Synthetic Glucocorticoids: Antenatal Administration and Long-term Implications

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Abstract: A clear relationship between intrauterine development and later life predisposition to long-term disease is well established. Weight at birth provides a surrogate measure for fetal development and low birth weight predicts changes in most endocrine axes in adulthood. The exposure of the fetus to elevated levels of either endogenous or synthetic glucocorticoids, pre and periconceptional nutritional status and immediate postnatal development including catch-up growth all contribute substantially to the development of adult onset disease. Fetal exposure to high levels of glucocorticoids has direct clinical relevance. Synthetic glucocorticoids (betamethasone/dexamethasone) are administered to women at risk of preterm delivery to advance fetal maturation and reduce neonatal morbidity and mortality. However, in human pregnancy, evidence suggests that fetal exposure to synthetic glucocorticoids has detrimental effects on birth outcome, childhood cognition and long-term behavior. Studies in animals have established a link between prenatal exposure to synthetic glucocorticoids and alterations in fetal development as well as changes in placental function. These developmental alterations appear to be permanent. Whether this is the case in humans awaits long-term follow-up of children enrolled in randomized controlled trials of prenatal glucocorticoid therapy. The research challenges in this field are now centered on uncovering the mechanisms by which glucocorticoids are involved in programming the fetus for its future life, and discovering ways in which the effectiveness and safety of antenatal glucocorticoids can be enhanced. The purpose of this mini-review is to provide a background into the use of antenatal synthetic corticosteroids and to highlight and summarize recently published clinical and animal-based studies.

Key Words: Betamethasone, dexamethasone, glucocorticoids, antenatal, growth, programming, disease, fetus.

CLINICAL PHARMACOLOGY OF GLUCOCORTICOIDS

Glucocorticoids are essential for life and have a wide spectrum of effects on many organ systems. Physiologically, glucocorticoid levels rise in response to a threat in homeostatic balance. In mammals, the primary glucocorticoids are cortisol (primates, guinea pigs, sheep) and corticosterone (rodents). The major role of the hypothalamic-pituitary-adrenal (HPA) axis is to control the synthesis and secretion of glucocorticoids from the adrenal cortex and glucocorticoids in turn regulate their own release through the action of a negative feedback system. The adrenal cortex secretes cortisol in a pulsatile fashion and exhibits a diurnal circadian rhythm; normal concentrations in humans peak early in morning (8am; 100-250ug/L) and reach nadir at midnight [1].

Given their importance in regulating most organ systems, glucocorticoids have widespread application in clinical practice and are administered for a wide variety of medical conditions. Synthetic glucocorticoid compounds can function either as glucocorticoids binding to the type 2 glucocorticoid receptor (GR), or have mineralocorticoid activity binding to type 1 glucocorticoid receptors (MR). Glucocorticoids have effects on carbohydrate, lipid and protein metabolism; on body and fat mass and energy expenditure; on gastrointestinal, renal, pulmonary and cerebral function; on the HPA axis itself; and are most often used for their anti-inflammatory actions with regards to immune system function [2]. Synthetic steroids such as beclomethasone and prednisolone share the same biochemical backbone as endogenous cortisol but have modifications at carbon 9 (C9) in addition to other structural changes (added methyl/ hydroxyl groups); fluorinated synthetics such as betamethasone and dexamethasone are structurally similar but fluorination at C9 is believed to prevent oxidative metabolism [3]; see below). Depending on the medical condition, a number of different glucocorticoid drugs can be used for management. Synthetic glucocorticoids have been designed to have higher glucocorticoid potency, reduced mineralocorticoid effects and a longer duration of action. Synthetic glucocorticoid products are selected on the basis of the route of administration and the desired duration and intensity of the effect [2]. Betamethasone for example can be taken orally as a free base; intravenously or for rapid intramuscular absorption as a sodium phosphate; and for slow intramuscular absorption as a combination of sodium phosphate (fast acting and absorbing) and betamethasone acetate (slow release). Under normal physiological conditions, 80-90% of circulating endogenous cortisol is reversibly bound to a high affinity binding protein (transcortin or corticosteroid binding
globulin; CBG) and 10-20% is bound to albumin (for review see [4]). The biologically active component in the circulation is that component of free unbound cortisol. CBG is primarily produced in the liver [5-7] and is well conserved throughout vertebrates. It binds glucocorticoids with high affinity [8, 9], and is considered a cortisol transport protein that maintains a constant readily accessible pool of protein bound cortisol, in addition to regulating cortisol tissue availability and increasing the half-life of cortisol [8]. Betamethasone and dexamethasone have poor binding affinity to steroid transport proteins thereby adding to their increased potency [10].

The major site of glucocorticoid metabolism is the liver. Most cortisol is reduced to dihydrocortisol and then to tetrahydrocortisol which is conjugated to glucuronic acid. These derivatives are freely soluble and enter the circulation where they are rapidly excreted in the urine. In addition, the liver and other tissues express the enzyme 11β-hydroxysteroid dehydrogenase (11βHSD). In mammals, at least 2 isozymes of this enzyme exist to catalyze the interconversion of biologically active 11-hydroxylated corticosteroids (cortisol/corticosterone) and inactive 11-keto metabolites (cortisone/11-dehydrocorticosterone) [11-13]. The type 1 and type 2 11βHSD isozymes share 14% homology and have distinctly different physiological roles and tissue distribution. 11βHSD2 is a high affinity (nM range) NAD-dependent enzyme that primarily functions as a dehydrogenase, acting unidirectionally inactivating biologically active glucocorticoids to inactive metabolites [11-13]. 11βHSD2 is present at high levels in the placenta [14] protecting the fetus from maternally derived glucocorticoids, and is found in many other organs such as the brain, pancreas and kidney. It was first identified in the kidney. By contrast, 11βHSD1 is a low affinity (µM range), NADP(H) dependant isozyme that is bidirectional, although it acts predominantly as a reductase enzyme in vivo converting inactive cortisone to biologically active cortisol [11-13, 15, 16]. 11βHSD1 is localized primarily to the liver but it is also present in the brain, pituitary, adrenal, lung, ovary and adipose tissue [17, 18]. 11βHSD1 has also been identified in human decidua and fetal membranes where it may act locally to increase bioactive glucocorticoid concentrations to facilitate the process of parturition [19]. Betamethasone and dexamethasone are poor substrates for 11βHSD and are not oxidized by these enzymes [3]. Therefore these synthetic glucocorticoids can gain direct access to glucocorticoid receptors without significant reduction in their circulating or tissue levels due to local oxidation.

