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REVIEWS

Review

Combined Antioxidant and Glucocorticoid Therapy for Safer Treatment of Preterm Birth

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Ante- and postnatal glucocorticoid therapy reduces morbidity and mortality in the preterm infant, and it is therefore one of the best examples of the successful translation of basic experimental science into human clinical practice. However, accruing evidence derived from human clinical studies and from experimental studies in animal models raise serious concerns about potential long-term adverse effects of treatment on growth and neurological and cardiovascular function in the offspring. This review explores whether combined antioxidant and glucocorticoid therapy may be safer than glucocorticoid therapy alone for the treatment of preterm birth.

Highlights

Antenatal glucocorticoid therapy accelerates fetal lung maturation and treatment of the preterm infant with glucocorticoids reduces the incidence of chronic lung disease and the baby's dependence on assisted ventilation. Therefore, perinatal glucocorticoid therapy has led to a significant reduction in morbidity and mortality of preterm infants.

Despite established beneficial effects, there is increasing evidence that exposure of the offspring to synthetic glucocorticoids during the perinatal period induces long term detrimental effects on growth, the brain, and cardiovascular system.

Glucocorticoid therapy in the perinatal period is here to stay. However, current therapy needs refining to maintain benefits but also limit detrimental effects.

Detrimental effects of glucocorticoid therapy are partly mediated by increased oxidative stress.

We propose combined antioxidant and glucocorticoid therapy may be the safer for the treatment of preterm birth.

Introduction

The clinical use of glucocorticoids both in mothers threatened with preterm labour and in preterm neonates has become common practice in the past 40 years [1,2]. This treatment is based on the pioneering work of Liggins who discovered that the development of the fetal tissues in preparation for extrauterine life was dependent upon the prepartum surge in fetal endogenous glucocorticoids and that exposure to synthetic glucocorticoids in premature offspring could aid the development of the pulmonary system [3–5]. It is now established that antenatal glucocorticoid therapy accelerates fetal lung maturation [6] and that treatment of the preterm infant with glucocorticoids reduces the incidence of chronic lung disease and the baby's dependence on assisted ventilation [7,8]. Ante- and postnatal glucocorticoid therapy has therefore significantly reduced the incidence of morbidity and mortality in the preterm infant [6], and it is one of the best examples of the successful translation of basic experimental science into human clinical practice [9].

Physiological Effects of Glucocorticoids in the Perinatal Period

In adults, glucocorticoids are well-known mediators of physiological responses to stress. Endogenous production occurs in the adrenal cortex, and exogenous synthetic analogues are frequently administered clinically as anti-inflammatory or immunomodulatory treatments [10]. During the fetal period, glucocorticoids take on a pivotal role as orchestrators of fetal organ maturation. This fetal maturation is stimulated by a prenatal surge in circulating levels of fetal plasma glucocorticoid in most species studied [11]. We now know that this prepartum surge in fetal plasma glucocorticoid is intricately linked with the appropriate preparation of many organ systems to ensure the successful transition from intra- to extrauterine life. Some of the key changes are summarised in Figure 1. For instance, the fetal lungs are filled with fluid and relatively inelastic [12]. However, the **neonate** (see Glossary) must be able to inflate the lungs with air to begin ventilation immediately after birth. As development progresses to term, fetal lung liquid begins to be reabsorbed [13] and elastin mRNA levels increase [14]. Furthermore, pulmonary surfactant protein expression increases and, histologically, surfactant granules can be seen in type II pneumocytes [15]. All these changes in the pulmonary system have been shown *in vivo* and *in vitro* to rely on the surge in fetal circulating levels of glucocorticoids [6,15].

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In the fetal brain, the relationship between the prepartum cortisol surge and the development of the central nervous system is complex, and the various cell populations and brain regions are affected differently. Neurogenesis occurs predominantly during early gestation; however, by late gestation there are still principal events occurring including multiplication of glia and astrocytes, programmed cell death, neuronal migration, synapse formation and pruning, as well as myelination [16]. Fetal glucocorticoids are thought to be involved in the physiological regulation of all of these processes, by altering cell number and size within specific populations, promoting changes in synaptic function [17], and balancing apoptosis in the developing brain [18].

