Early-Life Glucocorticoid Exposure: The Hypothalamic-Pituitary-Adrenal Axis, Placental Function, and Long-term Disease Risk

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An adverse early-life environment is associated with long-term disease consequences. Adversity early in life is hypothesized to elicit developmental adaptations that serve to improve fetal and postnatal survival and prepare the organism for a particular range of postnatal environments. These processes, although adaptive in their nature, may later prove to be maladaptive or disadvantageous if the prenatal and postnatal environments are widely discrepant. The exposure of the fetus to elevated levels of either endogenous or synthetic glucocorticoids is one model of early-life adversity that contributes substantially to the propensity of developing disease. Moreover, early-life glucocorticoid exposure has direct clinical relevance because synthetic glucocorticoids are routinely used in the management of women at risk of early preterm birth. In this regard, reports of adverse events in human newborns have raised concerns about the safety of glucocorticoid treatment; synthetic glucocorticoids have detrimental effects on fetal growth and development, childhood cognition, and long-term behavioral outcomes. Experimental evidence supports a link between prenatal exposure to synthetic glucocorticoids and alterations in fetal development and changes in placental function, and many of these alterations appear to be permanent. Because the placenta is the conduit between the maternal and fetal environments, it is likely that placental function plays a key role in mediating effects of fetal glucocorticoid exposure on hypothalamic-pituitary-adrenal axis development and long-term disease risk. Here we review recent insights into how the placenta responds to changes in the intrauterine glucocorticoid environment and discuss possible mechanisms by which the placenta mediates fetal hypothalamic-pituitary-adrenal development, metabolism, cardiovascular function, and reproduction. (*Endocrine Reviews* 34: 885–916, 2013)

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Abbreviations: ABC, ATP binding cassette; AMPK, AMP-activated protein kinase; BCRP, breast cancer resistance protein; BNC, binucleate cell; BP, blood pressure; eNOS, endothelial NOS; GLUT, glucose transporter; GR, glucocorticoid receptor; HPA, hypothalamic-pituitary-adrenal; 11βHSD2, 11β-hydroxysteroid dehydrogenase type 2; IGFBP, IGF binding protein; IUGR, intrauterine growth restriction; LBW, low birth weight; MRP, multidrug-resistance-associated protein; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; NOS, nitric oxide synthase; oPL, ovine PL; P-gp, P-glycoprotein; PL, placental lactogen; POMC, proopiomelanocortin; RDS, respiratory distress syndrome; SGA, small for gestational age; VEGF, vascular endothelial growth factor.

I. Introduction

A clear relationship exists between events occurring during intrauterine development and later-life predisposition to long-term disease. Early-life adversity has been shown to alter the structure and function of distinct cells and organ systems; it has been shown to change the set point of homoeostatic pathways that together increase the risk of developing noncommunicable diseases including obesity and type 2 diabetes (1–4). Experimental studies have shown that fetal development can be modified either as an adjustment in response to adversity, including hypoxemia and nutritional compromise, or as a result of fetal exposure to excess glucocorticoids (5–11). These adaptations serve to prepare the fetus for the anticipated postnatal environment. In cases where the prediction and the actual environment do not match, these developmental modifications may be maladaptive. Therefore, it is hypothesized that early-life factors alter the capacity of the postnatal organism to react to its immediate environment. The period in which the adverse environment may have an impact on the developing organism appears to extend from conception until the neonatal period. Although there have been recent attempts to distinguish the fetal from the neonatal period, in this review, we consider both of these developmental periods to be part of a continuum of the vulnerable phase in which developmental trajectories can be set.

Intrauterine exposure to high levels of glucocorticoids during plastic developmental time windows has direct clinical relevance; synthetic glucocorticoids (betamethasone and dexamethasone) are routinely used in obstetrical practice in the management of women at risk of early preterm birth (Table 1), in treatment of asthma bronchiale, and in suspected cases of congenital adrenal hyperplasia (13–21). Adverse medical events in human newborns have raised concerns about the safety of repetitive glucocorticoid treatment; clinical data exist showing that fetal exposure to synthetic glucocorticoids is associated with detrimental effects on birth outcome, childhood cognition, and long-term behavior (Table 2) (11, 22–24). A recently published international collaborative placebo-controlled trial in 1858 women demonstrated, that compared with placebo, neonates in the repeated antenatal corticosteroids group, after controlling for gestational age at birth and confounding factors such as smoking and neonatal sex were born smaller (−33.5 g; 95% confidence interval, −66.3 to −0.7) with decreased body length (−0.34 cm; 95% confidence interval, −0.62 to −0.06) and decreased head circumference (−0.30 cm; 95% confidence interval, −0.46 to −0.13) in a dose-dependent manner (25). Experimental evidence supports a link between prenatal exposure to synthetic glucocorticoids and alterations in fetal endocrine development as well as changes in placental function, alterations that were thought to be permanent but now may be regarded as potentially reversible. Equally, transgenerational effects may also be evident (26), although human studies are not yet available.

Table 1. Generalization of the Effects of Antenatal Glucocorticoid Treatment in Preterm Gestation[a]

Advantages	Refs.
↓ Mortality	16, 17
↓ Morbidity	16, 17
↓ RDS	16, 24, 96–99, 395–398
↓ Intraventricular hemorrhage	399
↓ Periventricular leukomalacia	400
↓ Neonatal infections	17
↓ Necrotizing enterocolitis	88, 401, 402
↓ Need for respiratory support	17
↓ Disability or handicap	403
↓ Cerebral palsy	101
↑ ? ↓ ? Intelligence quotient	404

[a] The table summarizes some of the effects of antenatal glucocorticoid treatment in humans. The references contain an overview on original articles as well as review articles. Arrows indicate decrease (↓) or increase (↑).

The effectiveness and safety of antenatal glucocorticoids can be improved and should be the subject of long-term studies. The research challenges in this field are to determine the mechanisms underpinning the association between fetal glucocorticoid-induced physiological dysfunction and long-term disease risk. Experimental studies suggest that alterations in the fetal hypothalamic-pituitary-adrenal (HPA) axis may be one pathway linking early-life adversity with adult metabolic disease (12, 27–30). Because the placenta is the conduit between the maternal and fetal environments, it is not surprising that placental function plays a key role in mediating responses to fetal glucocorticoid exposure and is likely implicated in the disease process. Here, we review how the placenta responds to changes in the maternal glucocorticoid environment and discuss possible mechanisms by which the placenta mediates fetal HPA development, metabolism, cardiovascular function, reproduction, and long-term disease risk (Figure 1). We provide an overview of possible mechanisms of early-life programming, highlight the role of the fetal HPA axis and the placenta, and summarize recently published clinical and animal-based studies.

Table 2. Associated Effects of Antenatal Glucocorticoid Therapy in Animal Models and Human[a]

	Refs.
Animal models	
↓ Fetal growth (birth weight, head circumference, body length, etc)	39, 130–135, 138, 139, 165, 170, 236, 405, 406
↓ Placenta weight	43, 170, 231–241, 412
↑ Impairment of HPA axis	30, 39, 41, 42, 68, 69, 71, 131, 140, 143, 146, 158–164, 414
↓ Locomotion, motivation, cognition	30, 124
↓ Myelination	68, 140–142, 158, 163
↑ Metabolic impairment (adiposity, hyperinsulinemia, hyperglycemia etc.)	29, 42, 69, 143
↑ Cardiovascular (hypertension)	67, 102, 131, 143, 418
↓ Fecundity	159
Humans	
↓ Fetal growth (birth weight, head circumference, body length, etc)	17, 23–25, 107, 118–122, 260, 407–411 and Braun T, Sloboda DM, Tutschek B, et al (unpublished data, 2013)
↓ Placenta width	413
↑ Impairment of HPA	12, 27–29, 56, 70, 103, 145, 146, 148–157, 415, 416
↑ Neuropsychiatric and behavioral changes	56, 101, 123, 125
↑ Metabolic impairment (adiposity, hyperinsulinemia, hyperglycemia, etc)	107, 417
↑ Cardiovascular (hypertension)	5, 56, 102, 112, 129, 180, 419
↑ Fetal heart rate variation	223
↓ Renal function (glomerular filtration rate)	111

[a] The table summarizes some of the associated effects of antenatal glucocorticoid treatment in human and animal models on fetal growth and development and metabolic, neurodevelopmental, and cardiovascular changes. The references contain an overview of original articles as well as review articles. Arrows indicate decrease (↓) or increase (↑).