CLINICAL ADMINISTRATION OF SYNTHETIC GLUCOCORTICOIDS (CORTICOSTEROIDS): ANTENATAL ADMINISTRATION

Clinical administration of synthetic glucocorticoids has widespread application for a wide variety of medical conditions including autoimmune disease, allergic conditions, asthma, inflammatory bowel disease, and enhancement of fetal lung maturation; as in the case of threatened preterm delivery. In each case the administration of glucocorticoids can result in both desirable (i.e. immune suppression) and undesirable (changes in glucose homeostasis) effects [20]. No other clinical administration protocol for those compounds however, has become more controversial as that of administering synthetic glucocorticoids to pregnant women.

Over 30 years ago, Liggins (1969) demonstrated that lambs delivered prematurely (118-123 days of gestation where term is 150 days) after fetal infusions of adrenocorticotropic hormone (ACTH), cortisol or dexamethasone exhibited advanced alveolar stability in their lungs. He suggested that the maturational properties of glucocorticoids caused premature pulmonary development [21]. Subsequently, Liggins & Howie (1972) were the first to suggest the administration of synthetic glucocorticoids to women at risk of preterm delivery to advance fetal lung maturation and thereby reduce neonatal morbidity and mortality. In that study, women in premature labor between 24-34 weeks of pregnancy were admitted into the first controlled trial of antepartum glucocorticoid treatment for the prevention of respiratory distress syndrome (RDS) in premature infants [22]. The administration protocol consisted of an intramuscular injection of either a mixture of 6mg of betamethasone phosphate and 6mg of betamethasone acetate or a control injection, followed by a second injection 24 hours later. The choice of glucocorticoid preparation was based on products available at the time and there have been no studies optimizing dosing regimens. In premature infants, glucocorticoid treatment reduced the incidence of RDS by 50% and the incidence of neonatal death in the first 7 days of life. The maximum effects were seen if delivery occurred more than 24 hours and less than 7 days after treatment [22]. Subsequently in 1984 Bauer and colleagues reported a reduction in the incidence of necrotizing enterocolitis (NEC) and patent ductus arteriosus following antenatal corticosteroid administration [23].

The mechanisms regulating the maturational effects of glucocorticoids on lung and other organs are complex. The fetus is normally exposed to very high levels of endogenous glucocorticoids late in gestation and this exposure is essential for normal maturation and extra-uterine life. In the lung, glucocorticoids stimulate surfactant production, lung growth and structure, pulmonary growth factors, inflammatory mediators and regulate pulmonary fluid reabsorption (for review see [24]). The mechanisms that underpin the improvements in newborn lung function induced by antenatal GCs treatment after preterm birth have been explored in animal models, principally the sheep. Most of the benefit arises from structural changes in the anatomy of the lung, resulting in larger alveoli and less interstitial tissue [25]. A single injection of betamethasone given to a pregnant sheep doubles the lung volume in 48 hours [26, 27]. Surfactant production increased, but this effect was not long-lasting and was not the major contributor to improved function. Effects on the pulmonary vasculature have been studied through the analysis of endothelial nitric oxide synthase (eNOS) activity in the lung [28]. Nitric oxide contributes to the fall in pulmonary vascular resistance after birth but lung eNOS content increased only after multiple antenatal corticosteroid treatments. Further, the increase was observed at term but not preterm gestational ages, thus indicating that any effects of antenatal corticosteroids on the pulmonary vasculature are not a major component in the improved function of the preterm lung. Lung function in lambs up to four weeks postnatal age has been studied by our
research group. Our results have suggested that the profound changes in morphology induced by antenatal corticosteroids before birth are followed by unaltered function postnatally [29]. Doyle and colleagues measured lung function in a cohort of children at 14 years of age and also found that antenatal corticosteroids did not result in measurable differences in lung function during late childhood [30].

A number of studies have emerged since 1972 evaluating various aspects of antenatal glucocorticoid use. Placental metabolism of synthetic glucocorticoids such as dexamethasone and betamethasone was reported to be low or negligible (1.8% and 7.1% respectively) even before the discovery of 11βHSD2 in the placenta (Blanford 1977). Placental transfer of betamethasone was studied in a number of early reports [31-34]. After maternal intramuscular betamethasone administration, peak levels of betamethasone occurred within one hour in the maternal circulation and declined within 6 hours. In cord blood, betamethasone was detected within one hour and declined within 14 hours. Therefore, the half-life of betamethasone was presumed to be longer in the fetal circulation. Betamethasone was not detectable in infants delivered 40 hours or more after the last injection [31]. A parallel study has been recently performed using chronically catheterized fetal sheep [35]. The pharmacokinetics of betamethasone has also been recently evaluated in singleton and twin human pregnancies [36]. Not surprising, the half life of betamethasone in twin pregnancies was lower than that in singleton pregnancies due to an increase in clearance rate. It was suggested that these levels of betamethasone in twin pregnancies may be sub-therapeutic for lung maturation, although respiratory parameters were not reported [36]. This possibility however adds an additional variable into the clinical use of antenatal glucocorticoids.