Fetal plasma glucocorticoids are also involved in the development and maturation of the cardiovascular system. Fetal arterial blood pressure increases with advancing gestational age [19,20]. This change can be experimentally induced preterm by exogenous administration of synthetic glucocorticoids [21,22]. Similarly, the fetal arterial baroreflex undergoes changes in set point and sensitivity to accommodate the ontogenic rise in fetal arterial blood pressure with advancing gestation. These effects can be mimicked preterm by exogenous administration of synthetic glucocorticoids, promoting an accelerated rightward shift in the fetal cardiac baroreflex, thereby allowing a greater resting arterial blood pressure in the fetus without triggering sustained fetal **bradycardia** [22]. There are also significant developmental effects of glucocorticoids on the fetal heart [2]. They stimulate cardiomyocyte maturation, specifically hypertrophy and **binucleation** [23]. Left ventricular developed pressure also increases towards term [24] and the electrical conduction system in the heart is also matured in preparation for the increased cardiac workload at birth [23]. The capacity of the fetal cardiovascular system to respond to acute stress also changes with advancing gestational age in parallel with the prepartum surge in fetal plasma glucocorticoid [21,25]. In response to an acute period of fetal oxygen deprivation, a fall in fetal heart rate occurs and there is an increase in fetal **peripheral vascular resistance**, contributing to a redistribution of the fetal cardiac output away from peripheral circulations. Fetal bradycardia reduces myocardial oxygen consumption and the redistribution of blood flow is part of the well-known fetal brain sparing effect [26]. We now know that in the immature fetus, before exposure to the pre-partum increase in fetal plasma glucocorticoid, the fetal bradycardia is transient and the increase in peripheral vascular resistance modest [25]. Conversely, in the more developed fetus, bradycardia is sustained and there is a highly significant increase in peripheral vascular resistance in response to acute hypoxia [25]. These maturational changes give rise to improved myocardial oxygen sparing and more efficient redistribution of oxygen towards the fetal brain [27,28]. Treatment with exogenous synthetic glucocorticoids can switch the pattern of the fetal heart and circulatory responses to acute hypoxia from the immature towards the mature type, thereby enhancing the fetal defence to acute hypoxic stress [21,25,27,28].

Activation of the fetal hypothalamo–pituitary–adrenal (HPA) axis is also vital for acute stress responses, resulting in a rapid rise in fetal blood glucocorticoid levels [29]. The sensitivity of the fetal HPA axis to an acute stressor also increases with gestational age, including changes in the production of prohormones in the fetal pituitary, such as pro-opiomelanocortin [30], the bioactivity of adrenocorticotropic hormone (ACTH), and increased sensitivity of the fetal adrenal cortex to ACTH [29]. Treatment of the immature fetus with synthetic glucocorticoids can also promote an increase in the sensitivity of the adrenal cortex to ACTH and thereby increase the magnitude of the fetal plasma endogenous glucocorticoid response to acute stress [31].

During fetal life the kidneys do not act as the main osmoregulatory organ; a role attributed to the placenta. However, the fetal kidneys do produce large volumes of relatively hypo-osmotic urine,

Glossary

Antenatal: during pregnancy, the period prior to birth.
Aortic distensibility and pulse wave velocity: aortic distensibility and aortic pulse wave velocity are two parameters closely related to the elastic function of the aorta and the ability of this vessel to distend. The parameters serve as pathogenic markers in cardiovascular disease.
Binucleation: maturational change in which heart cells switch from a single nucleus to two nuclei per cell, which is a terminal differentiation event as cells no longer proliferate after undergoing this change.
Bradycardia: reduced heart rate in response to a physiological stressor.
FENa: fractional excretion of sodium ions by the kidney. Refers to the % of Na^+ that passes through the kidney, that is eventually excreted in the urine.
Neonate: a newborn child or mammal.
Perinatal: relating to the period shortly before and after birth.
Peripheral vascular resistance: resistance to blood flow in the peripheral circulation, which consist of responsive vessels that may rapidly alter their state from constriction to dilatation or vice–versa to modulate local blood flow. Key examples of peripheral circulations that contribute to peripheral vascular resistance are the mesenteric and femoral vascular beds.
Postnatal: the period after birth, the immediate postnatal period refers to the first 6 weeks following birth.
Uteroplacental: referring to the uterus and placenta as a functional unit.