II. Developmental Programming and the Fetal HPA Axis

Early studies described the dramatic exponential rise in plasma cortisol that occurs in the circulation of the sheep fetus during the latter part of normal gestation (31). We (32, 33) and others (26, 34, 35) have established that this rise in biological availability of cortisol to the fetus is associated with a progressive increase in the concentration of ACTH(1–39) in the fetal circulation in a manner that is consistent with the latter driving the increase in fetal adrenal function. Fowden et al (36) has provided a detailed review of the consistency of this change in circulating glucocorticoid across animal species. In the human, as in the sheep, the major glucocorticoid secreted is cortisol, whereas in rodents it is corticosterone. Activation of the fetal HPA axis in late gestation is a key developmental characteristic present in most animal species, with the increased output of fetal glucocorticoid contributing to the onset of parturition and maturation of organ systems required for extrauterine survival (36–38).

The fetus responds to early-life adversity with precocious HPA activation and premature upregulation of key regulatory genes at each level along the axis. Thus, in utero, the fetus may be exposed to sustained elevations of endogenous glucocorticoids during plastic developmental windows, with downstream effects on developing organ systems. Fetal glucocorticoid concentrations may be elevated in circumstances of maternal stress, particularly in association with diminished activity of placental 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) activity, or after maternal administration of synthetic glucocorticoids. We have shown, using sheep as an animal model, that glucocorticoid administration in late gestation results in intrauterine growth restriction (IUGR) and significant alterations in metabolic and HPA axis function and regulation in both the fetus and postnatal animals (39–41). Similar results have been shown across many species (42–45), thus suggesting that early-life exposure to high levels of glucocorticoids leads to long-term metabolic programming. Historical data from epidemiological studies have established that a suboptimal intrauterine environment is associated with an increased risk of developing cardiovascular disease, hypertension, type 2 diabetes, and the metabolic syndrome (46–52); any or all of these associations are underpinned by changes in the endocrine set point of the HPA axis (12, 53–57).

III. Activation of the Fetal HPA Axis and Its Role in Modifying Long-term Disease Risk

The molecular mechanisms regulating the increasing activity of the fetal HPA axis over the course of late pregnancy have been described in detail previously (12, 28, 33, 58), and much of what we know today regarding fetal HPA axis development has been established using a sheep model. In the fetal sheep in late pregnancy, increases in mRNA levels of CRH and arginine vasopressin from the

Figure 1.

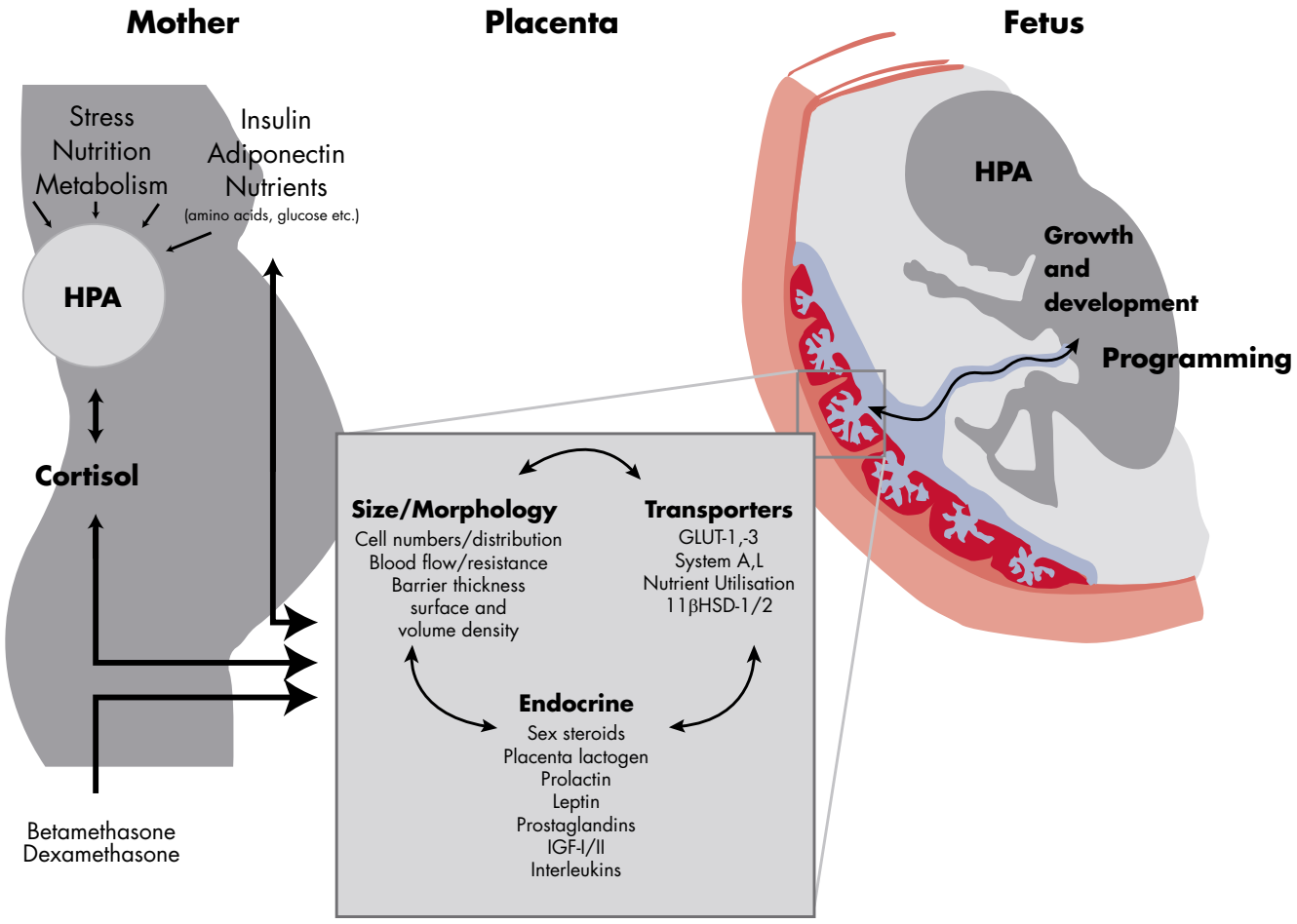

Figure 1. Perinatal programming: the HPA axis, placental function, and long-term disease risk.

paraventricular nucleus of the fetal hypothalamus, independently and together, stimulate a temporal increase in levels of proopiomelanocortin (POMC) in the fetal pars distalis of the pituitary (59, 60). Addition of CRH to fetal pituitary cells in culture increases accumulation of *POMC* mRNA and output of ACTH, consistent with a causal relationship (59, 60). In vivo the rise in circulating ACTH concentrations cause an upregulation in the expression of adrenal *MC2-R* (ACTH receptor) with concomitant increases in gene expression of key regulatory steroidogenic enzymes as well as enhanced coupling of the ACTH receptor through adenyl cyclase to steroidogenic enzymes (59, 60).

