Invited Review

Title

Antenatal glucocorticoids: where are we after forty years?

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Short title

Antenatal glucocorticoids

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Abstract

Since their introduction more than forty years ago, antenatal glucocorticoids have become a cornerstone in the management in preterm birth and have been responsible for substantial reductions in neonatal mortality and morbidity. Clinical trials conducted over the past decade have shown that these benefits may be increased further through administration of repeat doses of antenatal glucocorticoids in women at ongoing risk of preterm and in those undergoing elective caesarean at term. At the same time, a growing body of experimental animal evidence and observational data in humans has linked fetal over-exposure to maternal glucocorticoids with increased risk of cardiovascular, metabolic and other disorders in later life. Despite these concerns, and somewhat surprisingly, there has been little evidence to date from randomised trials of longer term harm from clinical doses of synthetic glucocorticoids. However, with wider clinical application of antenatal glucocorticoid therapy there has been greater need to consider the potential for later adverse effects. This paper reviews current evidence for the short and long-term health effects of antenatal glucocorticoids and discusses the apparent discrepancy between data from randomised clinical trials and other studies.

Key words

Antenatal glucocorticoids

Preterm birth

Cardiovascular risk factors

Developmental origins of health and disease

1. Introduction

After more than forty years since being first introduced, antenatal glucocorticoid therapy to promote fetal maturation remains one of the most important interventions for preterm birth and has been acknowledged as a "rare example of a technology that yields substantial cost savings in addition to improving health."[1] A key reason for this success is that synthetic glucocorticoids mimic the developmental maturational changes that normally occur in late gestation in response to rising fetal glucocorticoids. But it is this capacity of glucocorticoids to exert potent effects throughout gestation that makes exposure to excess maternal glucocorticoids, the transfer of which is normally tightly regulated by the placenta, an important candidate mechanism underlying known associations between an adverse fetal environment and risk of cardiometabolic and other diseases in adulthood.

Remarkably, despite the emerging body of evidence linking fetal glucocorticoid excess to permanent changes in homeostasis and organ function, there has been little evidence to date from randomised trials of harm following exposure to clinical doses of antenatal glucocorticoids. However, with wider use of glucocorticoids in an attempt to maximise neonatal benefits, including repeat doses and administration before caesarean at term, there is increased need to consider whether short term benefits could be outweighed by later adverse effects.

In this paper we outline the clinical benefits and actions of synthetic glucocorticoids, highlight areas of clinical uncertainty, review what is currently known about long-term effects of antenatal glucocortcoids, and discuss the apparent discrepancy between data from randomised clinical trials and other studies.

2. Clinical benefits of antenatal glucocorticoid treatment

The benefits of antenatal glucocorticoid treatment in women with threatened or planned preterm birth have been summarised in a Cochrane systematic review involving 21 trials (4269 infants), and include a reduced incidence of neonatal death, respiratory distress syndrome, intraventricular haemorrhage, early neonatal sepsis and necrotising enterocolitis, with numbers needed to treat to benefit of 30 or fewer (table 1).[2] These benefits were not associated with an increase in the incidence of intrauterine infection or puerperal sepsis. In these trials, efforts were made to expose fetuses to glucocorticoids for at least 48 to 72 hours,[3] though subgroup analysis showed that the incidence of neonatal death and respiratory distress syndrome were reduced even if exposure to glucocorticoids was within 24 and 48 hours of birth, respectively.[2]

Although the earliest trials were performed before many of the advances in modern neonatal intensive care, approximately a quarter of all data in the review came from trials that completed recruitment after 1990. In this subgroup, the relative and absolute benefits of treatment were at least as good as, if not better than, those for infants born in earlier decades,[2] possibly reflecting synergistic effects between antenatal glucocorticoids and other treatments such as surfactant.[4-7]

In the review, statistically significant reductions in the incidence of respiratory distress syndrome were seen only with administration of glucocorticoids before 35 weeks' gestation, though risk ratios at 35 to 36 weeks' were similar.[1] In two subsequent open-label trials, antenatal glucocorticoid treatment before caesarean at term reduced admission for respiratory distress, primarily at early-term gestation.[8,9] However, in another double-blind trial at late-preterm gestation betamethasone had no effect on the incidence of respiratory distress,

though the need for phototherapy was reduced.[10] Although selective use of antenatal glucocorticoids after 34 weeks' gestation has been recommended by some authorities,[11] there is concern that the balance of benefits and potential harms in the more mature fetus may be different, particularly given the low incidence of serious morbidity.[12,13] Indeed, a two-fold increase in teacher-reported low academic ability in children exposed to betamethasone at term suggests need for caution.[14] Further evidence from ongoing trials of glucocorticoid treatment in late gestation is awaited (NCT01222247, NCT01206946, NCT00446953, Khazardoust *et al*[15]), and long-term follow-up of these infants will be crucial in determining the overall effect of treatment.