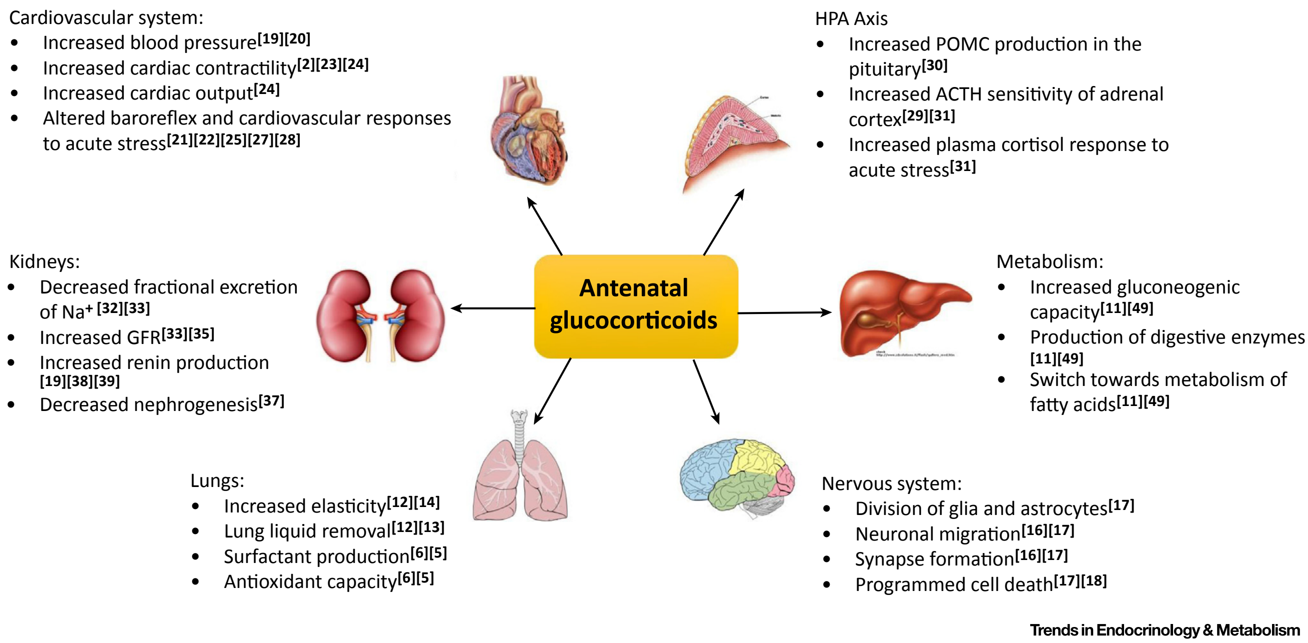


Figure 1. Roles of Glucocorticoids in Fetal Maturation. Key maturational events associated with the prepartum cortisol rise in: the cardiovascular system, the HPA axis, fetal metabolism, the nervous system, the fetal lungs, and the kidneys. For reference see [2,6,11–25,27–33,35,37–39,49].

which contributes to amniotic fluid generation [32]. The fetal kidneys are poor at retaining sodium and consequently have a large fractional excretion of sodium (**FENa**) [33]. This allows for the excretion of the large amounts of fluid that cross the placenta and must be lost from the fetal circulation. As gestational age increases, FENa is reduced as transporters increase in number and activity in the nephron [33]; effects that can be induced experimentally in the preterm fetus by exogenous glucocorticoid treatment [34]. This prepares the fetus for the need for sodium retention upon birth to help maintain blood volume. In fetal sheep, FENa decreases from around 15% of all sodium being excreted to 5% sodium excretion at term, and continues to decrease during early neonatal life [34]. In humans, FENa also decreases during neonatal life and glomerular filtration rate (GFR) in the fetal kidneys is also low. Again, this increases towards term, in part due to an increased cardiac output and renal blood flow [35], with the percentage of plasma being filtered through the kidneys increasing from 3% to 25% in the neonate [33]. Maturation of the kidney is also essential for renal endocrine pathways. The renin–angiotensin system (RAS) is vital for sodium homeostasis and long-term control of arterial blood pressure. Renin production increases with gestational age [36], concomitant with changes in expression of AT-1 and AT-2 receptors in various target organs [37]. The period of nephrogenesis occurs predominantly during late gestation in sheep and humans [37]. In humans, no nephrogenesis occurs after 36 weeks [37]. The prepartum increase in fetal plasma glucocorticoid has also been shown to be involved in mediating changes in fetal renin production [38], changes in the expression of AT-1 and AT-2 receptors in various target organs [39] and in mediating the decrease in nephrogenesis [36].

Clinical Benefits of Glucocorticoids in the Perinatal Period

Given the beneficial effects of glucocorticoid exposure on numerous physiological systems in the fetus during late gestation, it is not surprising that **antenatal** glucocorticoid therapy is now considered to be an indispensable treatment for preterm birth by many health institutions.

Antenatal glucocorticoids are recommended by the Royal College of Obstetricians and Gynaecologists for all pregnancies at risk of preterm birth between 26 and 34 weeks [40]. Similarly, since 1996, the National Institutes of Health have advised routine administration of synthetic glucocorticoids to all pregnant women at risk of delivery before 34 weeks of gestation [6]. Across the world, treatment for possible preterm birth involves maternal intramuscular injection of the synthetic glucocorticoids, betamethasone or dexamethasone, using a variety of dosing regimens [41]. Currently, the recommended protocols are two doses of 12 mg of betamethasone or of dexamethasone intramuscularly 24 h apart, or four doses of 6 mg of dexamethasone 12 h apart [41].

Endogenous glucocorticoids, such as cortisol, are synthesised in the zona fasciculata of the adrenal cortex by the conversion of cholesterol to 21C steroid hormones [42]. Betamethasone and dexamethasone are fluorinated synthetic analogues of cortisol (Figure 2). Fluorination at the 9C position enhances the biological activity of the synthetic glucocorticoids compared with cortisol, and insertion of the 1,2 C–C double bond selectively augments glucocorticoid over mineralocorticoid activity, decreasing the rate of metabolic clearance and prolonging the biological half-life in plasma. Mineralocorticoid activity is eliminated in synthetic glucocorticoids by methylation at the 16C position, and betamethasone and dexamethasone are stereoisomers, with the 16-methyl group located in the α or β configuration (Figure 2). Thus, while betamethasone and dexamethasone have negligible mineralocorticoid activity, their glucocorticoid potency is approximately 25-fold that of cortisol [42]. Furthermore, experiments in fetal sheep have shown that the clearance half-time from plasma is extended by 6–8 h compared to cortisol [27].