Since 1972, randomized trials examining the use of antenatal synthetic glucocorticoids for the prevention of respiratory distress syndrome (RDS) and other neonatal complications have reported positive results [23, 37-39]. Crowley et al. 1995 reported that of the 15 trials in their meta-analysis all but three demonstrated a significant reduction in the incidence of RDS following antenatal glucocorticoid exposure [40]. The authors also found a 50% reduction in the incidence of intraventricular hemorrhage (IVH), a 65% reduction in NEC and an overall reduction in neonatal mortality [40]. The National Institutes of Health Consensus Developmental Conference on the Effects of Corticosteroid for Fetal Maturation was held ~25 years after the first trial by Liggins & Howie in 1972 and concluded that antenatal corticosteroid therapy for fetal lung maturation reduced neonatal mortality, RDS and IVH in preterm infants [41]. Betamethasone or dexamethasone administration was recommended by the panel in a treatment protocol of 12mg of intramuscular betamethasone for 2 doses, 24 hours apart; or 6 mg of intramuscular dexamethasone every 12 hours for 4 doses. Corticosteroids were recommended between 24-34 weeks of gestation and in a treatment window of 24 hours to 7 days prior to delivery. The potential risk of repeated doses was highlighted. The concept of a 24 hour to 7 day treatment window was and remains, contentious (for review see [42]). The widespread belief that infants born later than 7 days after treatment would not receive the beneficial effects of corticosteroids led to repeated dosing of women at continuing or recurring risk of preterm delivery.

Despite the NIH recommendations [41], the use of synthetic glucocorticoids in the management of women in threatened preterm labor was not consistent and treatment protocols were either institution-based or dependant on physicians’ attitudes. Many early reports demonstrated that corticosteroid use could vary from 0%-55% [43-45]. Treatment was highly dependant upon the attitude of the physician [42, 45]. More recent surveys evaluating clinical corticosteroid use demonstrated that a high percentage of obstetricians (>85%) prescribed repeat doses and 50% of clinicians surveyed prescribed weekly doses in cases where women have a persisting risk of preterm delivery [46, 47]. Recently, Meadow et al. 2003 reported the frequency of antenatal corticosteroid use over the years 1985, 1990, 1995 and 2000. Published reports revealed that the use of antenatal corticosteroids increased steadily from 8% (1985) to 20% (1990) to 52% (1995) and 75% (2000) [48]. The clinical use of antenatal steroid administration in the management of preterm delivery by 2001 had therefore become routine practice. But what are the possible long term effects of fetal exposure to these highly potent long-acting synthetic glucocorticoids?

Prenatal Glucocorticoid Exposure and Fetal Programming of Long Term Disease (DOHaD)

The Developmental Origins of Health and Disease (DOHaD)

Over the last decade or so, many animal models have been used to highlight the potential adverse effects of fetal exposure to elevated levels of glucocorticoids. Many of these studies used treatment protocols designed to mimic clinical practice. A general pattern of results emerged that was consistent with antenatal glucocorticoids as causing or modulating fetal programming, the so-called Developmental Origins of Health and Disease (DOHaD) hypothesis. First proposed by epidemiologists David Barker and colleagues in the mid 1980’s (“Barker Hypothesis”; [49, 50]), this concept suggested that subtle changes in the intrauterine environment that were important in determining the health and development of the fetus resulted in permanent changes in specific organ development seen much later in adulthood. The mechanistic view of an intrauterine factor mediating cellular growth and development at a vulnerable time in gestation while subsequently resulting in permanent alterations in tissues and organs is one that has included heart disease and stroke, hypertension, diabetes, reproductive function including polycystic ovarian syndrome and alterations in the onset of menarche and menopause [50-55].

In most of the epidemiological studies, intrauterine growth restriction (IUGR) has served as a surrogate for fetal well being. In the first epidemiological studies conducted by Barker and colleagues birth weight was correlated strongly to the incidence of developing hypertension, heart disease and diabetes later in adulthood.

In early studies, Barker & Osmond [49] described associations between infant mortality and the risk of death from cardiovascular disease independent of adult lifestyle, suggesting that poor intrauterine conditions increased
sustainability to ischemic heart disease. They explored the link between growth in utero and the onset of cardiovascular disease in adulthood through a series of epidemiological studies based on the birth records of men and women in the UK. Barker et al. (1989) found that men delivered at term, whose birth weights were below 5.5 pounds had the highest death rates from ischemic heart disease. An association between death from ischemic heart disease and body weight at 1 year of age was observed suggesting that the combination of poor prenatal and postnatal growth led to the highest death rates from heart disease in this population [56]. This relationship was later confirmed in women as well [57], although the association was stronger in men than in women. Hales et al. (1991) found that the risk of developing glucose intolerance and diabetes later in life was 2 fold greater among men who had low birth weights. Similar trends were observed between weight at one year of age and the subsequent development of diabetes [58]. Later, Phipps et al. (1992) showed a strong negative relationship between birth size and the incidence of impaired glucose tolerance in adult life, independent of the gestational age at delivery [59]. The relationship between reduced fetal growth and insulin resistance was confirmed by studies showing that men and women who were thin at birth, as measured by ponderal index (weight/length$^3$), were more likely to be insulin resistant [60-62]. Furthermore, prepubertal children (between 8-10 years of age) who were short exhibited significant insulin resistance during prepuberty and this insulin resistance were increased during pubertal development [63].

Many studies have reported that low birth weight is associated with a higher incidence of the metabolic syndrome X, [64-66], a series of related variables including insulin resistance, glucose intolerance, hyperinsulinemia, hypertriglyceridemia, decreased high-density lipoprotein cholesterol and hypertension [67]. It is this syndrome that has been proposed to have importance in the genesis of coronary artery disease. More recently, low birth weight has been associated with elevated fasting and stimulated cortisol concentration in adults [66, 68, 69]. In each case, cortisol levels were positively associated with high blood pressure and, in some populations, associated with glucose intolerance [66, 69]. It is important that the results of Barker and colleagues included birth weights in the normal range and were not simply a reflection of severe intrauterine growth restriction. Recent studies have emphasized the fact that fetal adaptations to subtle changes in the intrauterine environment may not result in gross abnormalities in growth, but may still predispose to subtle changes in endocrine function later in life. These may lead to the onset of disease when combined with an inappropriate postnatal and adult lifestyle.

