individuals born in Sweden between 1987 and 2012, gestational age prior to 32 weeks at delivery was strongly associated with risk of heart failure in childhood and early adulthood. The risk was increased...
17-fold in those born at <28 weeks and 4-fold if born at 28–31 weeks of gestation (Carr et al. 2017). This suggests a vulnerability to heart failure with preterm birth, with a reduced threshold at which an insult will trigger heart failure. Reduced cardiomyocyte endowment with compensatory hypertrophy may explain this vulnerability, with subsequent greater stress on individual myocytes. Stress and/or ageing is associated with polyploidy in cardiomyocyte nuclei (Anatskaya et al. 2009, Senyo et al. 2013). Polyploidy may be a mechanism to allow larger cardiomyocytes to fulfil metabolic and functional demands (Schoenfelder & Fox 2015). In humans, polyploidy is likely to arise in childhood and is strongly associated with impaired cardiac function and pathological hypertrophy (Brodsky et al. 1991, 1994). Whether preterm hearts show higher levels of ploidy is unknown. However, myocyte ploidy is increased in preterm sheep (Bensley et al. 2010) consistent with polyploidy as an indicator of greater susceptibility to cardiac stress/load in the preterm-born adult. Fibrosis, seen in adults born preterm, may occur secondarily to cardiomyocyte loss.

**ACT: what are we doing to the heart?**

Although preterm birth is strongly associated with increased risk of cardiovascular disease in later life, the evidence to date suggests that effects of preterm birth on cardiac morphology and function are independent of antenatal corticosteroid exposure (Dalziel et al. 2005, Aye et al. 2017). While both are predicted to have similar effects (e.g. reduced cardiomyocyte endowment, cardiomyocyte hypertrophy), ACT is likely to be a minor contributor compared to preterm birth itself. Indeed, should preterm birth occur, in the short term, ACT may enhance cardiomyocyte function directly through rapid effects on metabolic and structural maturation and possibly indirectly by improving haemodynamic stability. Glucocorticoids increase haemodynamic load in the fetus as well as the neonate (Unno et al. 1999), likely to affect cardiomyocyte proliferation and/or maturation independently of direct effects of glucocorticoids in cardiomyocytes. Indeed, systemic haemodynamic instability (hypotension and low systemic blood flow) is inversely related to gestational age at birth (du Plessis 2009) and is a cause of mortality in some preterm infants (Ng et al. 2004, Kluckow 2005, Sehgal 2011, Rog-Zielinska et al. 2014). In fetuses >29 weeks gestational age, heart rate was rapidly (within 24 h) and transiently decreased following betamethasone (Mulder et al. 2004). This was ascribed to the baroreceptor reflex in which an increase in blood pressure very rapidly triggers a decrease in heart rate, causing blood pressure to fall. This did not occur in younger fetuses (Mulder et al. 2004). Thus, ACT has gestational age-specific effects on the developing fetus, consistent with the idea of developmental windows of susceptibility to the maturational effects of glucocorticoids, including in the heart, dependent on the stage of organ development at the time of glucocorticoid exposure: organogenesis, growth or maturation. This warrants further research.