It has been demonstrated repeatedly that early-life events modulate and disrupt this fine balance of developmental processes by modulating negative feedback thresholds of fetal HPA activity and altering the long-term function of those organ systems that rely on fetal cortisol for the process of maturation. This modulation is often underpinned by alterations in central receptor populations, enzyme activity and expression, and peptide expression (39, 61–65). Human studies have shown that fetal ACTH and cortisol concentrations, reflected in elevated maternal circulating levels of placenta-derived CRH, are increased in growth-restricted pregnancies (66). Cortisol infusion into the fetal sheep results in elevated fetal and adult blood pressure (BP) (67). Similarly, prenatal stress and fetal glucocorticoid administration restrict fetal growth, modify HPA activity, and impair glucose tolerance later in life, eg, in rats and rhesus macaques (68–71). In humans, low birth weight (LBW) is positively correlated with adult cortisol levels and is associated strongly with insulin resistance and elevated BP and risk of metabolic syndrome (54, 72, 73).

Given these associations, it is not surprising that studies in animal models have demonstrated adverse physiological effects of repetitive fetal exposure to glucocorticoids. Glucocorticoid exposure is a major player in developmental programming, a conceptual framework used to describe the association between environmental adversity

occurring during early-life windows of plasticity and long-term disease risk. Adverse events occurring during periods of high developmental plasticity impart critical cues that the fetus uses to adapt its physiology to its environment. Thus, prenatal events serve to modulate fetal development in a manner that facilitates long-term postnatal survival. For example, prenatal exposure to high levels of endogenous maternally derived glucocorticoids could indicate to the fetus that the postnatal environment is hostile (high predation or low resources), thereby facilitating the needed developmental adaptations to take place for future survival. These adaptations, however, may prove to be deleterious if the postnatal circumstances change (ie, are not hostile) or if the fetus misinterprets these cues. These early adaptations then confer increased disease risk where developmental changes become maladaptive (74). The newly coined International Society for Developmental Origins of Health and Disease (DOHaD) paradigm (50, 75) has provided a foundation based upon this mechanistic view of prenatal factors mediating cellular growth and development, inducing fetal adaptations that result in biological alterations in tissue and organ function (76). These prenatal factors may be endocrine-derived, as described above, relating to fetal exposure to high levels of glucocorticoids, or equally may be nutritionally based. A large recent meta-analysis, including 66 studies from 26 countries from 5 continents demonstrated that LBW was followed by a decreased long-term risk of overweight, whereas high birth weight predisposed for overweight in later life (77), suggesting that the nature of the adversity is critical in determining long-term outcome.

Many experimental models have been used to investigate effects of early-life adversity on HPA axis development and long-term function. Modifications in maternal care in rodents are associated with differences in HPA stress reactivity in the offspring (78). Different levels of maternal care during lactation are associated with altered expression of key genes that regulate stress reactivity and cognition (79–81). Underlying these events are epigenetic alterations including DNA methylation and histone modifications that regulate gene expression (82), which have been implicated as means by which prenatal environmental factors, including maternal behavior and nutrition, have consequences for long-term health (83, 84). These epigenetic events have been shown to be modifiable (79, 82, 83, 85–87).

IV. Exposure to Synthetic Glucocorticoids and Fetal HPA Axis Activation

A. Clinical background

Synthetic glucocorticoids such as betamethasone or dexamethasone are administered to women threatened with preterm delivery to promote fetal lung maturation (16–18, 88). Other circumstances of glucocorticoid treatment during pregnancy are maternal asthma therapy with the need for inhalative glucocorticoids or early maternal dexamethasone therapy in female fetuses in cases of suspected congenital adrenal hyperplasia (13–15, 21, 89–92). Synthetic glucocorticoids are fluorinated; they are poor substrates for metabolizing enzymes (11βHSD2) and do not bind to circulating corticosteroid binding proteins but do associate with tissue glucocorticoid receptors (GRs). Hence, many experimental studies have examined the long-term programming effects of fetal exposure to synthetic glucocorticoid. The use of synthetic glucocorticoids is grounded in historical evidence derived from animal studies. Liggins in 1969 (93) demonstrated that lambs that were induced to deliver prematurely after fetal administration of ACTH, cortisol, or dexamethasone exhibited advanced alveolar stability and premature pulmonary development and maturation. Based upon a series of experimental studies, Liggins and Howie (16) completed the first randomized controlled trial investigating the effectiveness of early glucocorticoid administration in improving neonatal respiratory outcomes in preterm infants. In this truly groundbreaking study, they demonstrated that in women at 24 to 34 weeks gestation, who were at risk of preterm delivery, the administration of synthetic glucocorticoids significantly enhanced fetal lung maturation and reduced neonatal morbidity and mortality by ~50% (Table 1). Since then, it has been established that fluorinated synthetic glucocorticoids such as betamethasone and dexamethasone are 25 to 30 times more potent than cortisol and have insignificant mineralocorticoid action (94). Because synthetics are poor substrates for metabolism by placental 11βHSD2 (95) the use of these pharmacological agents has directly improved clinical outcomes and significantly contributed to obstetrical practice acting as prime candidates for clinical management of women at risk of preterm delivery. The early work by Liggins and Howie (16), has been confirmed many times over in multiple trials (96–99), and the administration of synthetic glucocorticoids to women threatened with preterm delivery is routine practice (17, 18), although current recommendations are to limit administration to one course (18, 100) (Table 1).

Follow-up studies of preterm infants exposed to high levels of glucocorticoids in utero have shown an increased incidence of behavioral disorders (101), elevated BP, and increased insulin resistance (102) together with changes in HPA axis baseline and stress responsiveness (103) (Table 2). A recent systematic review has suggested that the benefits outweigh the cost (104). In their systematic review of randomized trials, McKinlay et al (104) found that the use

of repeat doses of antenatal glucocorticoids in women at risk of preterm delivery, 7 or more days after an initial course, reduced the risk of combined serious neonatal outcome. However, the suggestion that glucocorticoids may impact longer-term homeostatic regulation and endocrine set points beyond the neonate reinforces the fact that it is essential that well-designed long-term follow-up studies be performed. Some data in this regard are available, but it is still early days.

In the follow-up studies of the original Liggins cohort (105–110), 30 years later, adults exposed to betamethasone (24–48 mg total betamethasone dose) as fetuses exhibit more insulin resistance as measured by an oral glucose challenge (107), but the risk of impaired lung function, the prevalence of wheeze and asthma, or the risk of cardiovascular disease were not increased after a single course of betamethasone treatment (105, 110). In other follow-up studies, renal function at the age of 19 years was decreased after a single course of fetal betamethasone exposure, although this finding may be confounded by preterm birth rather than the early glucocorticoid exposure, because the risk of chronic renal failure is increased in prematurely born individuals (111). Recent evidence suggests that glucocorticoid-induced cardiovascular effects may be related to aortal stiffness. In a 25-year follow-up of a cohort of preterm-born individuals from 5 centers in the United Kingdom, Kelly et al (112) demonstrated that young people born preterm, whose mothers received antenatal glucocorticoids had a regional increase in aortic stiffness as well as changes in glucose metabolism that were independent of the impact of antenatal steroids on aortic function. This study, however, was relatively small, and results may be confounded by the fact that the participants were matched but not randomized.