There are insufficient data from randomised trials to evaluate effects of antenatal glucocorticoid treatment before 26 weeks' gestation, but in large cohort studies of extremely preterm infants antenatal glucocorticoid exposure has been consistently associated with significant clinical benefit, especially improved survival and a decreased incidence of intraventricular haemorrhage.[16-20] Furthermore, studies in animals and human lung explants have shown that glucocorticoid-induced pulmonary maturation occurs from the early saccular phase of lung development.[21-23]

2.1 Obstetric subgroups

Randomised trial data are available for a limited number of maternal subgroups, and have shown that antenatal glucocorticoids are effective in women with pre-clampsia and preterm prelabour rupture of membranes (PPROM), without increasing the incidence of intrauterine or neonatal infection.[2,24] Recent studies support the use of antenatal glucocorticoids in fetuses that may be affected by inflammation or infection,[25-28] though early administration appears to be important.[29]

In the Cochrane systematic review, antenatal glucocorticoids did not have a significant effect in multiple pregnancy,[2] raising concern that higher doses of glucocorticoid may be required.[30] However, this is likely to represent a type 2 error as there were few data available for this subgroup and subsequent studies have shown maternal and fetal glucocorticoid pharmacokinetics are not altered in multiple pregnancy.[31,32]

Cardiovascular responses to glucocorticoids may differ between normally grown and growth restricted fetuses, with growth restricted fetuses showing reduced rather than increased fetal and placental vascular resistance[33-35] and increased cerebral blood flow.[36-38] These observations have raised concern that glucocorticoids may disrupt fetal cardiovascular compensation for placental insufficiency[39-41] and increase cerebral oxidative stress and injury.[37,42] While randomised trials have not specifically addressed the use of antenatal glucorticoids in the presence of fetal growth restriction, key trials did include such pregnancies[3] and observational data, though limited, appear reassuring with respect to later neurological outcome.[43] One trial suggested that the glucocorticoids may be less effective in reducing respiratory distress syndrome in infants with lower birthweight percentile,[44] but in growth restricted fetal sheep, glucocorticoid-induced pulmonary maturation was similar to that of normally grown animals.[45]

Antenatal glucocorticoid therapy causes a transient increase in maternal glucose concentrations,[46-48] often more pronounced in women with diabetes,[46,49] and some[47] but not all[50] randomised trials have shown an increase in the incidence of glucose intolerance in women following glucocorticoid administration. While hyperglycaemia and hyperinsulinism can impair glucocorticoid action,[51-53] outcomes appear similar for preterm infants of mothers

with and without diabetes when there is adequate maternal glycaemic control and a high rate of antenatal glucocorticoid exposure.[54,55] Consequently, antenatal glucocorticoid therapy remains indicated in women with diabetes,[11] although increased insulin therapy may be required.[56]

2.2 Mechanism of action and pharmacology

Fetal glucocorticoids have a key role in late gestation in preparing the fetus for extra-uterine life and are important in achieving synchrony between maturation and parturition.[57-59] They induce a wide range of proteins and enzymes with morphological and functional maturational effects in most fetal tissues, especially the lung, liver, and intestine (table 2). However, this tends to occurs at the expense of ongoing cell division.[60]

Glucocorticoid action is mediated primarily by activation of the cytosolic glucocorticoid receptor with subsequent effects on transcription,[61] mRNA stability,[62] and post-translational processing.[63] The activated glucocorticoid receptor induces a limited number of genes directly via nuclear response elements within the gene promoter,[62,64-68] but for most genes transcription is induced indirectly through interactions with nuclear transcription factors that coordinate expression of multiple genes.[61] At higher concentrations, glucocorticoids may have a variety of non-genomic effects, including altered cell membrane permeability, mitochrondrial function and intracellular signalling.[69-71]

The success of glucocorticoid therapy is due in large part to the fact that clinical doses of synthetic glucocorticoids accelerate a similar sequence of coordinated organ development in the preterm fetus to that which normally occurs in late gestation in response to the rise in endogenous fetal glucocorticoids.[57,58,63] Glucocorticoid receptor expression is high in fetal

tissues from mid-gestation, especially in the lung, intestine, pituitary and thymus,[72] though glucocorticoid action may be influenced by fetal expression of the 11β-hydroxysteroid dehydrogenases (11β-HSD) that determine local glucocorticoid concentrations,[73] chromatin conformation, [61] the developmental stage of tissues,[74,75] and glucocorticoid receptor polymorphisms.[76]