Babies born preterm have a high risk of developing bronchopulmonary dysplasia (BPD); a chronic lung disease affecting both the airways and parenchyma, which is defined as the need for oxygen supplementation for babies >36 weeks of age [43]. Significant inflammation is seen in the lungs of neonates suffering from BPD, which may lead to scarring and cellular abnormalities and it is predictive of future neurological impairment and long-term respiratory dysfunction [43]. BPD is resistant to many interventions; however treatment with synthetic steroids, which have anti-inflammatory as well as maturational effects, is one possible treatment option. Use of synthetic glucocorticoids in the immediate **postnatal** period has well-known clinical benefits; most notably, facilitating weaning from mechanical ventilation, thereby reducing mortality and morbidity associated with BPD [43].

In the UK, women at risk of preterm birth, who are between 24 and 34 weeks of gestation, have a target coverage for antenatal glucocorticoid therapy of 85% [44]. Postnatal steroid use still

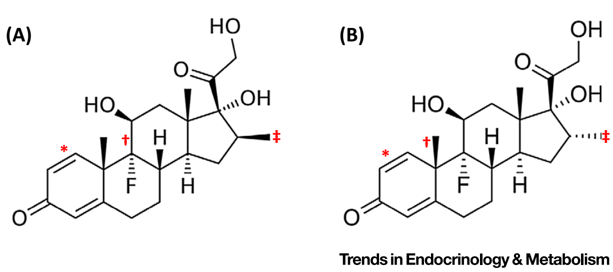


Figure 2. Chemical Structures of the Synthetic Glucocorticoids: Betamethasone and Dexamethasone. (A) Betamethasone, where * highlights the C1–C2 double bond, † highlights the fluorination of C9, and ‡ highlights the stereoisomeric methyl group. (B) Dexamethasone, where * highlights the C1–C2 double bond, † highlights the fluorination of C9, and ‡ highlights the stereoisomeric methyl group.

affects around 8% of preterm neonates, with many in this group having also been exposed to antenatal therapy. A retrospective analysis in North America estimated antenatal steroid coverage of infants also receiving neonatal steroids at 61–75% [45], meaning that many babies will have been exposed to both ante- and postnatal steroid therapy. In parallel to increasing treatment coverage, the rate of preterm birth globally does not seem to have decreased in recent years, being maintained at approximately 10%; 11.4% being reported in 2010 [44]. Clearly, with the maintained number of fetuses at risk of preterm birth and the expected coverage, it is reasonable to predict that vast numbers of infants will continue to receive ante- and/or postnatal glucocorticoid therapy.

Adverse Effects of Glucocorticoids in the Perinatal Period

Despite clear life-saving beneficial effects of ante- and postnatal glucocorticoid therapy in the preterm infant, accruing evidence derived from human clinical studies [46,47] and from experimental studies in animal models [48,49] raise serious concerns about potential adverse long-term consequences for growth and neurological and cardiovascular function in the offspring (Table 1).

Postnatal glucocorticoid treatment can stunt growth when administered in preterm human infants [50] and in animal models of preterm birth [51]. In animal models, both single and repeat doses of antenatal glucocorticoids lead to a growth restriction that persists to term [52]. This effect is not seen if the fetus is injected directly compared with maternal treatment [52]. Therefore, fetal growth restriction resulting from maternal injection with synthetic glucocorticoids may partially be related to an increase in **uteroplacental** vascular resistance. Accordingly, Jellyman and colleagues reported that human clinically relevant doses of dexamethasone administered to sheep in the last third of pregnancy led to a significant increase in uteroplacental vascular resistance measured *in vivo* directly by means of a chronically implanted transonic flow probe around one of the main uterine arteries [53]. Antenatal glucocorticoids are also associated with decreased placental weight, and alterations in placental amino acid transport [49]; further factors that affect fetal growth. There is evidence in humans for alterations to placental amino acid transport, specifically that related to the system A transporters [54]. Studies in nonhuman primates also show intrauterine growth restriction following one or multiple doses of antenatal glucocorticoid therapy [52].

Table 1. Evidence for Detrimental Effects of Perinatal Glucocorticoid Exposure[a]