Since the first trial of Liggins and Howie (16) in 1972, there has been ongoing discussion about the minimal effective dose of glucocorticoid, the best gestational age at which to treat, whether to use repeated courses, and whether a rescue dose is effective in reducing neonatal respiratory distress syndrome (RDS) (24, 113–115). A dose-response trial in humans has never been conducted. Studies in sheep investigating lung maturation demonstrated in fetuses born at 0.75 time point in gestational length after treatment with half the clinical dose of betamethasone currently recommended (twice in total at 42.5 μg/kg ewe weight corresponding to twice in total at 6 mg/70 kg in humans) similar pulmonary maturity with maximal improvement in fetal lung pressure-volume curves as compared with a full betamethasone dose (116). These studies, however, were conducted using a mixed group of twin and singleton fetuses and thus should be interpreted with caution. Studies on antenatal glucocorticoid treatment aimed at reducing neonatal preterm morbidity and mortality have shown that babies born more than 7 days after a single course of prenatal glucocorticoid treatment had no reduction in risk of RDS (17, 117). Observational studies in humans have reported varying effects of antenatal glucocorticoids on birth weight and head circumference (107, 118–122). Repeated glucocorticoids have been associated with decreased birth weight (118) and delayed development in early childhood (123). Others have reported a reduction in cerebral palsy after repeated glucocorticoid treatment (101), however, treatment intervals, age at treatment, and maternal complications might have biased those outcomes. Antenatal glucocorticoid treatment in clinical doses leads to postnatal behavioral changes in baboons with decreased motivation and cognition (124) and in humans with acute change in higher cortical functions in the exposed fetus (125). A recent Cochrane database analysis in 2011 on repeated doses of prenatal glucocorticoid for women at risk of preterm birth analyzed 10 trials with low to moderate bias (126). It still remains unclear whether and under which conditions repeat doses of prenatal glucocorticoids might be beneficial in preventing neonatal health outcomes. The Prenatal Repeat Corticosteroid International IPD Study Group (PRECISE) was formed in 2012 to assess the effects of repeat doses of glucocorticoid treatment for maximizing benefit and minimizing harm (127). The aim of this study group is to evaluate how, using an individualistic approach, women with different clinical backgrounds will benefit from antenatal glucocorticoid treatment with a maximum reduction in harm. The study aims to evaluate whether repeat prenatal corticosteroids should be recommended, the optimal gestational ages for administration, the optimal number of repeat treatments that should be given, and what dose and timing could be recommended (127). The results of this study will provide valuable information on the dosing and will no doubt contribute to improve clinical glucocorticoid therapy.

B. Clinical evidence

There is substantial experimental evidence demonstrating that fetal exposure to elevated levels of glucocorticoids impairs fetal growth and is associated with cardiovascular, HPA, and metabolic compromise (5, 102, 128, 129). Significant fetal growth restriction has now been shown in many experimental models (39, 130–134). Early studies in nonhuman primates demonstrated that maternal betamethasone administration at 120 to 133 days of gestation (term is 167 days) resulted in fetal growth restriction as well as impaired growth and function of specific organs including the brain, pancreas, adrenal, and pituitary (135–137). We have reported previously that maternal admin-

istration of synthetic glucocorticoids to pregnant sheep resulted in fetal growth restriction (39, 133, 138) that persisted until 3 months of postnatal age (139) and was associated with reductions in whole brain and cerebellum weights as well as reductions in the myelination of axons located in the optic nerve and the corpus callosum (140–142). In rats and primates, exposure to high glucocorticoid levels in utero resulted in permanent elevated basal cortisol/corticosterone levels in the offspring (70, 131, 143, 144).

There are only limited data on the effects of antenatal glucocorticoid treatment and persisting effects on the HPA axis in humans (145). Tegethoff and colleagues (146) have demonstrated in a systematic review that antenatal glucocorticoid administration resulted in a reduced HPA activity in the neonates without changing baseline glucocorticoid levels in later life. The authors suggested that timing of glucocorticoid treatment during pregnancy in relation to the fetal development may be a key factor that influences the direction of HPA change observed in neonates but not in adulthood. This was demonstrated in a cohort of 1-year-old infants who showed lower salivary cortisol levels only if the mother had developed posttraumatic stress disorder after exposure in the last third of pregnancy to the September 11 World Trade Center catastrophe in 2001 (147). Others have demonstrated that in the first week of neonatal life after antenatal glucocorticoid exposure, basal cortisol levels were suppressed but returned to normal afterward (148–152).

Early studies from Ballard et al (148, 152) suggested that there is a persistent effect of antenatal glucocorticoid administration on postnatal HPA axis function. A suppression of a cortisol response to a painful heel-prick blood draw after antenatal glucocorticoid treatment was found in preterm infants, and this effect persisted for several weeks after birth (153–156). In term infants who had received antenatal glucocorticoid treatment but were not exposed to the other multiple sources of stress found in the neonatal intensive care units and that are experienced by preterm infants, significantly higher cortisol responses were observed in response to the painful heel-prick blood draw (155).

Recently, a follow-up study in children 6 to 11 years of age reported significantly higher cortisol reactivity to acute psychosocial stress in term-born children exposed to antenatal synthetic glucocorticoid treatment compared with controls, with more profound effects in females, independent of the glucocorticoid used (157). Long-term consequences on the HPA axis function are not well understood. At 30 years of age after antenatal glucocorticoid treatment, basal morning cortisol levels in glucocorticoid-exposed individuals were 7% higher as compared with controls, although after adjusting for multiple variables, these differences were no longer significant (107). This observation, however, leaves the possibility open that antenatal glucocorticoid treatment not only may affect fetal and infant HPA function but also may induce long-term endocrine changes consistent with other studies investigating this possibility (145, 146).

C. HPA programming in animal models

In a variety of species including sheep, rats, guinea pigs, and nonhuman primates, antenatal glucocorticoid administration resulted in alterations of HPA function (30, 41, 42, 131, 143, 158–161). The magnitude of the HPA response varied according to the type of steroid used (betamethasone or dexamethasone), the time of gestation when glucocorticoid was administered, the total dose, acute and chronic effects, and the sex of the fetus (140, 146, 159, 161–163). In sheep, antenatal betamethasone treatment in the last third of pregnancy resulted in elevated basal cord levels of plasma ACTH and corticosteroid binding capacity, but these changes were not reflective of changes in steady-state concentrations of *POMC* and *CRH* mRNA in the fetal pituitary or hypothalamus (39). At 1 year postnatal age in the same model, a previous single injection of betamethasone to the mother resulted in significantly elevated basal and stimulated cortisol levels without significant changes in the ACTH response (41). Higher doses of antenatal betamethasone abolished the difference in peak ACTH response between animals at 6 months and 1 year postnatal age (41).

Last-trimester antenatal dexamethasone treatment in rats resulted in offspring with hypertension and also increased basal plasma corticosterone levels with reduced sensitivity to glucocorticoid feedback due to permanently attenuated *GR* and *MR* mRNA expression levels in specific hippocampal subfields (131). In guinea pigs, antenatal betamethasone treatment changed HPA axis activity in a sex- and cycle-dependent manner compared with controls. In luteal-phase females, basal salivary cortisol levels were significantly decreased after betamethasone treatment and hippocampal *GR* mRNA and adrenal *MC2R* mRNA expression were significantly increased compared with controls (159). Long-term reproductive success was impaired; females showed reduced fecundity after antenatal betamethasone treatment (159). Dexamethasone resulted in the same reduction in basal salivary cortisol levels during luteal-phase testing, whereas estrous-phase testing demonstrated elevated salivary basal cortisol levels compared with controls (159). In this same model, male offspring had reduced basal and activated plasma cortisol levels after antenatal dexamethasone treatment (164).