The clinical benefits of glucocorticoids in preterm infants result from combined maturational effects on multiple organ systems and pathways. For example, prevention of intraventricular haemorrhage is likely due to increased circulatory stability and vascular resistance,[6,77-79] maturation of cerebral microvasculature [80,81] and improved lung function reducing the need for mechanical ventilation. Initial improvements in lung function are due to enhanced absorption of fetal lung fluid and thinning of alveolar septae, followed by increased surfactant proteins and phospholipids, the concentrations of which are not significantly altered until at least 48 hours after glucocorticoid exposure.[82-87]

Dexamethasone and betamethasone are the only parenterally administered glucocorticoids that reliably cross the placenta due to their limited affinity for placental 11β-HSD-2, which metabolises maternal cortisol into inactive cortisone.[88] Fetal serum concentrations of dexamethasone and betamethasone are approximately one-third that of maternal.[89,90] Hydrocortisone and prednisone do reach the fetus if given in sufficient amounts, but are rapidly cleared from the fetal circulation and thus have limited fetal effect.[75] Although dexamethasone and betamethasone are stereoisomers of the same fluorinated steroid, meta-analysis of several small trials suggested that dexamethasone was more effective at preventing intraventricular haemorrhage.[91] This may be due to dexamethasone having slightly greater affinity for the glucocorticoid receptor than betamethasone, the longer

duration of increased fetal glucocorticoid activity achieved with current dexamethasone dosing regimens,[75,92,93] and greater potency for non-genomic effects.[70] Results of a large clinical trial comparing the effect of dexamethasone and betamethasone for preterm birth on later neurodevelopment are awaited.[94]

One trial found that doubling the dose of betamethasone administered to women (two doses of 24 mg) did not result in any additional neonatal benefit,[95] which is not surprising given that the glucocorticoid receptor is saturated at low nanomolar concentrations of glucocorticoids.[75] The molecular action of glucocorticoids suggests that a small but sustained increase in fetal glucocorticoid activity would be sufficient to induce premature maturation. Indeed, in sheep a single maternal injection of betamethasone in a depot form (betamethasone acetate) was as effective as serial bolus dosing (betamethasone phosphate), despite considerably lower fetal plasma concentrations.[96]

2.3 Repeat doses

The use of repeat doses of antenatal glucocorticoids was originally proposed because subgroup analysis of the first and largest trial showed that infants born seven or more days after glucocorticoid treatment did not experience respiratory benefit and may have actually been at increased risk of respiratory distress syndrome.[95,97] Subsequently, nine placebo-controlled trials allowed use of weekly repeat doses of glucocorticoids but the effect on the incidence of respiratory distress syndrome and neonatal death in these trials was similar to those permitting only a single course of glucocorticoids.[2]

However, experimental animal studies have shown that repetitive maternal dosing or longer courses of glucocorticoids result in greater biochemical and structural maturation in the

preterm fetal lung than a single dose or course.[23,86,98-104] This may be partly due to the reversibility of glucocorticoid action in fetal tissues and declining effect over time.[105] However, improvements in lung function and architecture persist in the fetus for at least two to three weeks before gradually reverting to the baseline developmental state.[98,100,106,107] Overall, these studies suggest that a maximal maturational response in the preterm fetus requires repeat exposure to glucocorticoids.

More recent trials have investigated the effect of giving repeat dose(s) of glucocorticoids to women at risk of preterm birth seven or more days after an initial course of glucocorticoids. Though treatment regimens were quite variable and not all trials showed neonatal benefit, a Cochrane systematic review (10 trials, 5700 infants) found that repeat dose(s) of betamethasone were associated with a reduced incidence of respiratory distress syndrome and combined serious neonatal morbidity compared with a single course (table 3).[108] Other related benefits included decreased use of oxygen, surfactant, mechanical ventilation and inotropes, and reduced treatment for patent ductus arteriosus.[108] Most trials included women with PPROM and there was no significant increase in incidence of intrauterine infection or puerperal sepsis (table 3). Importantly, the absolute benefit of repeat dose(s) was similar to that of an initial course (numbers needed to treat to prevent respiratory distress syndrome [95% CI]: single course 12 [10, 19][2]; repeat dose[s] 17 [11, 32][108]).