**Fetal Exposure to Glucocorticoids and the Programming of Disease**

Glucocorticoids late in gestation provide the signals necessary for the maturation of most fetal organ systems and are imperative for the onset of parturition in most species [70, 71]. Inappropriate fetal glucocorticoid exposure could potentially disrupt the balance of HPA development and function. There are lines of evidence supporting a role for glucocorticoids in the programming of adult disease [72]. Studies in humans have shown that cord blood levels of ACTH and cortisol are increased in association with IUGR [73]. Glucocorticoids increase blood pressure in adults [74] and cortisol infusion into the fetal sheep results in elevated fetal blood pressure [75]. Prenatal stress or glucocorticoid administration has been shown in numerous studies to alter growth and HPA activity as well as glucose tolerance [76-79]. As mentioned previously, low birth weight in humans correlates with increased adult cortisol levels as well as insulin resistance and elevated blood pressure [66, 69, 80].

Given these relationships, it becomes crucial to ascertain the role of antenatal administration of synthetic glucocorticoids in fetal programming. Since synthetic glucocorticoids are administered to women threatened with preterm delivery the fetus would be exposed to high levels of potent glucocorticoids at a time in gestation when endogenous cortisol levels may be quite low. Furthermore, if preterm delivery has not occurred within 7 days, fetuses may be exposed to repeat doses. Because the mechanisms regulating the onset of preterm labor are poorly understood and preterm labor is difficult to diagnose, women who are not in preterm labor may be receiving unnecessary synthetic glucocorticoid treatment.

**Animals Models of Glucocorticoid Administration**

The improvements observed in neonatal lung function after antenatal glucocorticoid administration in humans also occur in animals. A recent review demonstrated significant improvements in lung function when compared to the effects of a single dose [81]. Some time ago, our research group developed a model to investigate the effects of maternal synthetic glucocorticoid administration on the developing fetal lung [26, 27]. In this model intramuscular injections of betamethasone (0.5mg/kg) are administered to pregnant sheep, beginning at 104 days of gestation (term is 150 days in the sheep), with repeated injections given again at 111, 118 and 125 days, mimicking clinical administration. This dose has been shown to be the minimal dose required for maximal fetal lung maturation in sheep. Ikegami et al. (1997) demonstrated dose dependant improvements in lung compliance, ventilation efficiency index and lung volumes after 2, 3 and 4 doses of maternally administered betamethasone in preterm (125 day) lambs. But improvements do not come without consequences and most animal studies, while demonstrating improvements in respiratory function, also demonstrate deleterious consequences of antenatal glucocorticoid exposure on many other organ systems. In the report by Ikegami et al. (1997), although pulmonary function was improved, birth weights decreased in a dose dependant manner [27].

**Growth Effects**

There is substantial evidence in animals demonstrating that fetal exposure to elevated levels of glucocorticoids alters fetal growth and has long term effects on cardiovascular, HPA and metabolic function. A recent review demonstrated that 9 of 11 animal studies found evidence of growth restriction following repeated doses of antenatal glucocorticoids [81]. Early studies using rhesus monkeys demonstrated that maternal intramuscular betamethasone administration at 120-133 days of gestation (term is 167 days) resulted in
significant reductions in fetal body weight of ~23% at 133 and 167 days of gestation. In addition, brain, cerebellar, pancreatic, adrenal and pituitary weights were all significantly reduced with treatment [82]. Growth restriction has also been shown in rats and rabbits treated with maternal dexamethasone [83-86]. More recently, studies in sheep and mice demonstrated that clinically relevant doses of maternal betamethasone caused significant fetal growth restriction and reductions in the weight of most organs [87-91]. In our sheep model of antenatal glucocorticoid administration, there were significant dose dependant reductions in fetal weight at 125 and 146 days of gestation [87, 89]. These alterations in whole body weight have been associated with significant reductions in whole brain and cerebellum weights in preterm and term fetal sheep [92] and this effect on brain weight persisted until 3 years of postnatal age [93]. Reductions in brain weight have important implications in the “hard-wiring” of the brain and postnatal brain function and behavior (see below).

Few studies have evaluated the mechanisms regulating fetal growth restriction following glucocorticoid administration, but the fetal insulin-like growth factor (IGF) axis is likely implicated [86, 94, 95]. IGFs, their receptors and binding proteins (IGFBPs) are regulated by glucocorticoids in fetal tissues [94-96]. Maternal IGF1 concentrations rise progressively with advancing gestation and are thought to promote placental and fetal growth. Birth weight is associated with maternal and fetal concentrations of IGF1 and inversely related to IGFBP1 [97]. Cortisol infusion in the fetal sheep significantly reduced IGF2 mRNA levels in the fetal liver and skeletal muscle by 20-55% at 145 days of gestation [95]. In the rat, maternal dexamethasone administration resulted in a 32% decrease in fetal body weight in addition to significant reductions in fetal liver and lung weights [94]. In this rat model, fetal concentrations of IGFBP1 and maternal IGFBP1 mRNA were elevated following maternal dexamethasone treatment. An increase in circulating IGFBP-1 binds circulating IGF and reduces its efficacy. Jensen et al. 2002 demonstrated that maternal cortisol infusion in the sheep reduced fetal growth rate by 35% and significantly decreased fetal IGF1 concentrations. Fetal IGF binding proteins were negatively correlated with fetal weight [98]. The regulation of fetal and placental growth by IGFs and their binding proteins has been extensively reviewed [96, 99] but the mechanisms regulating the effects of antenatal glucocorticoids on fetal growth remain poorly understood.

**Metabolic Effects**

The mechanisms linking intrauterine growth restriction and metabolic function may target specific developing organ systems such as the liver and the pancreas [78, 85, 100]. Treatment with carbenoxolone, a placental 11βHSD inhibitor in the rat, allows increased passage of maternal glucocorticoids to the fetus [101], and resulted in reduced birth weight [78], an effect similar to that observed with dexamethasone treatment [84, 85, 94]. Adult offspring had altered glucose tolerance. Maternal adrenalectomy prevented this effect, supporting the role of fetal exposure to maternally derived glucocorticoids in the programming of metabolic function [78]. Similarly, in the sheep maternal administration of dexamethasone early in pregnancy (40-41 days of gestation), resulted in elevated fetal basal and stimulated insulin levels at 135 days of gestation [102]. Our group has shown previously that as little as one dose of maternally administered betamethasone to the pregnant sheep results in insulin resistance to a glucose challenge in the offspring at 6 months and one year of postnatal age [90].