Alterations in behavior, particularly locomotion, after antenatal glucocorticoid treatment have also been demonstrated (30). In the African vervet, oral dexamethasone given to the mother during pregnancy dose-dependently reduced maternal cortisol levels without affecting maternal BP, glucose, electrolytes, or weight gain (143). In 8-month-old offspring, this glucocorticoid treatment resulted in impaired glucose tolerance and hyperinsulinemia, associated with a 25% reduction in pancreatic β-cell number at 12 months (143). Long-term programming of HPA axis function with an exaggerated cortisol response in the offspring was also observed (143). In juvenile nonhuman primates, antenatal dexamethasone administration resulted in an irreversible deficiency of the hippocampal neurons detected with magnetic resonance imaging in 20-month-old offspring. This deficiency was associated with high plasma cortisol levels at the circadian baseline and poststress levels (158). Hauser et al (42) demonstrated that early dexamethasone treatment in marmosets significantly reduced maternal plasma cortisol levels during treatment. Showing characteristics of the metabolic syndrome, infant body weight was increased postnatally and was associated with changes in food intake behavior (42).

Changes in the IGF axis are likely to be one route by which excessive fetal glucocorticoids restrict growth (134, 165, 166) (Table 3). IGFs and their receptors and IGF binding proteins (IGFBPs) are regulated by glucocorticoids (165–168), and birth weight is correlated with maternal and fetal concentrations of IGF-1 and inversely related to IGFBP1 (169). Cortisol infusion to the fetal sheep significantly reduced IGF2 mRNA levels in the fetal liver and skeletal muscle by 20%–55% at 145 days of gestation (166). In the rat and sheep, prenatal glucocorticoid administration resulted in overall growth restriction in addition to the restriction of specific organ growth (165, 170). The regulation of fetal and placental growth by IGFs and their binding proteins has been extensively reviewed (168, 171), but the detailed relationship to the effects of antenatal glucocorticoids remains poorly understood.

In rats, exposure to dexamethasone or betamethasone at midgestation reduced plasma IGF-1 and increased plasma IGFBP1 concentrations in the fetus at term (172). Longer courses of antenatal DEX treatment with higher total glucocorticoid doses increased IGF2, type 1 IGF receptor, and IGFBP2 mRNA expression levels in fetal rat tissues (165). IGFBP3 mRNA expression levels were significantly decreased, whereas placental IGF2 mRNA expression was significantly increased (165, 173). In humans, antenatal treatment with a single course of betamethasone did not influence IGF-1 or IGFBP3 in umbilical cord serum or umbilical vein plasma in infants born at <37 weeks but significantly decreased IGF2 and IGFBP1 levels (174, 175). These differences persisted even when betamethasone exposure occurred 2 weeks before delivery. A dose response may exist; higher doses of glucocorticoid increased cord plasma IGF-1 and IGFBP3 concentrations and decreased those of IGF2 and IGFBP1 (176). Antenatal administration of DEX in rabbits resulted in similar IGF axis changes compared with humans, particularly in naturally growth-restricted fetuses, with

Table 3. Antenatal Glucocorticoid Treatment and Associated Molecular Changes in Animal Models and Human[a]

Animal models	Refs.	Humans	Refs.
Fetal liver			
↑ Gluconeogenic enzymes (PEPCK)	132		
↑ 11βHSD1	40		
↑ IGF-2, IGF-1R, IGFBP2	165		
Placenta			
↓ VEGF	278, 280		
↓ eNOS	276, 277		
↓ Barrier thickness, ↑ surface area	240		
↓ Blood space volume densities	235		
↓ IGFBP3, ↑ IGF-3	173		
↓ BNCs, oPL	236, 243		
Impairment of glucose transport	232, 241, 291		
↓ System A	302	↑ System A	301
↑ MRP1	324, 333	↓ DEPTOR	357
↓ BRCP	332		
↑ 11βHSD2	203, 300		
Circulation			
↓ IGF-1, ↑ IGF-2	172	↓ IGF2, IGFBP1	174, 175
↑ IGFBP-1	172	↑ IGF-1, IGFBP3	176

Abbreviations: PEPCK, phosphoenolpyruvate carboxykinase; DPTOR, DEP-domain-containing and mTOR (mammalian target of rapamycin)-interacting protein.

[a] The table summarizes some the associated molecular changes of antenatal glucocorticoid treatment in human and animal models found in the placenta, fetal liver, etc. The references contain an overview of original articles as well as review articles. Arrows indicate decrease (↓) or increase (↑).

increased plasma IGF-1 and tissue IGF-1 mRNA expression levels (134). Long-lasting effects of antenatal glucocorticoid exposure on the IGF axis beyond the perinatal period have yet to be evaluated.

Early-life exposure to glucocorticoids results in metabolic compromise that is likely to be associated with adaptations in key fetal organs such as the liver and the pancreas (69, 132, 177). Early studies in rodents demonstrate that treatment of the pregnant rat with carbenoxolone, a placental 11βHSD inhibitor, results in reduced birth weight, hypertension, and impaired glucose tolerance (69), an effect similar to that observed with dexamethasone treatment (131, 165). Maternal adrenalectomy prevented this effect, strengthening the hypothesis that maternally derived glucocorticoids participate in programming of the offspring's metabolic function (69). In a similar model, maternal dexamethasone administration late in gestation restricted fetal growth, resulted in fasting hyperglycemia, elevated insulin response to glucose, and increased hepatic expression of gluconeogenic enzymes (phosphoenolpyruvate carboxykinase) in the offspring, an effect that persisted up to 8 months of postnatal age (132). Similarly, in sheep, maternal dexamethasone treatment early in pregnancy elevated fetal basal and stimulated insulin levels later in gestation (178). We have shown that as little as one injection of maternal betamethasone in sheep results in insulin resistance to a glucose challenge in the offspring at 6 months and 1 year of postnatal age (139). In similar studies, fetal betamethasone exposure increased fetal hepatic 11βHSD1 (40), suggesting that glucocorticoids may not only directly impact metabolic enzymes but also modulate intrahepatic levels of glucocorticoid-metabolizing enzymes, thus potentiating a feed-forward loop of glucocorticoid effects. In this regard, *11βHSD1*-knockout mice demonstrated reduced gluconeogenic responses when stressed and were resistant to high-fat feeding-induced hyperglycemia, supporting the hypothesis that 11βHSD1 may act as a modulator of intrahepatic glucocorticoid action in vivo (179).

In a retrospective, clinical case-control study data from betamethasone-treated (n = 1799) and nontreated (n = 42 240) pregnancies, fetal growth measures, neonatal anthropometrics, placental weight, cord blood gases, and Apgar scores were analyzed regarding the effect of antenatal betamethasone treatment given for the induction of lung maturation in cases of preterm birth (Braun T, Sloboda DM, Tutschek B, et al, unpublished data, 2013). In all betamethasone dosage regimens analyzed, birth weight was significantly reduced, independent of major confounders (eg, hypertension, smoking, maternal weight, and body mass index gain during pregnancy), sex, or time of treatment compared with controls. Higher betamethasone (betamethasone) dosages were associated with greater growth reduction without further improvement in neonatal mortality, morbidity, and Apgar scores. Follow-up ultrasound-estimated fetal weight measurements before and after betamethasone treatment of normally grown fetuses at the time of betamethasone exposure showed reduced fetal weight gain after betamethasone exposure in both male and female fetuses (Braun T, Sloboda DM, Tutschek B, et al, unpublished data, 2013).

Differences in the observed effects between betamethasone and dexamethasone are believed to be due, at least in part, to the different binding affinities of the molecules, with dexamethasone having a greater affinity to the GR (30, 159). Furthermore, multiple doses of glucocorticoids, with higher total dose of the glucocorticoid, are associated with greater and more profound changes in the fetus and in postnatal offspring (140, 163).