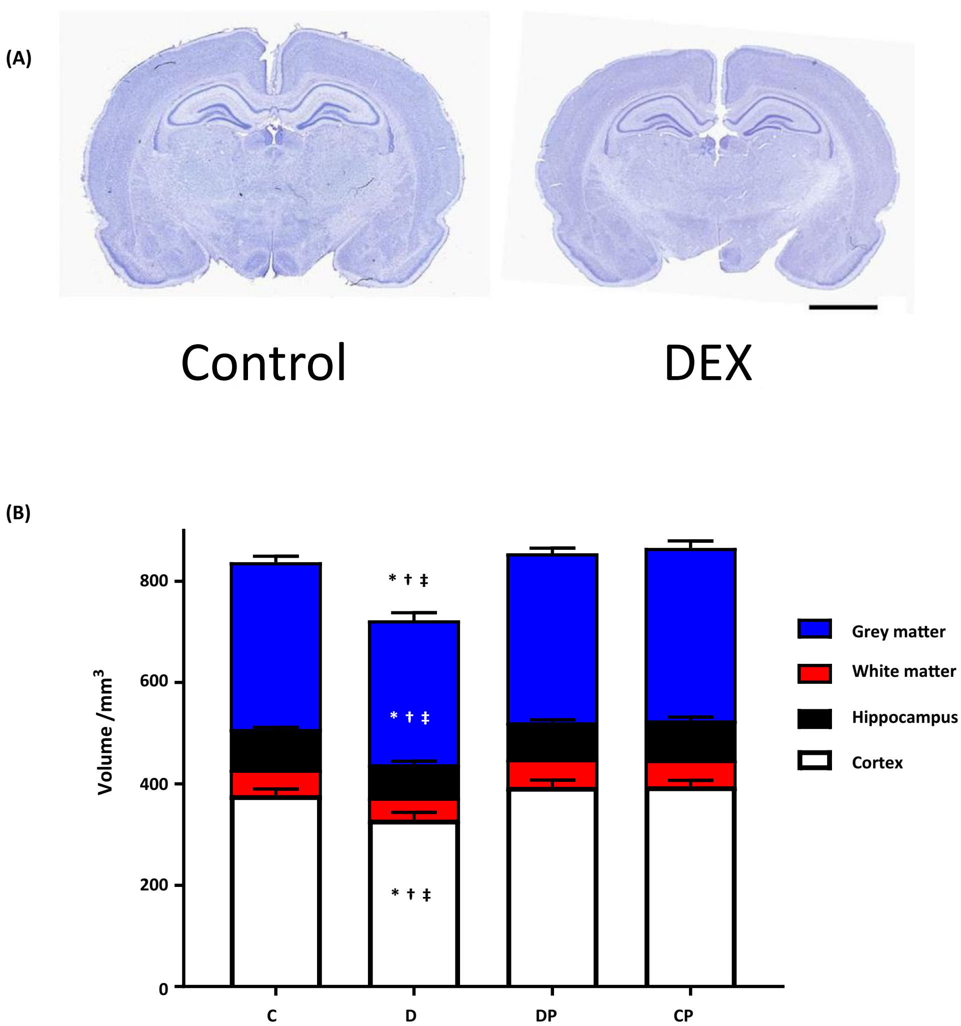
Species	Nervous system	Cardiovascular system	Renal system	Refs
Rodent	Reduced brain weight, reduced volume of cortex, hippocampus and deep grey matter, impaired motor development	Hypertension, cardiac diastolic dysfunction, impaired Starling mechanism, endothelial dysfunction	Impaired renin production, increased urine angiotensin II/creatinine levels and reduced renal 11β-HSD2 activity	Nervous system: [17,18,55,63,69,86] Cardiovascular system: [76,77,83,87,88,91] Renal system: [71,72]
Sheep	Altered HPA axis activity, decreased brain weight, increased sympathetic activity, delayed myelination, altered gap junctions	Hypertension, endothelial dysfunction, left ventricular hypertrophy	Decreased nephron number, increased glomerular volume, altered renin production, altered RAS sensitivity	Nervous system: [22,57,58] Cardiovascular system: [21,24,25,27,28,31,53,74,75] Renal system: [19,34,38,39]
Human and non-human primates	Unfavourable behaviour scores, altered HPA activity, more likely to be in lowest achievement group at school, increased stress responses	Increased aortic stiffness, increased blood pressure and altered glucose metabolism	Lower GFR at 19 years	Nervous system: [56,62,64–67] Cardiovascular system: [46,47] Renal system: [36]

[a]Key findings in different systems demonstrating detrimental effects following glucocorticoid administration in the human clinical data as well as in experimental animal models: rodents, sheep, non-human primates. Supporting references are linked to the various physiological systems.

Several animal studies investigating physiological consequences of **perinatal** glucocorticoid exposure have reported a reduction in brain weight following glucocorticoid exposure. Specifically, this appears to be associated with a decrease in the volume of the cortical and deep grey matter as well as of the hippocampal neuronal soma [55,56]. Cellular alterations include delayed myelination [57], alterations in gap junction proteins [58], and specific changes in gene expression [57]. These are worrying reports since the use of steroids in the immediate postnatal period in infants born preterm rose during the 1990s, with predominantly dexamethasone being administered [59]. However, it was soon observed that those children who had received postnatal glucocorticoids demonstrated immediate and long-term adverse effects from this treatment, including neurological impairment. In response, in 2002, the American Academy of Pediatrics released a consensus statement advising against the use of steroids for treatment of BPD [60]. The result was a sharp decrease in use of this therapy, except in the most severe cases of BPD. However, since then, the DART study has indicated no strong association with long-term adverse consequences when low-dose dexamethasone treatment was trialled [61]. Consequently, postnatal treatment with glucocorticoids in neonates born preterm continues as routine practice.