Fetal glucocorticoid exposure regulates normal pancreatic development [100, 103], although almost all evidence is derived from studies in the rat. Glucocorticoid receptors (GR) have been localised to β cells in the rat [104] and glucocorticoids have been shown to regulate insulin secretion [105]. In addition to acting directly on ducial epithelial cells and β cell proliferation [100, 103], glucocorticoids regulate transcription factors regulating pancreatic growth and remodelling, such as pancreatic duodenal homeobox-1 (Pdx-1) [106, 107]. Glucocorticoids regulate fetal IGF2, which in turn regulates growth and pancreatic apoptosis and remodelling [100, 107, 108]. The availability of glucocorticoids in the pancreas may be regulated by pancreatic 11βHSD2 and altered expression or activity of this enzyme would permit control of local tissue levels of glucocorticoids. Recently, Blondeau et al. (2001) have shown that fetal rat glucocorticoid concentrations were negatively correlated with pancreatic insulin content, and β cell mass increased when fetal steroid production was impaired [109]. Whether glucocorticoids affect pancreatic development by way of influencing the differentiation of precursor cells or affect β cells directly remain to be determined. Our group has recently demonstrated that 3 doses of maternal betamethasone result in significant alteration in fetal β cell morphology. In our model, fetuses exposed to maternal betamethasone did not demonstrate the same shift from large intra-lobular pancreatic islets to small compact islets with advancing gestation compared to control fetuses. Further, treated fetuses demonstrated changes in islet insulin and the transcription factor, pancreatic duodenal homeobox-1 (Pdx-1) protein expression ([110] and unpublished observations). Our observations suggest that maternal betamethasone administration resulted in the impairment of normal development of pancreatic islets.

Another key target for glucocorticoid programming may be the fetal liver, where glucocorticoids regulate several key enzymes involved in carbohydrate and fat metabolism. Expression of hepatic gluconeogenic enzymes (phosphoenolpyruvate carboxykinase, PEPCK) in offspring of dexamethasone treated pregnant rats was increased, an effect that persisted up to 8 months of postnatal age. These rats demonstrated a significant reduction in birthweight as well as fasting hyperglycemia and elevated glucose and insulin responses to glucose loading [85]. These observations may have important relevance in terms of hepatic insulin resistance, since transgenic mice over-expressing PEPCK exhibit increases in hepatic glucose output, increases in glucose–6 phosphatase levels, decreased insulin receptor substrate 2 (IRS-2) levels and decreased P3_1 kinase activity. We have shown previously that repeated exposure of the fetal sheep to betamethasone results in significant increases in fetal hepatic 11βHSD1 [111]. These data suggest that glucocorticoids may not only directly program metabolic enzymes, but also program intra-hepatic levels of glucocorti-
Glucocorticoids are critical for normal brain development exerting effects on neuronal growth, to cell to cell interactions and neuronal reorganization (for review see [119]). However, exposure of the developing brain to inappropriate levels of glucocorticoids at critical developmental time windows can modify both the structure and function of neuronal cells. In sheep, fetal exposure to repeated doses of maternal betamethasone results in significant reductions in fetal brain weight at 125 or 145 days of gestation [88, 92], reductions that have recently been shown to persist until 3 years of age [93]. Fetal betamethasone exposure also resulted in reduced myelination in the optic nerve and corpus callosum of fetal sheep [92, 120, 121] and a reduction in neuronal cytoskeletal microtubule associated proteins (MAPs) and synapse-associated protein, (synaptophasin) in the frontal cortex fetal baboons [122]. Cellular proliferation in the brain of neonatal rats is acutely decreased by betamethasone treatments and reductions in brain weight persist until at least 3 weeks of postnatal age. Maternal administration of dexamethasone in the rhesus monkey at 132 and 133 days of gestation (term is 165) resulted in significant alterations in the cytoarchitectural development of hippocampal neurons at 135 days of gestation [76]. Degeneration of neurons and a significant reduction in the size of the whole hippocampal formation were observed in dexamethasone treated fetuses at 135 and 162 days of gestation and those fetuses that received multiple injections showed more severe damage [76].

Given that glucocorticoids induce gross changes in brain growth and also specific alterations in neuronal development and cell death, it is not surprising that long term changes in the developing hippocampal hypothalamic pituitary axis have been observed following glucocorticoid exposure (for review see [53, 123]). The major role of the HPA axis is to control the synthesis and release of glucocorticoids from the adrenal cortex. Activation of the HPA axis causes the synthesis and release of corticotrophin-releasing hormone (CRH) and/or arginine vasopressin (AVP) from neurosecretory cells of the paraventricular nucleus (PVN) of the hypothalamus into the hypothyseal portal system to target corticotroph cells within the anterior lobe region of the pituitary gland. Here, CRH and AVP stimulate the synthesis of a polypeptide precursor pro-opiomelanocortin (POMC), which is then cleaved by processing enzymes to produce adrenocorticotropic hormone (ACTH) in addition to smaller molecular weight peptides [124, 125]. ACTH stimulates the synthesis and release of glucocorticoids from the zona fasciculata of the adrenal cortex [124]. Circulating glucocorticoid levels are maintained through the action of a negative feedback system present within the brain (hippocampus and hypothalamus) and pituitary via GR [126]. In several species, normal fetal HPA axis function is essential for growth, development and for the onset of birth [70]. Glucocorticoids in general promote tissue and organ maturation at the expense of proliferation, and are therefore responsible for the maturational changes of a variety of organ systems preparing the fetus for extrauterine life [70, 127]. Most of these changes can be induced prematurely by exogenous glucocorticoid administration [70, 127]. In all species studied, there is an increase in circulating glucocorticoid concentrations in the fetus towards term, although the timing and the magnitude of this increase may vary [127] and it is this increase in circulating fetal cortisol...
concentrations that provides the stimulus for organ maturation and the trigger for parturition [70, 128].