V. Role of the Placenta in Mediating Glucocorticoid-Induced Long-term Disease Risk

The materno-placental-fetal unit plays an important role in regulating fetal adaptations to prenatal adversity, thereby contributing to the organism's phenotype and susceptibility to disease (181). The placenta is the conduit between the maternal and fetal environment; it constitutes the interface between maternal and fetal circulations, serving as an immune barrier as well as an organ for exchange and metabolism. During pregnancy, the placenta maintains fetal homeostasis by performing a wide array of physiological functions, which after birth are carried out by the kidney, lungs, gastrointestinal tract, and endocrine glands. It is therefore not surprising that long-term health outcome and disease risk are determined by the functional ability of the placenta. Prenatal adversity, including nutritional compromise, stress, or glucocorticoid treatment, either is transmitted across the placenta to have an impact on the immediate fetal intrauterine environment or has direct influences on the development and function of the placenta. Together these regulatory pathways serve to mold and modify fetal adaptations to the in utero environment (181–185). In experimental models, most maternal perturbations associated with the programming of disease risk also affect several aspects of placental morphological structure, transport capacity, and function, thus providing a direct link between prenatal adversity and the triggering of fetal adaptive responses, potentially contributing to changes in long-term health status (182).

A. Interplay between the placenta and the fetal HPA axis: exposure to high levels of glucocorticoids

In most models of early-life adversity including maternal stress and high levels of glucocorticoids, experimentally induced placental insufficiency, hypoxemia, and/or neonatal handling, glucocorticoid concentrations act as signals of stress and their concentrations increase in mothers, fetuses, and/or neonates (128, 186, 187). In animal species, glucocorticoids rise as a result of the prepartum activation of the fetal HPA axis (58, 188), so that the placenta is exposed to glucocorticoid signals from both the mother and the fetus (Figure 2) (14, 36, 181, 189, 190). It remains highly likely that the antenatal increase in glucocorticoids occurs in the human fetus as well, but to date there is no firm information on this. The lipophilic nature of glucocorticoids allows them to pass through the placenta quite readily. Therefore, before the normal rise in fetal HPA activity near term, up to 80% of circulating fetal cortisol is of maternal origin (191). As such, for much of pregnancy, circulating cortisol levels in the mother are higher than in the fetus, but transplacental transfer is restricted because of the presence of the placental glucocorticoid-metabolizing enzyme 11βHSD2, which converts cortisol into inactive metabolites (79, 187, 192–194). 11βHSD2 is also expressed in various fetal organs such as liver, kidney, adrenal, and lung, thereby affecting local tissue cortisol concentrations as well (95, 195, 196). This activity maintains low placental and fetal exposure to elevated levels of maternally derived glucocorticoids (197). Compared with the mouse and rat placenta, where 11βHSD2 activity drops toward term, facilitating later gestational fetal organ maturation (198, 199), human placental 11βHSD2 activity steadily increases throughout gestation (200). As a result, human placental 11βHSD2 plays a pivotal role in the prenatal programming of disease risk through its regulation of fetal exposure to endogenous glucocorticoids.

Prenatal events that attenuate 11βHSD2 activity may expose the fetus to inappropriate levels of glucocorticoids; a reduction in its activity is associated with LBW in most (199–201) but not all (202) studies. In early gestation, the placenta may be able to overcome decreased 11βHSD2 levels and increase local GR signaling by increasing nutrient transfer from the mother to the fetus, and birth weight changes may not be obvious. Close to term when rapid growth requires an increased supply of nutrients, this compensatory mechanism fails, and in placentas with suppressed 11βHSD2 activity, birth weight changes become obvious (5). Placental 11βHSD2 levels are influenced by a variety of different factors. Decreased levels of 11βHSD2 are associated with maternal stress, dietary protein restriction, and changes in oxygen levels, catecholamines, and inflammatory cytokines (203–208). In rats, reduced placental 11βHSD2 activity is associated with increased BP in adult offspring (194). Treatment of pregnant rats with carbenoxolone, a potent inhibitor of 11βHSD2, results in reductions in birth weight, significantly higher fasting basal glucose levels, elevated insulin responses to a glucose challenge, and elevated basal corticosterone levels in offspring associated with increased anxiety-like behavior in adverse situations (69, 144, 209). These outcomes were abolished by maternal adrenalectomy; thus, maternally derived glucocorticoids are key in modulating metabolic compromise in the offspring (69).

Figure 2.

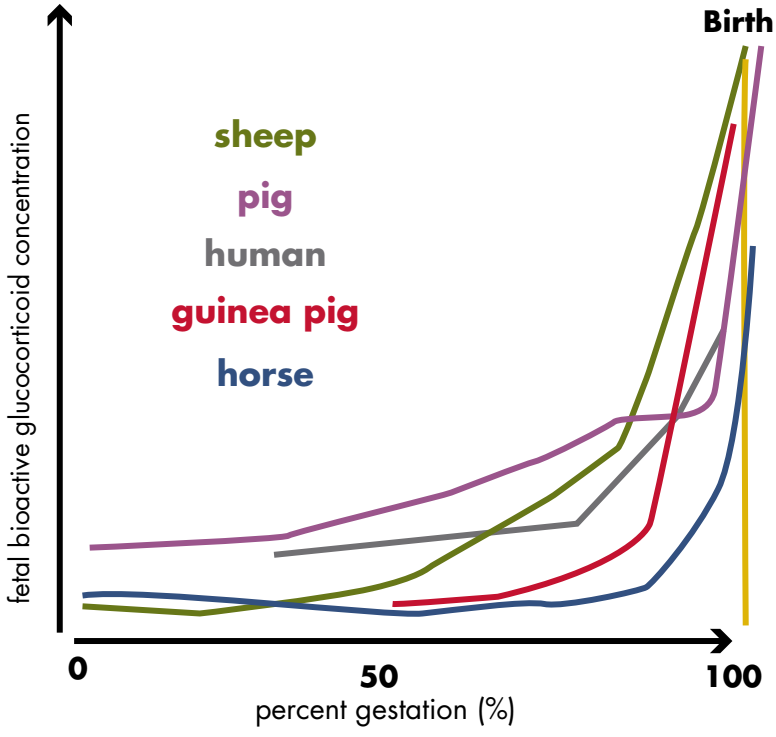

Figure 2. Representative diagram of fetal glucocorticoid levels over the course of gestation. There is an increase in bioactive fetal glucocorticoid levels with advancing gestation across many different species, although the timing and the magnitude of the increase varies considerably. [Derived from A. L. Fowden et al: Glucocorticoids and the preparation for life after birth: are there long-term consequences of the life insurance? *Proc Nutr Soc*. 1998;57:113 (36)].

Placental 11βHSD2 also mediates fetal growth in human pregnancy, such that decreased expression of placental 11βHSD2 is associated with intrauterine growth restriction (200, 210). Humans homozygous for genetic mutations in 11βHSD2 have been shown to have reduced birth weight (211). Excessive maternal consumption of licorice-containing food is not recommended during pregnancy; glycyrrhizin, an active constituent of licorice, inhibits placental 11βHSD2. Its consumption is associated with a shorter gestational length and with a significant decrease in verbal and visual-spatial abilities and narrative memory, rule-breaking behavior, and aggression (5, 212–215). Interestingly, there are also sex-related differences in human placental 11βHSD2 activity (216). Enzyme activity is greater in the trophoblast of term placentas associated with a female compared with a male fetus. These differences in enzyme activity may suggest that the female fetus could be exposed to lower maternally derived cortisol and thus escapes negative feedback regulation, facilitating autonomous development of fetal HPA function (89). Whether differences in placental 11βHSD2 activity account for sex-specific differences in long-term disease risk and behavior are unknown, but seem likely.