Studies have now begun to report impaired IQ and altered behaviour in young adults born preterm who were exposed to synthetic glucocorticoids during the perinatal period [62]. There are also reports of altered stress and anxiety responses in children [63,64]. This may be secondary to alterations in the HPA axis [65]. Follow-up studies have reported that adolescents between age 14 and 17 years who have been exposed to postnatal dexamethasone to have adverse motor function, impaired neuropsychological test scores, and females present in these cohorts are more likely to need special educational measures [66]. This human group also displays decreased brain volumes, specifically of the white matter, thalami, and basal ganglia, with a potential dose-dependent relationship [67], and altered HPA axis function. Evidence derived from human clinical studies also shows altered stress responses following antenatal glucocorticoid exposure; for example, an increased plasma cortisol response to stress tests in adolescents [68]. In addition, there is accumulating animal evidence for programming of the fetal hippocampus by antenatal glucocorticoid exposure, including alterations in the fetal hippocampal methylome and acetylome [69], hippocampal glucocorticoid receptor DNA binding patterns, and expression levels of both the glucocorticoid and mineralocorticoid receptors [70]. These alterations occur in a highly sex-specific manner, with a greater impact on male offspring, and have been observed to lead to functional changes in hippocampal function [69].

With regard to cardiovascular function, adult offspring, whose mothers have received antenatal steroids, have decreased **aortic distensibility** and increased aortic arch **pulse wave velocity**. Aortic stiffness in these individuals is similar to that of individuals who are a decade older [46]. In rodent studies, antenatal glucocorticoid exposure in animals born at term results in hypertension at adulthood [71,72]. However, due to the significant differences in maturation of the cardiovascular system and the longer and greater glucocorticoid exposure in these studies when compared to human clinical practice, the clinical relevance of these results has been called into question. In the sheep model of antenatal glucocorticoid exposure, in which the temporal development of the cardiovascular system is more similar to that in humans [73], there is also a resulting hypertension in adulthood in offspring born at term [74]. However, these studies have typically used an earlier window of glucocorticoid exposure than one would expect to see clinically (ranging from the equivalent of 7–8 to 22 weeks of human pregnancy). Clinical follow-up studies have also demonstrated elevated blood pressure [48,49] and impaired systolic and diastolic function in exposed individuals born preterm when compared to term-born adults. In such studies, it is clearly difficult to disentangle the partial adverse effect on blood pressure and cardiac function of being born preterm with those triggered by glucocorticoid therapy itself. Hence, the relationship between antenatal glucocorticoid exposure alone and raised blood pressure in adulthood remains unclear. Furthermore, multiple independent studies in animal models have reported endothelial dysfunction [75,76] and cardiac ventricular wall remodelling [77] resulting from perinatal glucocorticoid therapy. Importantly, these investigations have included studies in experimental animal models with human clinically relevant dosing regimens or actual studies in humans [47].



Trends in Endocrinology & Metabolism

Figure 3. Effect of Postnatal Dexamethasone on the Brain. (A) Coronal sections of control and dexamethasone-treated rat pups on postnatal day 22. Dexamethasone was injected intramuscularly into rat pups during the first 3 days of life using a human clinically relevant dose. Scale bar, 2.5 mm. (B) Total brain volume and volumes of deep grey matter, hippocampus, white matter, and cortex, at postnatal day 21 in control (n = 8), dexamethasone (n = 7), dexamethasone with pravastatin (n = 8), and control with pravastatin (n = 7) pups. Blue bar: deep grey matter; black bar: hippocampus; red bar: white matter; white bar: cortex. *$P < 0.05$ vs control; †$P < 0.05$ vs control with pravastatin; ‡$P < 0.05$ vs dexamethasone with pravastatin (analysis of variance + Student–Newman–Keuls). Redrawn, with permission, from [55].

A Way Forward?

It is clear that the evidence supporting the life-saving clinical benefits of ante- and postnatal glucocorticoid treatment of the preterm infant is overwhelming. Nevertheless, it is also clear that despite these beneficial effects, there is accumulating evidence for long-term detrimental adverse effects of ante- and postnatal glucocorticoid treatment. Therefore, there is interest in understanding the physiological mechanisms via which glucocorticoids promote these adversities in order to fine-tune current clinical therapy to maintain benefits while diminishing detrimental effects, thereby achieving the best of both worlds. This is no trivial task. Because of the pleiotropic nature of glucocorticoids in different tissues and at different gestational ages, it is difficult to isolate specific mechanistic pathways. This problem is compounded by the use of multiple experimental animal models with varying ranges of temporal developmental milestones as well as use of several doses, routes, and timings of administration. Furthermore, in humans, it is difficult to dissect the long-term detrimental effects of preterm birth compared to the effects from glucocorticoid exposure alone.