The hippocampus exerts an inhibitory influence on basal, circadian and stress-induced HPA activity [129]. Central corticosteroid receptors in the hippocampus are thought to play a critical role in the regulation of HPA activity [130-132]. Two corticosteroid receptors are present in the hippocampus: MR, identical to the kidney MR; and the classic GR. In most species, the hippocampus exhibits the highest levels of corticosteroid receptors of any brain region [129, 131] and is one of the few brain regions to express both MR and GR [133]. Under most circumstances MR are thought to regulate basal or circadian trough levels of ACTH and cortisol. GR mediate the effects of circadian peak or stress-induced increases in HPA activity [129, 133]. Alterations in MR and GR expression therefore influence basal and stress induced increases in HPA activity.

In many animal models, programming of the HPA axis has been associated with alterations in hippocampal corticosteroid receptor populations [84, 134, 135]. Negative feedback at the level of the hippocampus results in an inhibition of HPA activity, therefore reduced glucocorticoid feedback through alterations in receptor number would elevate HPA activity [129]. HPA hyperactivity has been demonstrated following prenatal undernutrition [136], prenatal stress [77, 137] and maternal synthetic glucocorticoid administration [84, 135]. In the rat, maternal dexamethasone administration (1000 µg/kg/day) over the last few days of pregnancy resulted in a reduction in birthweight and offspring demonstrated significantly elevated levels of basal corticosterone and blood pressure and a significant decrease in GR and MR mRNA levels in specific hippocampal subfields [84]. Maternal administration of dexamethasone in the rhesus monkey at 132 and 133 days of gestation (term=165) resulted in significant alterations in the cytoarchitectural development of hippocampal neurons at 135 days of gestation [76] and at 10 months of postnatal age. Dexamethasone treated offspring also demonstrated higher basal cortisol levels and higher plasma cortisol levels following stress [135]. Maternal dexamethasone treatment in the guinea pig resulted in significant changes in fetal basal cortisol and alterations in MR and GR mRNA in the hippocampus [134].

Since fetal glucocorticoid exposure can alter the expression of GR, synthetic glucocorticoids can potentially impact at every level of the axis including the hypothalamus, pituitary and/or the adrenal. In the fetal sheep, maternal administration of dexamethasone early in gestation (40-41 days) resulted in significant elevations in basal ACTH and cortisol levels in fetuses at 131 days of gestation, in addition to elevated ACTH responses to an exogenous CRH challenge [102]. Nevertheless, our understanding of the mechanisms remains superficial. Benediktsson et al. (1993) demonstrated that dexamethasone treatment in pregnant rats resulted in offspring that demonstrated a significant reduction in birth weight and significant elevations in systolic blood pressure [138]. Bakker et al. (1995) showed that administration of maternal dexamethasone on day 17 and 19 of pregnancy in the rat significantly decreased birth weight, did not alter basal HPA function but significantly decreased the ratio of CRH to AVP in the hypothalamus of offspring at 20 days postnatal age [83]. This may reflect subtle long-term effects on HPA regulation at the level of the hypothalamus. Our group has previously shown that in the ovine fetus maternal betamethasone administration results in HPA hyperactivity before birth [89] without associated changes in fetal hypothalamic and pituitary neuropeptide expression. We have also demonstrated that offspring of pregnant sheep treated with maternal betamethasone show HPA hyperactivity in early adulthood [139], but later in life the adrenal appears incapable of sustaining cortisol output and relative adrenal insufficiency develops [140]. In these animals we observed a dose dependant increase in basal ACTH levels in adulthood, associated with significant reductions in basal cortisol levels. These alterations in HPA function were most pronounced in offspring that were exposed to multiple doses [140].

Programming of HPA axis function may also be regulated through the serotonergic system. Nuclei within the hypothalamus that secrete CRH receive input from the forebrain through serotonergic inputs that are regulated by serotonin receptors 5-HT1A and 5-HT2A (for review see [141-143]) and alterations in serotonin receptors and transporters have been linked to hippocampal glucocorticoid receptor programming [144, 145].

**Placental Effects**

The mechanisms by which prenatal glucocorticoid exposure programs placental endocrine function is not entirely clear. Previous studies have shown that prenatal stress alters maternal HPA function, including changes in ACTH, cortisol and catecholamines. Placental 11βHSD2 acts as a dehydrogenase enzyme, rapidly converting active glucocorticoids to inactive metabolites [13, 146] and represents a barrier reducing fetal exposure to elevated levels of maternally derived glucocorticoids [147]. As a result, placental 11βHSD2 plays a pivotal role in fetal programming through its regulation of fetal exposure to endogenous glucocorticoids. In rats, reduced placental 11βHSD2 activity is associated with increased blood pressure in adult offspring [146], substantiating the role that glucocorticoids play in programming adult disease and supporting the importance of the placenta in regulating fetal adaptations. Treatment of pregnant rats with carbenoxolone, a potent inhibitor of 11βHSD2, results in significant reductions in birth weight, and significantly higher fasting basal glucose levels, elevated insulin responses to a glucose challenge and elevated basal corticosterone levels [78, 148, 149]. These results were abolished by maternal adrenalectomy [78]. Evidence from animal studies exists to suggest that synthetic glucocorticoid administration decreases placental 11βHSD2 expression and results in a reduction in fetal weight [150]. Evidence exists in humans that decreased expression of placental 11βHSD2 is associated with intrauterine growth restriction [151, 152]. Kajantie et al. (2003) demonstrated that relative birth weight in small preterm infants is correlated with placental 11βHSD2 activity and infants with increased umbilical artery resistance had lower total placental 11βHSD2 activity [153]. These observations suggest that after synthetic glucocorticoid administration the fetus may not only be
exposed to exogenous glucocorticoids, but also may be chronically exposed to increased levels of maternally derived endogenous glucocorticoids. This chronic exposure therefore may have important implications in the long-term resetting of the fetal HPA axis persisting into adulthood and influencing the offspring’s capability of maintaining homeostasis and stress responsiveness.