B. Placental neuropeptides

Decades of both clinical and experimental studies have suggested there are interactions between the maternal HPA axis, regulation of bioactive glucocorticoid levels, and altered HPA axis function in the fetus. Human placentas produce neuropeptide hormones in amounts that have suggested a placental HPA axis. Placental production of the neuropeptide CRH was discovered 4 decades ago and was shown to have profound influences on function (217). Placental CRH output increases in late pregnancy and is higher in women at risk of preterm delivery (218). Placental CRH is regulated by glucocorticoids; cortisol increases placental *CRH* gene expression and peptide secretion (217). Placental CRH is also regulated by cytokines, nitric oxide, and oxytocin and downregulated by progesterone (217). It is thought to have direct actions on the myometrium and is a potent vasodilator (219). Thus, it is plausible that in the face of early-life adversity, increased production of bioactive cortisol from the fetal HPA axis in turn upregulates placental expression of CRH, a placental vasodilator, compensating for this adversity, increasing placental blood flow and nutrient transfer.

Similarly, urocortins are members of the corticotrophin family and are present in placental tissue from very early in gestation (220). In vitro, trophoblast cells produce increasing amounts of urocortin 2 and 3 in response to lowered oxygen tension (221). Urocortin increases placental aromatase, inducing the production of placental estradiol (222). This may be an alternative pathway to increase placental blood flow in the face of intrauterine adversity (223). In this regard, the interaction between urocortin and lipopolysaccharide stimulates the output of the proinflammatory cytokines, including TNFα, by placental cells in vitro (224), turning on inflammatory pathways. Placenta from women in preterm labor with chorioamnionitis demonstrate higher levels of CRH, urocortin 2, and CRH receptor 1 compared with tissue from women delivered preterm without infection (224), consistent with this pattern of in vitro responses. Thus, urocortins and their family members may be signaling molecules that exert local regulation of placental inflammatory responses, responses that in turn may be influenced by local oxygen tension. It has been suggested that low-grade placental inflammation may compromise fetal growth and predispose to preterm labor (217, 223); an altered inflammatory pathway could present a common mechanism between these pathologies.

C. Mechanisms and principles of placental adaptations to glucocorticoid exposure

a. Placental size and morphology

Epidemiological data have suggested that placental weight may be a marker of the long-term health outcome of the fetus (225). Although the size, weight, and shape of the placenta is variable between women as well as between pregnancies, in general, low placental weight at birth is associated with small babies and predicts hypertension and coronary disease in offspring (226, 227). Equally, a high placental to birth weight ratio, also described as placental efficiency, has been shown to be associated with coronary heart disease and hypertension (226, 228), indicating that two disparate placental phenotypes can both confer increased disease risk in the offspring (226, 229). Barker et al (228) have suggested that the association between placental surface area and the offspring's risk of hypertension depends on the mother's nutritional state and that poor maternal nutrition may compound the adverse effects of small placental size. In better-nourished mothers, the placental surface may expand to compensate for varying degrees of nutrition during fetal development. Indeed, among men in the Helsinki Birth Cohort Study (103) who were thin at birth, different materno-placental-fetal phenotypes (defined by mother's height and body mass index) were associated with coronary heart disease and were thought to be suggestive of fetal undernutrition (230). Whether placental area associates with changes in circulating glucocorticoids is presently unknown but is an area of great research interest.

The mechanisms by which glucocorticoids impact placental growth and development and modify its phenotype still remain unclear. Many studies in different animal species exist, describing placental growth restriction and functional modifications in circumstances of elevated glucocorticoid exposure (43, 170, 231–239). The degree of placental growth restriction depends on the type of glucocorticoids as well as the dose, timing of treatment, and duration of administration (240). In most studies, placental weight is restricted to a lesser extent than fetal weight, which may be indicative of increased placental efficiency despite a modest reduction in placental weight (170, 238). Experimentally in the sheep, maternal betamethasone administration late in gestation alters the distribution of placentome subtypes by reducing the numbers of the more everted types (241, 242); it appears that this morphological remodeling of the ovine placenta may to be due to changes in migration of fetal binucleate cells (BNCs) (Braun, T., unpublished observations). We and others have shown that BNCs are reduced after maternal or fetal glucocorticoid administration in the sheep (236, 243). Intriguingly, maternal dexamethasone administration in sheep increased transplacental glucose gradient (244) in pregnancies with a higher number of everted placentomes, but fetal cortisol administration reduced the placental delivery of glucose and lactate to the fetus (241). It appears therefore that intrauterine conditions that raise fetal exposure to glucocorticoids and modify placentome subtype distribution may help to maintain placental glucose supply to the fetus in an adverse environment (245).

b. Placental lactogen

The placenta produces a wide range of hormones including steroids, eicosanoids, and glycoproteins, all of which are sensitive to glucocorticoids (186). Placental lactogen (PL) is found in both the maternal and fetal circulations (246) and plays an important role in the regulation of maternal carbohydrate, lipid, and protein metabolism (247). PL is thought to affect fetal growth, but this effect may be indirect through alterations in the maternal metabolic environment or maternal-placental nutrient transfer or through stimulated release of IGFs (248, 249). The ovine equivalent (ovine placental lactogen [oPL]) is somatogenic, stimulating amino acid uptake, glycogen synthesis, and IGF secretion (250). It stimulates development of the mammary glands (243) and redirects substrate utilization to make glucose available for transport to the fetus (251). The oPL is produced by BNCs, which are formed from uninucleated cells in the fetal trophectoderm (252). The number of BNCs declines near term, concomitant with a fall in maternal plasma oPL concentrations (253) and in parallel with the late gestational increase in fetal cortisol levels (37, 188, 254), suggestive of a link between circulating cortisol and oPL levels.

Infusion of cortisol to the sheep fetus decreased the number of BNCs in the fetal trophectoderm (243), but there are few data available regarding the effects of maternal administration of glucocorticoid on placental BNC distribution and function. We have demonstrated that BNC number, placental oPL protein levels, and circulating maternal and fetal oPL levels correlated positively with fetal weight (236) (Figure 3). We also demonstrated that maternal betamethasone administration changed the distribution and localization of BNCs within ovine placentomes. Placental oPL protein levels as well as circulating fetal and maternal oPL levels were decreased after late maternal betamethasone treatment owing to the de-

Figure 3.

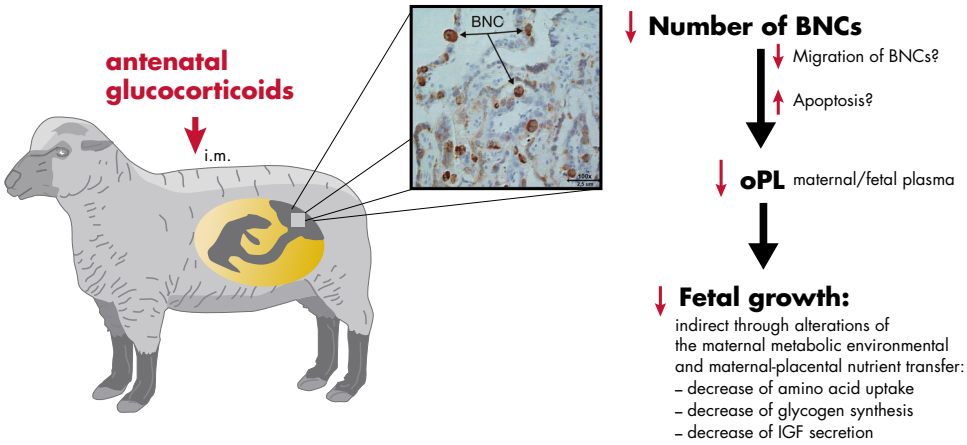

Figure 3. Overview on the effect of antenatal glucocorticoid treatment in sheep and the possible mechanism involving PL in the regulation of fetal growth.

creased number of BNCs that synthesize this hormone (236). Consistent with these observations, variations in fetal weight have been attributed to changes in maternal serum oPL and fetal cotyledonary oPL mRNA concentrations (252, 255). We suggest that the demonstrated growth-restricting effects of maternal betamethasone (39, 135, 256) may be associated with alterations in BNC formation and placental production of oPL.