Of concern, around half of women exposed to a single course of ACT do not deliver their babies within the optimal 2- to 7-day time window (Kemp et al. 2016). If delivered more than 7 days after a single course of ACT, the risk of perinatal death is increased two-fold and neonatal death three-fold (McLaughlin et al. 2003). The reasons may be multi-factorial, but studies in sheep support the idea that heart function is compromised by excessive glucocorticoid exposure in utero. A modest but chronic maternal hypercortisolaemia in late gestation sheep altered the trajectory of myocyte maturation and increased expression of calcium signalling genes (Richards et al. 2014), consistent with a short-term benefit. However, continuation of maternal hypercortisolaemia to term resulted in a high incidence of stillbirth (Keller-Wood et al. 2014). This was associated with reduced cardiac mitochondrial number and function and increased cardiac expression of hypoxia-response genes, though not the hypoxia master regulators, HIF1α or ARNT themselves (Richards et al. 2014). Chronic maternal hypercortisolaemia also altered the fetal ECG and depressed aortic pressure and heart rate immediately prior to delivery (Antolic et al. 2018). The authors speculate that glucocorticoid-induced alterations in fetal cardiac metabolism and/or ion homeostasis contribute to cardiac dysfunction, creating vulnerability to death during labour and/or delivery. In mice, a number of ion channels are direct targets of GR in cardiac cardiomyocytes (Rog-Zielinska et al. 2015) and transgenic over-expression of GR in cardiomyocytes (Rog-Zielinska et al. 2015) and transgenic over-expression of GR in cardiomyocytes of adult mice causes ion-channel remodelling (Sainte-Marie et al. 2007). MR over-expression in cardiomyocytes also causes ion-channel remodelling (Ouvrard-Pascaud et al. 2005). Teasing apart the relative contributions of GR and MR (and indeed, the balance between MR and GR (Richardson et al. 2016)) to the complex effects of endogenous and exogenous glucocorticoids in the heart will be important in understanding how excessive glucocorticoid exposure in utero increases risk of perinatal death. Further research in this area is needed.
There are longer-term concerns. The association between excessive exposure to glucocorticoid in utero (in term birth) and cardiovascular disease in adulthood is well known (Seckl & Holmes 2007). Indeed, excessive antenatal glucocorticoid exposure is considered as a key factor in the fetal origins of disease hypothesis (Seckl & Holmes 2007, Fowden & Forhead 2015, Fowden et al. 2016). It is, as yet, unclear if the short-term exposure to high concentrations of potent glucocorticoids used in ACT adversely affects the heart if birth does not occur within 7 days. We and others have reviewed a body of (sometimes conflicting) evidence from sheep and rodents suggesting glucocorticoid treatment reduces cardiomyocyte proliferation and promotes cardiomyocyte hypertrophy in the fetal and/or neonatal perinatal heart (Porrello et al. 2008, Rog-Zielinska et al. 2014, Richardson et al. 2016), beneficial in the short-term in promoting heart function and survival, but possibly at the expense of adult cardiovascular resilience. As discussed earlier, cardiomyocyte endowment is a key factor in susceptibility to cardiac disease particularly in heart failure where cardiomyocyte loss contributes to development and progression of the disease (Rayment et al. 1999). People born at term who are exposed to excess glucocorticoids in utero may start life with fewer cardiomyocytes, contributing to risk of cardiovascular disease. More information on this vital question is required, in particular whether and how endogenous and exogenous glucocorticoids increase or reduce cardiomyocyte number at birth.