In this systematic review, subgroup analysis of trials based on planned glucocorticoid dose and frequency did not identify any particular treatment regimen as being superior to another.[108] However, a meta-analysis of individual patient data, currently in progress, may help to determine optimal dose and frequency of repeat doses, and pregnancies in which

benefits are likely to be maximised.[109] For example, secondary analysis of one trial suggested that benefits were greatest when repeat doses were commenced before 29 weeks'.[110]

3. Adverse effects and long-term outcomes after antenatal glucocorticoid treatment

3.1 The glucocorticoid hypothesis of the developmental origins of disease

Fetal over-exposure to glucocorticoids is an important potential mechanism underlying the known associations between fetal growth restriction or under-nutrition and adult cardiometabolic diseases, such as diabetes, hypertension, stroke and ischaemic heart disease.[111] Key evidence includes the demonstration in animals that both fetal growth restriction and components of metabolic syndrome can be induced by administration of exogenous glucocorticoids[112-114] or by manipulations that increase placental transfer of maternal glucocorticoids, such as inhibition of placental 11-β-HSD-2.[115] Moreover, maternal protein restriction reduces placental 11-β-HSD-2 activity and induces hypertension in offspring,[116] which can be prevented by blockade of maternal corticosteroid synthesis.[117] In humans, maternal and fetal glucocorticoid concentrations are correlated with birthweight[118-121] and offspring blood pressure,[122] adiposity[123] and cortisol concentrations,[124] and may be related to risk of later cognitive impairment,[125,126] psychiatric disorders[127-131] and osteoporosis.[132]

3.2 Risk factors for cardiometabolic disease

In animals, antenatal exposure to synthetic glucocorticoids has been associated with increased risk factors for cardiometabolic disease including enhanced fat deposition,[133,134] impaired metabolism of visceral fat,[135,136] higher blood pressure, [112,137-145] and decreased insulin sensitivity,[113,114,141,146,147] which in some cases occurred without associated effects on fetal

growth.[145] In several studies, there were more marked effects on physiological function with larger or repeat doses of glucocorticoids.[114,145,148,149]

These pertubations in later cardiometabolic function may be due to permanent changes in organ structure, such as reduced nephron mass,[137,143,150-153] or altered neuro-hormonal regulation, including increased expression of peripheral glucococorticoid [113,136] and central mineralocorticoid receptors, [154,155] altered tissue activity of 11β-hydroxysteroid dehydrogenases,[140,156,157] and increased reactivity of sympathetic,[158-160] renin-angiotensin-aldosterone[139,141,144,161,162] and hypothalamic-pituitary-adrenal (HPA) systems.[138,141,145,163,164] In addition, impaired glucose tolerance may result from altered insulin signalling,[165,166] upregulation of hepatic gluconeogentic enzymes, such as phosphophenolpyruvate carboxykinase [113,145] and glucose-6-phosphate,[167] and reduced β-cell function.[114,145,168-170]

It is important to note that these findings have not been universal[135,167,171-174] and effects have often varied between sexes,[141,163,175] among species, with the stage of fetal development, and over time. In general, adverse effects on cardiometabolic function have been seen more consistently in smaller animals, possibly reflecting a longer duration of glucocorticoid exposure relative to gestation length. Nevertheless, in rodents glucose intolerance only occurred with prolonged but not short courses of antenatal dexamethasone.[113,141,148] However, rodents appear particularly susceptible to glucocorticoid-induced hypertension,[112,137,141,150,151] whereas the association is more variable in sheep[135,167] and primates,[145,173] perhaps due to the earlier onset of metanephric development in higher species.[176] In some studies, antenatal glucocorticoid exposure was associated with increased HPA axis activity in juvenile animals but decreased activity in older animals, emphasising the importance of assessments throughout the life course.[149,177]

In contrast to animal studies, long-term follow-up of adult subjects in one clinical trial found that those exposed antenatally to betamethasone compared with placebo had similar blood pressure, adiposity, blood lipids and morning cortisol concentrations.[178] Betamethasone-exposed subjects did have a slightly increased insulin response to oral glucose challenge, with some evidence of greater effect in the higher dose arm of this trial.[178] However, the clinical significance of this result is uncertain as the differences were small and fasting insulin concentrations and glucose tolerance did not differ between groups. In another trial, antenatal betamethasone exposure was actually associated with slightly lower adult systolic blood pressure.[179]

Results from human observational studies have been conflicting, with many showing no association between antenatal glucocorticoid exposure and later risk factors for cardiometabolic disease, including blood pressure,[180-182] insulin sensitivity,[180,181] cortisol concentrations,[180] peripheral arterial function,[180] blood lipids and adiposity.[180,181] However, in others there was a small increase in blood pressure,[183] slight decrease in renal clearance[181] and beta-cell function,[184] and evidence of increased aortic stiffness.[184] In addition, two studies demonstrated increased stress reactivity in term-born children exposed antenatally to glucocorticoids.[185,186]