Glucocorticoids regulate the onset of parturition in most species and therefore the effects of antenatal glucocorticoid administration on the activation of factors mediating parturition should not be ignored. Placental derived prostaglandin E_2 (PGE_2) has been shown to play a role in the activation of fetal HPA function and is central in the initiation of parturition (for review see [154]). Fetal plasma PGE_2 concentrations rise progressively in late gestation with a time course that is similar to that seen in fetal plasma cortisol [155]. Infusion of PGE_2 into catheterized fetal sheep resulted in a significant elevation in circulating ACTH and cortisol concentrations [156, 157]. At term cortisol can act directly on placental prostaglandin H_2 synthase type 2 (PGHS2) to further increase PGE_2 output [158]. Placental PGE_2 may represent a unique positive feed-forward mechanism whereby an increase in fetal glucocorticoids stimulates placental prostaglandin (PG) production and PGs further stimulate an increase in fetal HPA activity [159]. Glucocorticoids can also act on prostaglandin metabolising enzymes (15-OH prostaglandin dehydrogenase; PGDH) to alter local levels of PGs. Yeshaya et al. (1996) reported increased uterine activity after betamethasone administration in women between 26-34 weeks' gestation without the initiation of labor [160]. Based on underlying principles from other species it should be expected that glucocorticoids could potentially increase the incidence of preterm labour. However the randomized controlled trial evidence both individually and collectively, provide compelling evidence that the gestational age at birth is not significantly altered by glucocorticoid treatment. This does not however explain the mechanisms by which glucocorticoid exposure could alter placental function.

**Effect of Fetal Glucocorticoid Exposure: Human Studies**

Whether antenatal glucocorticoids have long term effects on human endocrine function is a question that cannot be answered until the results of large randomized controlled trials evaluating antenatal corticosteroid use are available and follow-up of the offspring is completed. Until then, evidence exists that antenatal administration of synthetic glucocorticoids both significantly decrease [161, 162] or have no effect [163, 164] on fetal growth. Modi et al. 2001 using magnetic resonance imaging demonstrated that infants exposed to 3-11 courses of prenatal glucocorticoids exhibited lower whole cortex convolution indices and a smaller surface area [165]. French et al. (1999) have reported a decrease in head circumference with exposure to increased number of antenatal glucocorticoid courses [161]. Alterations in brain growth have important implications for the “hard-wiring” of the brain and given the importance of the limbic system (hippocampus) in subsequent cognition, memory and behavior, brain function is certainly highly susceptible to programming. A recent report has demonstrated that individuals who had larger head circumference as adults gained significantly higher scores on intelligence tests and were less likely to show a decline in memory performance with age [166]. Further, small head circumference at birth and head circumference growth velocity during the first year of life have been strongly associated with learning difficulties and cognition in school aged children [167]. Nilsson et al. (2001) demonstrated that men with lower birth weight and a small head circumference at birth scored poorly on psychological assessment surveys compared to their heavier counterparts. It was suggested that impaired fetal growth was predictive of suboptimal psychological functioning and increased stress susceptibility [168]. French et al. (2004) have recently evaluated a subset of children in their original study (1999) and demonstrated that children exposed to 3 or more courses of antenatal glucocorticoids exhibited significantly higher relative risks of externalizing behavioral disorder and distractibility at 3 and 6 years of age, without any changes in intelligence quotient [169]. Stress (high glucocorticoid levels) has been associated with changes in memory and behavior and disorders such as depression, anxiety, chronic fatigue syndrome and schizophrenia (for review see [143, 170, 171]). Further, stress and anxiety during pregnancy have recently been reported to be associated with alterations in fetal growth and neonatal behavior. Fetuses of high anxiety women were noted to be more active and to experience growth delays. Newborns of high anxiety mothers spent more time in deep sleep and less time in quiet and active alert states and showed more state changes and less optimal performance on the Brazelton Neonatal Behavior Assessment Scale (motor maturity, autonomic stability and withdrawal) [172]. Jackson et al. (2003) have recently reported that 2 doses of maternal betamethasone significantly decreased biophysical profile scores in 28% of their study population and 44% of the patients reported a decrease in fetal movement [173]. The overall effects of antenatal glucocorticoids administration in humans however are still controversial; other studies have reported that antenatal glucocorticoid therapy is not associated with adverse effects on growth or on sensori-neural and cognitive function in adolescence and early adulthood [30, 174].

Although most human studies have shown significant neonatal benefits from antenatal treatment with synthetic glucocorticoids in terms of improvement in the incidence of RDS, IVH and NEC, knowledge of the effects of repeated antenatal doses of long acting synthetic glucocorticoids on the function of important fetal endocrine systems (such as HPA axis function) is still lacking. The principal product of the fetal adrenal gland in humans is dehydroepiandrosterone sulfate (DHEAS) which is hydroxylated in the fetal liver (16-OH DHEAS) and forms the primary substrate for aromatization to estriol in the placenta. Fetal DHEAS and maternal estriol concentrations late in gestation are therefore good indicators of fetal adrenal function. Hendershott and colleagues (1999) administered weekly betamethasone treatments between 24 and 32 weeks of gestation to women at high risk for preterm delivery and collected salivary samples before and 1 to 2 days after each betamethasone dose. In this study women were administered treatments until 32-34 weeks of gestation or until delivery. The authors
demonstrated a mean decrease in maternal salivary estriol of 23% from pre-treatment to post-treatment values with each dose of betamethasone. In the 7 days between treatments, maternal estriol levels recovered to the previous value but among patients who received greater than 6 doses there was a tendency for the estriol levels not to recover [175]. In a prospective cohort study Parilla and colleagues (1999) demonstrated no significant long-term suppression in fetal steroidogenesis (fetal DHEAS levels) with multiple doses of betamethasone. Although these studies indicated no dramatic effects in fetal steroidogenesis, the studies were small (n=10; [175] and n=34 [176]) and did not address the question of long-term postnatal adrenal function. As stated earlier in this review, animal studies have demonstrated that the fetal HPA axis is very vulnerable to changes in glucocorticoid levels during gestation. Given that glucocorticoid receptors are present at every level of the HPA axis, it remains possible that synthetic glucocorticoid administration subtly alters fetal HPA axis development and/or negative feedback function in humans.