One likely mechanistic pathway leading to detrimental consequences of synthetic glucocorticoids may relate to their capacity to induce oxidative stress. Reactive oxygen species (ROS), such as the superoxide anion ($O^{\cdot}-$) perform essential cellular signalling processes in the brain

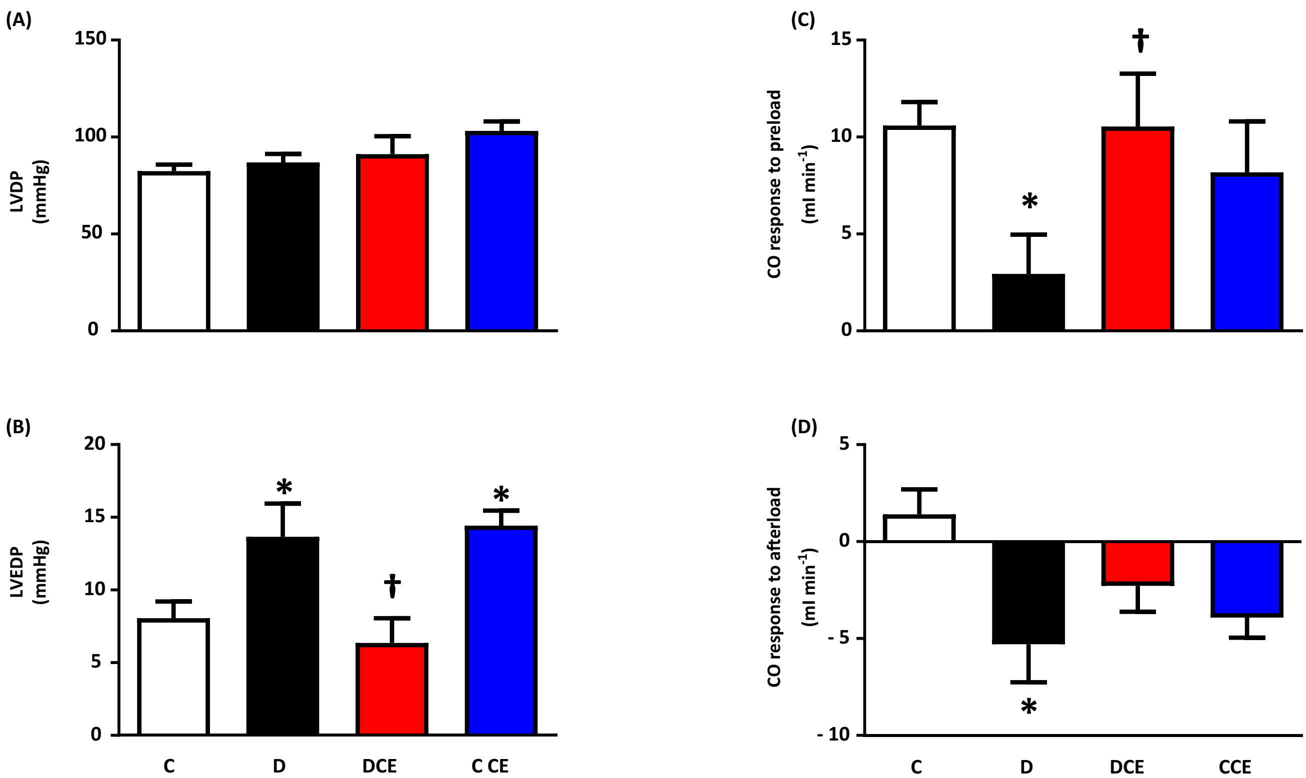


Figure 4. Effect of Postnatal Dexamethasone on the Heart. Values are mean ± standard error of the mean for (A) systolic function (LVDP), (B) diastolic function (LVEDP), (C) CO in response to preload, and (D) CO in response to afterload in control pups (n = 7), pups treated with dexamethasone (D, n = 6), pups treated with dexamethasone combined with vitamins C and E (DCE, n = 7), and control pups treated with vitamins C and E (CCE, n = 7). Significant differences ($P < 0.05$) are: * vs C; † vs D, (analysis of variance + Tukey test). Redrawn, with permission, from [88]. Abbreviations: CO, cardiac output; LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure.

and circulatory system [78]. These effects are predominantly mediated by interaction with a plethora of other cellular molecules, in a highly integrated system. ROS also contribute to vascular resistance, principally by reacting with NO and H_2S, establishing a resultant oxidant tone in the circulation [79]. Excessive ROS production or a decrease in antioxidant defences induces oxidative stress and increases vascular resistance to blood flow [79]. This vascular oxidant tone is functional in fetal life and it can be manipulated so that an increase in the $O^{\bullet}-$:NO ratio promotes vasoconstriction, while a decrease leads to vasodilatation in several vascular beds, including the umbilical circulation [80,81]. Accumulating evidence suggests that one pathway by which glucocorticoids may promote their deleterious effects is through the inappropriate generation of ROS and consequent decreased NO bioavailability. For instance, glucocorticoids are known to activate pro-oxidant systems, such as xanthine oxidase, and the antioxidant tempol reverses dexamethasone-induced hypertension in the adult rat [82,83]. Once generated, ROS may subsequently damage cellular components, affect signalling pathways, and alter the oxidant tone of the vasculature by decreasing NO availability. There is also evidence of oxidative stress in the cardiovascular system and brain in humans and in animal models of antenatal glucocorticoid exposure [57,82] and human umbilical vein endothelial cells treated with dexamethasone show an increase in hydrogen peroxide production and decreases in cellular NO, secondary to ROS production via NAD(P)H oxidase, xanthine oxidase, and the mitochondrial electron transport chain [82]. In humans, there is also evidence that glucocorticoids may cause an acute repression of the key antioxidant glutathione peroxidase 3 [84]. It is therefore possible that the adverse effects of glucocorticoids on the developing brain and cardiovascular system are secondary to the generation of oxidative stress, with a subsequent decrease in the bioavailability of NO. In a series of studies, Giussani and colleagues have tested the hypothesis that combined antioxidant and glucocorticoid therapy is safer than glucocorticoid treatment alone for the treatment of preterm birth.