There are currently few data from randomised trials on the long-term effects of repeat doses of antenatal glucocorticoids on cardiometabolic function, apart from blood pressure, which did not differ between children exposed to repeat doses or a single course.[187-189] Results of a detailed investigation of the effects of repeat doses on later physiological function in one trial are awaited.[190]

3.3 Growth, bone mass, and reproduction

In many animal studies antenatal glucocorticoids have been associated with a dose-related reduction in birthweight, primarily due to decreases in soft tissue and solid organ mass with less effect on skeletal size.[191] However, in primates birthweight is not invariably affected, even after prolonged exposure.[145] Several mechanisms may contribute to glucocorticoid-induced slowing of fetal growth, including altered placental function and nutrient transfer,[146,191,192] decreased DNA synthesis and cell division,[75] reduced fetal tissue water content,[193] and increased protein catabolism.[194-196] It is likely that altered expression and action of insulin-like growth factors underlie many of these changes, with an overall shift from paracrine to centrally regulated secretion.[196-201] In both small and larger animals glucocorticoid-induced fetal growth restriction was followed by rapid catch-up growth,[103,141,146,197] and did not affect adult body size, even after repeat doses.[167,173,202]

In a Cochrane systematic review, exposure to repeat doses of antenatal glucocorticoids compared with a single course was also associated with a small reduction in birth size (table 3), though anthropometric measures that adjusted for gestational age were similar between groups.[108] It is important to note that even a single course of antenatal glucocorticoids may decrease birthweight, but this effect is not seen until at least 48 hours after treatment.[2] Nevertheless, secondary analysis in one trial suggested that placental growth was reduced in a dose-dependent manner.[203] However, as in other animals, effects on human fetal growth are transitory,[204] and antenatal glucocorticoid treatment has not been associated with altered body size, in either childhood [108,187,205,206] or adulthood.[178,179]

There are few data on the long-term effects of antenatal glucocorticoids on bone development. In one study, adult female rats exposed to antenatal dexamethasone had

decreased femoral cortical thickness.[207] However, in a human randomised trial antenatal betamethasone treatment did not have any effect on bone mineral density or femoral geometry in early adulthood.[208]

Similarly, the long-term effects of antenatal glucocorticoids on reproduction have, until recently, received little attention. In female guinea pigs, antenatal exposure to repeat doses of betamethasone led to reduced fertility.[209] Several studies have also demonstrated that antenatal glucocorticoids can alter physiological function in second generation offspring, including decreased stress reactivity[210] and altered beta-cell function.[147] In humans, one trial found a trend towards delayed pubarche in boys exposed to antenatal betamethasone,[205] which was not observed in another trial,[178] though the finding in this study of increased appendicular growth relative to axial growth[208] may be suggestive of a delay in onset of puberty.

3.4 Neurodevelopment

In animal studies, exposure to antenatal glucocorticoids has been associated with reduced brain mass, [211] [164,212] delayed myelination, [213-215] decreased maturation of the retina and peripheral nerves[213-215] and impaired programmed apoptosis.[216,217] Many of these effects were dose-related and some persisted into adulthood,[167] raising concern that antenatal glucocorticoid therapy may have adverse effects on long-term neurodevelopment. However, in clinical trials a single course of antenatal glucocorticoids has not been associated with adverse effects on later cognitive and academic ability in childhood[218-221] or adulthood,[179,222] and may reduce the incidence of developmental delay and cerebral palsy (table 1).[2]

Similarly, in a Cochrane systematic review, pre-school children randomly exposed to repeat doses of antenatal betamethasone compared with those exposed to a single course of

glucocorticoids did not differ in cognitive function or incidence of neurosensory disability, including cerebral palsy, despite a possible small negative effect on head circumference at birth (4 trials, table 3).[108] Subsequently, one of the trials included in this review also found that by early school-age intellectual ability was similar between groups but there was a trend towards a decrease in the incidence of severe cerebral palsy in those exposed to repeat doses.[187] Despite several reports suggesting adverse effects on emotional regulation,[77,223] in longer term follow-up of clinical trials antenatal glucocorticoid exposure has not been associated with clinically significant disturbances in early childhood behaviour, executive function or adult psychiatric illness, even after repeat doses.[187,189,222,224] Additional outcome data at school age are awaited from another large trial.[225]

While these data are reassuring, a small observational study suggested that infants who were born at term after exposure to repeat doses of antenatal glucocorticoids may have less mature brain development.[226] Unlike preterm infants in whom potential adverse effects on brain growth may be mitigated by reduced neonatal morbidity, outcomes may be different if pregnancy continues to term. In a secondary analysis of infants born at term in one trial, those exposed to repeat doses of betamethasone compared with a single course of glucocorticoids had increased risk of sensory disability. However, very few children were affected and the impairments in vision and hearing were not severe.[187,227] Furthermore, this effect was not seen in the main trial and the potential for bias in subgroup analyses based on post-randomisation variables is well recognised.[228] It should be noted that in the first and largest trial that played such a key role in establishing the long-term safety of antenatal glucocorticoid treatment, approximately 40% of subjects were born at or after 36 weeks' gestation.[2]