 Fetuses exposed to excess glucocorticoids are growth restricted [177], and growth restricted babies have elevated levels of cord plasma corticotropic releasing hormone (CRH), adrenocorticotropic (ACTH) and cortisol [73, 178]. Increases in urinary glucocorticoid metabolites in children 9 years of age were associated with reduced birth weight [179] and twelve-year-old children born small for gestational age had increased DHEAS and catecholamine levels [180]. Epidemiological studies have established a strong association between circulating cortisol levels and the incidence of hypertension and diabetes. Phillips et al. (1998) have shown that fasting plasma cortisol levels in men aged 64 years were inversely related to birth weight, independent body mass index, and that elevated cortisol levels were significantly associated with higher blood pressure, plasma glucose levels, fasting triglyceride levels and insulin resistance [80]. This observation has recently been demonstrated in children as well, suggesting that the relationship is not just a function of adult pathology [181]. Low birth weight has been associated with elevated fasting and stimulated cortisol concentrations in adults [66, 68, 69] and also with significant changes in sympathetic-adrenomedullary system activity and behavior in school aged children [182]. Cortisol levels were positively associated with high blood pressure and in some populations, associated with glucose intolerance [66, 69]. These observations support a role for altered HPA axis function in the predisposition of adult disease. The long-term specific effects of synthetic glucocorticoid administration on HPA axis development and adrenal function in humans will not be addressed however, until the results of large randomized controlled trials become available.

**CURRENT PRACTICE AND THE FUTURE OF ANТЕNATAL CORTICOSTEROID USE**

Given the overwhelming amount of evidence from animal experiments and the increasing number of human reports suggesting that excessive fetal exposure to elevated glucocorticoids has serious long-term effects on the risk of developing postnatal disorders, the practice of administering synthetic glucocorticoids in humans remains extremely controversial. In animal models, multiple doses of antenatal glucocorticoids significantly improved lung function compared to single doses [27] but not without serious adverse consequences [88, 90, 92, 135, 139, 183]. Limited data are available however for a full evaluation of the use of multiple versus single doses in humans. Many reports have suggested that weekly doses of glucocorticoids may not be advisable [184-186]. In 2001, Guinn et al. published the first multicentre trial evaluating the safety of single versus multiple courses of antenatal glucocorticoids and reported little benefit and the potential for adverse effects from repeated courses of antenatal glucocorticoids [187]. In 2001 the National Institutes of Health Consensus development panel revisited the issue of repeated doses of antenatal glucocorticoid administration and published a revised statement [188]. The panel concluded that data from available studies were inadequate to argue for or against the use of repeat or rescue courses of antenatal glucocorticoids but recommended that all pregnant women between 24-34 weeks of gestation at risk of preterm delivery within 7 days should be considered candidates for a single course of antenatal glucocorticoids [188]. The recommended treatment regimen was the same as that recommended previously by the NIH in 1994 [41]. The panel recommended that repeat or rescue courses be reserved for those women currently enrolled in randomized controlled trials and should not be administered routinely.

Since the new recommendations from the NIH consensus panel in 2001, some reports have published meta-analyses assessing the safety of single versus multiple doses of antenatal glucocorticoid to women threatened with preterm delivery. Most reports suggest that there is insufficient evidence on the benefits and risks of repeat doses and recommend single doses to be administered only ([189, 190], for reviews see [191, 192]). Clearly the results of large randomized controlled trials are needed to fully assess the safety of antenatal corticosteroid use. Large randomized controlled trials (RCT) are currently underway in Canada, Australia, the United States and the United Kingdom. Most studies have been initiated or are in the planning phase and results will not become available for quite some time. In each RCT, the intervention includes repeating the courses of antenatal glucocorticoids at 7-14 day intervals with long term follow-up of up to 2 years in some trials. The sample sizes vary from 980-4000 women and the outcome measures include death or adverse neonatal outcome, lung disease, growth, neurodevelopment and overall cost (for details of the Canadian study and other RCTs see http://www.utoronto.ca/miru/macs/). Unfortunately, there are likely to be problems conducting these trials effectively. Aghajafari et al. 2002 recently published the results of a study evaluating multiple versus single courses of antenatal corticosteroids. In this study the authors demonstrated that 85% of the women who met the criteria refused to participate in the trial and of those women who had refused 21% of them had physicians who did not allow them to participate [193]. Clearly, these trials need to be supported by clinical staff and their patients in order for the trials to be successful.

**CONCLUDING REMARKS**

Although nearly 30 years have passed since Liggins and Howie first published their results on the use of antenatal glucocorticoids for women at risk of preterm delivery, the
medical and scientific communities have only limited understanding of the long-term effects of this treatment protocol. Animal models have begun to unravel some of the mechanisms regulating antenatal glucocorticoids exposure on fetal, neonatal and adult outcomes. These models have provided and continue to provide important insight into the potential harm that fetal exposure to synthetic glucocorticoids may have on the health of the human population. The time has come to seriously evaluate the effectiveness and safety of administering synthetic glucocorticoids to women at risk of preterm delivery. Research needs to be accompanied by a greater awareness amongst practitioners of the need for critical clinical judgment in identifying those pregnancies truly at risk of preterm delivery. The evolution of the clinical use of glucocorticoids in pregnancy is an outstanding example of the improved health care outcomes resulting from basic laboratory and animal research. It is clear that ongoing refinements are required and these will result from successful partnership between laboratory and clinical scientists.

There remains the possibility that in some women presenting with preterm labor, that preterm delivery is not eminent. Given our current lack of understanding of the labor process we are unable to identify these women and set them apart from those who will in fact deliver early. Not only does our understanding of the effects of glucocorticoids on long-term outcome need to develop but equally our understanding of the process of parturition need to advance to a point where we are able to identify and diagnose preterm delivery. The continuation of longitudinal animal studies as well as the results of large RCTs currently underway will, in the future, provide invaluable information regarding the safety of antenatal glucocorticoid use and its effects on long-term health.

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