Combined Glucocorticoid and Antioxidant Therapy in the Perinatal Period

The newborn rat is an established experimental model of human prematurity, as postnatal development of respiratory, cardiovascular and neuronal function in this species compares with prenatal milestones in the human [85]. A first series of studies showed that postnatal treatment of newborn rat pups with a human clinically relevant tapering course of dexamethasone induced multiple indices of increased cerebral oxidative stress and decreased total brain volume and the soma volume of neurons in the CA1 region and in the dentate gyrus of the hippocampus, when measured at weaning [86]. Furthermore, neonatal dexamethasone treatment in the rat increased cardiac oxidative stress, induced left ventricle wall thinning with aortic wall remodelling, and increased constrictor reactivity to phenylephrine and thromboxane, while it impaired endothelium-dependent vasorelaxation in the femoral circulation, when measured at weaning [76,87]. Investigation of the longer-term effects of postnatal dexamethasone treatment in the adult rat revealed lower circulating plasma NOx, and left ventricular wall hypertrophy with significant diastolic dysfunction, and these hearts failed to adapt output to increased preload or afterload, indicating a compromised cardiac Starling mechanism [88]. Combined treatment of newborn rat pups with dexamethasone and the antioxidant vitamins C and E protects against the shorter- and longer-term detrimental effects of dexamethasone on the brain, heart, and peripheral vasculature [76,86–88] (Figures 3 and 4).

Further studies have tested whether agents that are not antioxidants *per se* but are known to increase NO bioavailability could also be used to diminish the adverse effects of postnatal glucocorticoid therapy. In addition to their established cholesterol-lowering effects, statins have additional beneficial actions by increasing NO bioavailability [88]. Indeed, clinical and experimental evidence suggests that the pleiotropic effects of statins, by improving endothelial

Clinician's Corner

Synthetic glucocorticoids are routinely administered during the perinatal period in instances of preterm birth. Despite clear life-saving beneficial effects, growing evidence suggests long-term detrimental consequences for the offspring growth, brain and cardiovascular development.

If we can better understand the physiological mechanisms resulting in these adverse side effects of synthetic glucocorticoids, we could fine-tune current clinical treatment to minimise adverse effects whilst maintaining the clinical benefits.

Combined antioxidant and glucocorticoid therapy may be safer for the treatment of preterm birth and the hypothesis the Giussani laboratory proposes needs to be tested in human clinical trials.

function, might be useful for the treatment of neurological disorders, such as Parkinson's and Alzheimer's diseases, ischaemic stroke, and vascular dementia [89,90]. Recent experiments have confirmed that postnatal treatment of rat pups with dexamethasone in human clinically relevant doses decreases regional brain and hippocampal soma volumes, and reduces cortical neuronal number, while increasing the density of white matter GFAP-positive astrocytes when measured at weaning [55]. Dexamethasone combined with pravastatin treatment restored circulating NOx and prevented the adverse effects of dexamethasone on the developing brain at weaning [55]. In an elegant study by Wyrwoll and colleagues, it was also reported that pravastatin normalised placental vascular defects, fetal growth, and cardiac function in a murine model of glucocorticoid excess [91].

Collectively, these studies provide strong evidence to support that combined antioxidant and glucocorticoid therapy may be safer than glucocorticoid therapy alone for the treatment of preterm birth. Future work will determine if these beneficial effects of postnatal glucocorticoid and antioxidant therapy may also be advanced to the antenatal period (see Outstanding Questions). Since antenatal glucocorticoids are administered more or less at the point of diagnosis of preterm birth, there is a limited window for intervention. This means that the design of combined glucocorticoid and antioxidant therapy to be administered simultaneously could be of significant clinical value. More generally, these studies provide evidence of proof-of-principle supporting that it is possible to minimise the detrimental effects of glucocorticoid treatment while maintaining their clinical benefits in the perinatal period. The ultimate goal in the field is to be able to translate these findings to the human clinical setting and to move towards a combined antioxidant and glucocorticoid therapy for the safer treatment of preterm birth.

Outstanding Questions

Which mechanisms in addition to increased oxidative stress contribute to the detrimental effects on the offspring of glucocorticoid exposure during the perinatal period?

Does combined antioxidant and glucocorticoid therapy maintain beneficial effects and minimise detrimental effects on the offspring when administered antenatally?

In contrast to rodents, which are mostly altricial species, humans and sheep share similar temporal developmental milestones in terms of cerebral, cardiovascular, and pulmonary maturation. Does combined antioxidant and glucocorticoid therapy maintain beneficial effects and minimise detrimental effects on the offspring when administered in ovine models of preterm birth?

Does combined antioxidant and glucocorticoid therapy maintain beneficial effects and minimise detrimental effects on the offspring when administered in human pregnancy threatened with preterm birth?

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