3.5 Respiratory function

Glucococorticoids induce many beneficial changes in fetal lung architecture but also cause slowing of secondary septation and alveolar formation.[229-231] Although the effect on alveolarisation is reversible,[23,231-233] rats exposed to antenatal glucocorticoids had larger and fewer alveolar air spaces in adulthood,[231,234] raising concern that later lung growth may be impaired. However, a single course of glucocorticoids did not affect spirometric measures of lung volume or expiratory flow in childhood[2,205,235] and adulthood.[236] The effect of repeat doses on later lung function is currently unknown, but data from one trial are awaited.[190]

4. Discussion

The neonatal benefits of antenatal glucocorticoid therapy are now well established and are recommended for all women at <35 weeks' gestation with threatened or planned preterm birth, with few exceptions.[2] While a single course of antenatal glucocorticoids has been associated with several subtle late physiological effects, including mildly increased stimulated insulin and altered body segment proportions, these are unlikely to be of major clinical significance. Importantly, in randomised cohorts there has been no evidence of clinical side effects across a range of organ systems, including neurocognitive, cardiovascular, endocrine and respiratory function, through into early adulthood.

This is somewhat surprising given the wider animal literature in which the potential for long-term adverse effects after fetal glucocorticoid exposure has been well documented. Furthermore, in humans, as with most species, antenatal glucocorticoid treatment can have a small negative effect on fetal growth, a potential marker of altered organ development. There are several possible reasons for the apparent discrepancy in outcomes between clinical trials

and animal experimental studies. Firstly, fetal effects can vary among species due to differences in the timing of organ development and expression of glucocorticoid receptors and the 11β-hydroxysteroid dehydrogenases, which determine local glucocorticoid concentrations. Second, dosage schedules in animals have tended to be longer relative to gestation length and there have been few pharmacological studies involving measurement of fetal glucocorticoid concentrations to establish appropriate physiological doses.[96,237,238] In humans, the increase in fetal glucocorticoid activity with current clinical treatment regimens is similar to that seen in preterm infants with respiratory distress syndrome, but effective fetal doses are likely to be higher in many animal studies.[238] Thirdly, there are few comparative animal models of current neonatal intensive care practice. Finally, publication guidelines for animal studies have not, until recently, required the same methodological rigour as clinical trials, with greater potential for bias.[239]

While clinical trial data are reassuring, several observational studies have reported adverse cardiometabolic effects in adulthood. However, studies in which exposures are not randomly allocated are at greater risk of bias. This was well illustrated by an observational study that was performed in parallel with a clinical trial by the same investigators using a similar protocol; infants who were exposed to repeat doses of betamethasone in the observational study demonstrated cardiac hypertrophy whereas those in the trial did not.[240,241] Thus longitudinal study of trial cohorts is essential for reliable estimates of long-term risk.

Evidence from clinical trials has shown that there is opportunity to achieve additional neonatal benefit through extended use of antenatal glucocorticoid therapy, including administration of repeat doses in women at risk of preterm birth at <34 weeks' gestation and before elective early-term caesarean. However, given the range of dose-dependent effects that

have been observed in animal studies and the possibility that outcomes may be different at term, it cannot be assumed that long-term safety data derived from earlier trials apply equally to these newer clinical applications. Thus short-term benefits and their clinical importance need to be weighed against the uncertainty about later health outcomes.

Infants of women who are considered eligible for repeat doses continue to have high neonatal morbidity despite exposure to a single course of glucocorticoids seven or more days earlier, including an incidence of respiratory distress syndrome of 35%, severe lung disease of 13% and combined serious neonatal complications of 20%.[108] Thus, use of repeat doses to maximise fetal maturation and decrease this level of morbidity would seem justified, particularly given the relatively high absolute benefits and that there is high quality evidence showing absence of harmful effects on neurodevelopment and general health in early to mid-childhood. However, more data are needed on longer term cardiometabolic and respiratory outcomes, and the influence of different obstetric risk factors on the benefits achieved.