What are the effects of ACT upon endogenous glucocorticoid production and how might this affect the fetal and/or neonatal heart? Maternal stress, perinatal adversity and exposure to inappropriately timed or excessive concentrations of glucocorticoids prenatally are all associated with life-long alterations in HPA axis activity (Seckl & Holmes 2007, Waffarn & Davis 2012, Moisiadis & Matthews 2014). Relative adrenal insufficiency may occur in preterm neonates (especially if delivered prior to the initiation of de novo adrenocortical glucocorticoid synthesis at 28 weeks gestational age) or could result from ACT. The adrenal gland undergoes extensive remodelling immediately after term birth (Keene 1927) with regression of the fetal zone and maturation of the zona fasciculata. How this is affected in preterm infants and by ACT is unclear. However, what is clear is that both the maternal and the fetal HPA axis are suppressed following ACT (reviewed in Waffarn & Davis 2012). Corticosteroids administered in ACT persist in the fetal circulation for up to 3 days following the last dose (Ballard et al. 1975, Kajantie et al. 2004, Nykanen et al. 2007). However, cortisol concentration in the neonate remains depressed well after clearance of administered steroid from the circulation: a meta-analysis of 49 studies concluded that both basal cortisol and stress-induced cortisol release are suppressed in the preterm infant (Tegethoff et al. 2009). Furthermore, and while basal cortisol concentration recovers within 2 weeks of delivery, the cortisol response to pain is suppressed for at least 4 weeks and possibly considerably longer (Tegethoff et al. 2009). In sheep, three courses of betamethasone reduced late gestation fetal cortisol concentration, which remained suppressed 6 weeks postnatally (Li et al. 2013). Pituitary expression of POMC (encoding the precursor to ACTH) was reduced up to 12 weeks postnatally (Li et al. 2013). ACT also affects stress reactivity and HPA axis regulation in those infants who are delivered at or near term. In one study, newborns exposed to ACT but delivered near or at term were unable to mount a cortisol stress response, in contrast to non-exposed newborns (Schaffer et al. 2009). On the other hand, another study reported an increase in stress reactivity of cortisol in term-born neonates after ACT exposure (Davis et al. 2011). The reasons for the differences are unclear, but may relate to the conditions of testing or gestational age at ACT exposure. In an animal model, the ability of neonatal rats to mount a glucocorticoid response to stress depended on developmental age and was stressor specific (Walker 1991). HPA axis abnormalities persist: school-age children exposed to ACT but born at term had abnormal diurnal cortisol release: they lacked the normal cortisol awakening response and had a flattened diurnal rhythm in salivary cortisol concentration compared with non-exposed term-born children (Edelmann et al. 2016). These effects of ACT upon endogenous glucocorticoid production will have two consequences in the fetal and neonatal heart: First, in the short-term the HPA axis suppression will deprive cardiac MR of their glucocorticoid ligand. This will severely alter the cardiac MR/GR balance in favour of GR, for example, favouring the anti-proliferative effect of GR activation over the pro-proliferative effects of MR activation (Richardson et al. 2016). A similar consideration applies to MR in the hippocampus: here, the severe adverse psychological/psychotic effects of dexamethasone in children treated for acute lymphoblastic leukaemia can be ameliorated by co-administration of cortisol, for replacement at the MR (Warris et al. 2016). Secondly, persistent suppression of the fetal HPA axis following ACT may also deprive GR as well as MR of stage-appropriate levels of its endogenous ligand. ACT, even in the term born, may therefore alter the trajectory of fetal and neonatal heart maturation
through changes in endogenous glucocorticoid production.

**Concluding remarks**

ACT is an established and effective therapy to improve lung function in preterm infants, to reduce neonatal morbidity and mortality. However, increasing evidence suggests it is not always harm-free, particularly in infants delivered at or near term (Althabe et al. 2015). The jury is still out on whether ACT alters the risk of late-onset cardiac disease, but given the complex effects of corticosteroids on haemodynamics and fetal and neonatal heart maturation, it would be surprising if it does not impact on the trajectory of perinatal heart maturation, subsequent cardiac growth and vulnerability to insult.

More knowledge is urgently needed to inform future refinements to ACT. Current treatment protocols were largely established decades ago and are based on limited knowledge regarding optimal formulation, timing of dosage and efficacy at different gestational ages. A ‘one size fits all’ dosage applies, irrespective of maternal weight or gestational age. It seems likely that different processes during cardiac development may be affected by ACT at different gestational ages. With the increasing use of ACT in preterm infants as young as 22 weeks, it is important to elucidate how glucocorticoids may impact heart structure and function at different times. The manner of cardiomyocyte proliferation shapes the heart. If ACT alters cardiomyocyte proliferation, particularly in a region-dependent manner, then both shape and function will be affected (Foglia & Poss 2016). There may be sex differences in cardiac susceptibility to ACT. Certainly evidence from sheep supports a sex difference in lung maturation, with a lower dose of betamethasone required for survival of female lambs compared to male (De Matteo et al. 2010). Mice with GR knockout in cardiomyocytes show sex differences in cardiomyocyte hypertrophy (Richardson et al. 2017) and in susceptibility to cardiac disease (Oakley et al. 2013). Answers to these questions should help inform and refine future use of ACT.


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