In contrast, only about 5% of infants born at term by elective caesarean require admission for respiratory distress, and serious morbidity and severe disease is uncommon.[8] Although preventing these admissions is desirable, the finding of lower teacher-reported academic ability in those exposed to glucocorticoids raises serious concern about whether glucocorticoid treatment in this group may actually be harmful, and long-term follow-up with psychometric testing is required before the balance of benefits and risks can be accurately defined. In addition for many women there is an equally effective alternative, namely, delaying elective caesarean until 39 weeks'.[8]

Research is ongoing into the most effective type of synthetic glucocorticoid and whether there are benefits from antenatal glucocorticoid treatment at late preterm gestations. Other areas of uncertainty include the use of different preparations of betamethasone, the minimally effective glucocorticoid dose and the optimal timing of glucocorticoid administration prior to preterm birth.

It is important that these and future questions are investigated in randomised trials powered to assess clinically relevant effects on both short and long-term outcomes, especially neurodevelopment but also cardiometabolic and respiratory effects. A recent study that showed increased risk of necrotising enterocolitis with a simple change in the timing of betamethasone administration from 24 to 12 hourly is a reminder that the effects of antenatal glucocorticoids on the fetus are complex and clinical practice must remain firmly based on evidence from clinical trials.[242]

In summary, the introduction of antenatal glucocorticoid treatment for preterm birth remains one of the most important discoveries in perinatal medicine and has been responsible for substantial reductions in neonatal mortality and morbidity. Remarkably, despite the evidence linking fetal glucocorticoid exposure with adverse long-term health outcomes, in randomised trials a single course of antenatal glucocorticoids has not been associated with clinical harm up to early adulthood. More recent evidence has shown that there is opportunity to maximise neonatal benefit through extended use of antenatal glucocorticoids, including administration of repeat doses in women at risk of preterm birth and before elective caesarean. However, the longer term effects of these newer applications are less certain and more longitudinal research is needed to determine the overall effect of treatment in these situations. More than forty years ago, the investigators of the first antenatal glucocorticoid trial concluded that “it would

be surprising if there were no scope for improved results from therapeutic regimens based on a better understanding of the mode of action of glucocorticoids…[and] better selection of patients."[3] To this we must also add the need for a better understanding of the effects of antenatal glucocorticoid therapy throughout the life-course.

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Conflicts of interest

None

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Tables

Table 1: Perinatal effects of antenatal glucocorticoids compared with placebo or no treatment in women at risk of preterm birth.

Outcome	Total infants or women	Risk ratio, fixed effects (95% CI)	Number needed to treat to benefit (95% CI)
Perinatal			
Perinatal death	3627	0.77 (0.67, 0.89)	23 (16, 48)
Neonatal death	3956	0.69 (0.58, 0.81)	22 (16, 36)
Respiratory distress syndrome			
All	4038	0.66 (0.59, 0.73)	12 (10, 15)
Moderate or severe	1686	0.55 (0.43, 0.71)	14 (11, 21)
Ventilatory support	569	0.69 (0.53, 0.90)	10 (7, 31)
Surfactant use	456	0.72 (0.51, 1.03)	NA
Bronchopulmonary dysplasia	818	0.86 (0.61, 1.22)	NA
Intraventricular haemorrhage			
All	2872	0.54 (0.43, 0.69)	21(17, 30)
Severe (grade 3 or 4)	572	0.28 (0.16, 0.50)	7 (8, 12)
Necrotising enterocolitis	1675	0.46 (0.29, 0.74)	30 (23, 29)
Early neonatal sepsis	1319	0.56 (0.38, 0.85)	27 (19, 78)
Proven neonatal sepsis	2607	0.83 (0.66, 1.04)	NA
Birthweight (g)	2588	-17 (-62, 27)	NA
Small for gestational age	378	0.96 (0.63, 1.44)	NA
Maternal chorioamnionitis	2485	0.91 (0.70, 1.18)	NA
Maternal puerperal sepsis	1003	1.35 (0.93, 1.95)	NA
Early childhood			
Developmental delay	518	0.49 (0.24, 1.00)	NA
Cerebral palsy	904	0.60 (0.34, 1.03)	NA

Adapted from meta-analysis of Roberts and Dalziel.[2] NA, not applicable; CI, confidence interval.

Table 2: Maturational effects of glucocorticoids on the fetus in late gestation.

Organ	Morphological effects	Functional effects (protein or enzyme induced)
Lung	Epithelial cytodifferentiation Thinning of alveolar septae Increased alveolar airspace Increased elastin and collagen content Maturation of alveolar capillaries	Increased tissue and alveolar surfactant (surfactant proteins A, B, C, D; fatty acid synthetase, phosphatidyl acid phosphatase, lyso PC acyl CoA acyltransferase; fibroblast pneumocyte factor) Increased antioxidant activity (superoxide dismutase, catalase, glutathione peroxidase) Enhanced clearance of fetal lung fluid (sodium-potassium ATPase subunits, epithelial sodium channel subunits) Increased glycogenolysis which provides substrate for phospholipid synthesis Increased catecholamine induced surfactant synthesis and clearance of lung fluid (beta adrenergic receptors) Reduced vascular permeability
Liver	Increased bile canaliculi	Increased glygcogen deposition (glycogen synthetase) Increased gluconeogenesis (phospho-phenolpyruvate carboxykinase, glucose-6-phosphatase) Enhanced protein and lipid metabolism (fatty acid synthetase, aminotransferases) Increased synthesis of plasma proteins (cortisol binding globulin) Induction of hepatic receptors (growth hormone, beta adrenoreceptors) Increased conversion of thyroxine (T4) to triidothyronine (T3) (5'-monodeiodinase) Decreased expression of some hormones (Insulin-like growth factor 2, angiotensinogen)
Kidney		Increased renal blood flow Increased glomerular filtration rate Increased tubular sodium reabsorption (sodium-potassium ATPase, sodium-hydrogen exchanger) Enhanced sodium regulation (increased secretion of renin)
Gut	Increased villus height and density Maturation of glands in stomach and small intestine	Increased stomach acid secretion (gastrin) Enhanced digestive activity of intestine (pancreatic amylase and trypsin; brush border hydrolases) Reduced permeability to large proteins
Pancreas		Enhanced insulin response to glucose
Adrenal		Increased adrenaline content of medulla Increased cortical response to adrenocorticotrophic hormone
Skin	Keratinisation	
Blood	Regression of lymphoid	Switch from liver to bone marrow as primary site of

	tissue in thymus and spleen	haematopoiesis
Brain	Enhanced blood-brain barrier Maturation of microvascular circulation	
Heart	Myocyte differentiation	Increased cardiac output (myocardial adenylyl cyclase, sodium-potassium ATPase alpha-isoforms) Enhanced closure of ductus arteriosus

Adapted from Ballard and Ballard,[243] Fowden and Li,[58] Grier and Halliday[244] and Liggins.[57]

Table 3: Effects of repeat dose(s) of antenatal betamethasone compared with placebo or no treatment given to women at risk of preterm birth seven or more days after an initial course of glucocorticoids.

Outcome	Total infants or women	Relative risk or mean difference, fixed effect (95% CI)	Number needed to treat to benefit (95% CI)
Perinatal			
Perinatal death	5554	0.94 (0.71, 1.23)	NA
Neonatal death	2713	0.91 (0.62, 1.34)	NA
Respiratory distress syndrome	3206	0.83 (0.75, 0.91)	17 (11, 32)
Severe lung disease*	4826	0.83 (0.72, 0.96)	NA
Ventilatory support	4918	0.84 (0.71, 0.99)	22 (13, 368)
Surfactant use	5525	0.78 (0.65, 0.95)	19 (12, 86)
Bronchopulmonary dysplasia	5393	1.06 (0.87, 1.30)	NA
Intraventricular haemorrhage			
All	3065	0.94 (0.75, 1.18)	NA
Severe IVH	4819	1.13 (0.69, 1.86)	NA
Composite serious infant outcome†	5094	0.84 (0.75, 0.94)	33 (20, 83)
Necrotising enterocolitis	5394	0.74 (0.51, 1.08)	NA
Early neonatal sepsis	1544	0.93 (0.79, 1.11)	NA
Proven neonatal sepsis	5002	1.00 (0.83, 1.20)	NA
Birthweight (g)	5626	-76 (-118, -34)	NA
Small for gestational age	3975	1.18 (0.97, 1.43)	NA
Maternal chorioamnionitis	4261	1.16 (0.92, 1.46)	NA
Maternal puerperal sepsis	3091	1.15 (0.83, 1.60)	NA
Early childhood			
Death or neurosensory disability	3164	0.99 (0.87, 1.12)	NA
Cerebral palsy	3800	1.03 (0.71, 1.50)	NA
Mental developmental index (Bayley Scales of Development II)	1162	1 (-1, 3)	NA
Score 1 to 2 SD below mean	1595	1.00 (0.83, 1.20)	
Score >2 SD below mean	3496	0.94 (0.77, 1.15)	

Adapted from meta-analysis of Crowther *et al*.[108] CI, confidence interval; NA, not applicable. *Statistically significant heterogeneity (I^2=76%); average relative risk (random effects) 0.80 (95% CI 0.56, 1.14); 95% prediction interval 0.4 to 1.56 (τ^2=0.12). †Variously defined but includes severe lung disease, chronic lung disease, severe intraventricular haemorrhage, periventricular leukomalacia, necrotising enterocolitis, retinopathy of prematurity, proven sepsis, patent ductus arteriosus requiring treatment, and perinatal death.