Although early studies in human pregnancies did not demonstrate significant changes in maternal human PL levels after glucocorticoid treatment (257, 258), others report a significant reduction in maternal human PL after maternal dexamethasone treatment (259). In humans, betamethasone-exposed (two doses of × 12 mg were given as treatment in normally grown fetuses between 23 completed wk of gestation + 5 d through 34 completed wk of gestation + 0 d) women who delivered between 23 completed weeks of gestation + 5 days through 42 completed weeks of gestation + 0 days weeks were compared with gestational age-matched controls. Betamethasone use was associated with decreased birth weight (−18.2%), head circumference (−8.6%), body length (−6.0%), and placental width (−5.5%) as compared with controls who did not receive betamethasone treatment (260). However, these growth-restricting effects were not associated with altered placental or maternal plasma PL levels. Altered expression of PL appears not to be causal for betamethasone-induced fetal growth restriction in the human (260), denoting perhaps species difference in the biological actions of placental PL.

Together these data suggest that early-life exposure to glucocorticoids induces fetal growth restriction through effects on placental size and function as well as through the inhibition of PL synthesis, thereby altering essential pathways used for the provision of maternal metabolic substrates to the placenta and ultimate transfer to the fetus. The exact mechanism by which glucocorticoids act on BNCs and regulate placental lactogen secretion requires further investigation.

VI. Placental Vascular Development

The relationship between the uterine vasculature, maternal-placental blood flow, the subsequent remodeling and development of the definitive placenta, and programming of long-term disease risk is complex (261). Key events take place during the early stages of placental development after implantation that are vulnerable to compromise. Anemia in early pregnancy can increase fetal-placental angiogenesis (262, 263). A high altitude or hypoxic environment results in dilatation of fetal capillary sinusoids and thinning of the placental exchange barrier (263–265). Placental villi of growth-restricted fetuses are poorly branched with low capillarity, and the fetal placental exchange area is thickened (266, 267). These growth-restricted fetuses demonstrate increased umbilical artery resistance, and the resultant adaptations in the fetal heart to an increased pressure work load has been suggested to contribute to cardiovascular disease postnatally (268). Clearly, placental vascular development may be affected by inappropriate glucocorticoid exposure. Dexamethasone treatment in mice decreased the barrier thickness of the placental diffusion area in the labyrinthine zone and concomitantly increased the surface area for nutrient exchange (240). In contrast, dexamethasone treatment in rats late in gestation reduced maternal and fetal blood space volume densities and the relative surface area of the fetal capillaries in the labyrinthine zone (235). Clifton and colleagues (89) have shown that in the placentas of asthmatic women taking high doses of glucocorticoids, fetal villi are hypovascularized at term with reduced placental blood flow.

Inappropriate exposure to high levels of glucocorticoids in utero affect vascular regulatory systems at different key regulators, including nitric oxide synthase (NOS) (269, 270), endothelin (271), and the renin-angiotensin system (272). Endothelial NOS (eNOS) is an important regulator of endothelial-dependent arterial vasodilatation and plays an important role in regulating vessel diameter including in the placental circulation (273, 274). In human cells, in vitro dexamethasone downregulates eNOS (275). In primates, betamethasone treatment reduced eNOS in cultured baboon endothelial cells (276), which may contribute to the cardiovascular long-term changes observed after antenatal glucocorticoid treatment (277).

The signaling pathways that regulate glucocorticoid-mediated changes in placental vasculature are no doubt multifactorial but likely involve vascular endothelial growth factor (VEGF) and its receptors Fms-like tyrosine kinase 1 (*Flt1*) and kinase domain region (*KDR* or *Flk1*). VEGF is expressed in amnion epithelial and cytotrophoblast cells throughout pregnancy, although the density of expression increases toward term. In the first trimester, VEGF is responsible for placental angiogenesis and development of the villous system, whereas near-term VEGF is responsible for vascular permeability and the regulation of endothelial cell migration and protease synthesis (278). Glucocorticoid administration has been shown to reduce VEGF expression and protein synthesis in keratinocytes, retina cells, chondrocytes, and ischemic muscle cells (279), and in rats, maternal dexamethasone administration prevented the normal increase in placental VEGF expression near term (235). Hypoxia in animal models induced a

reversible increase of VEGF expression (278, 280), an effect that may be mediated by glucocorticoids. Similarly, undernutrition reduced *VEGF* and *KDR* expression with an associated reduction in placental weight (281), an effect likely mediated by glucocorticoids but not yet proven.

VII. Placental Nutrient Transport

Placental nutrient transporter activities are regulated by a variety of factors, including nutrient concentration, oxygen, and endocrine signaling pathways such as leptin and glucocorticoids. In general, fetal growth restriction has been associated with limitations in oxygen and nutrient transport, whereas fetal overgrowth has been attributed to excess nutrient delivery, particularly in circumstances of maternal hyperglycemia (255, 282). Nutrient transport may be reduced to match availability or upregulated to compensate for reduced (283) availability through the modification of placental nutrient transporter expression (284–287).

A. Glucose transport

In the human placenta, the syncytiotrophoblast constitutes the primary barrier to the transport of molecules. The transport of glucose across the placenta is mediated by the glucose transporter (GLUT) family of facilitated-diffusion glucose transporters. Different isoforms of GLUT transporters (GLUT1, -3, and -4) are expressed at various locations in the human placenta; GLUT1 is found mainly in the syncytiotrophoblast but also in the vascular endothelium and primary cytotrophoblast cells (283). GLUT3 is less abundant and is expressed only in the vascular endothelium (283, 288). GLUT4 expression, the insulin-responsive isoform of the glucose transporter, is restricted to intravillous stromal cells and appears to be colocalized with insulin receptors (283).

The expression of glucose transporters in the placenta appears to be regulated by nutritional and endocrine factors, and their expression levels and activities fluctuate depending on maternal status. In type 1 diabetic women, fetal overgrowth was associated with increased glucose transporter activity (289), and the relationship between maternal glucose status and fetal growth is complex and does appear to be linearly related. In this regard, fetal overgrowth is also observed in diabetic women whose glucose levels were rigorously controlled throughout gestation (182). In cases of gestational diabetes, placental glucose transporter activity is not different from controls, again highlighting the complexity of glucose transport regulation in the placenta in circumstances of fluctuating maternal glucose status (290).

Glucocorticoids can influence placental hormone production and alter the placental transport capacity and therefore may modify the partitioning of nutrients between maternal, fetal, and placental tissues. Glucocorticoids are known mediators of glucose transporter expression and function (232, 291). The direction and degree of change in glucose transport is, however, highly dependent upon the species and the model used to study it. In *11βHSD2*-knockout mice, fetoplacental overexposure to glucocorticoids resulted in a reduction in placental glucose delivery to the fetus, associated with reduced placental expression of the GLUT3 but not the GLUT1 isoform (292).Chronic maternal stress in rodents attenuated placental 11βHSD2 and GLUT1 expression and reduced fetal plasma glucose levels (203). In the rat placenta, ip injections of glucocorticoids resulted in a significant reduction of both GLUT1 and GLUT3 transcripts and protein levels (232). However, it has been reported that synthetic glucocorticoid exposure (dexamethasone) in a rodent model in fact increased GLUT1 protein expression in a dose-dependent manner. This was accompanied by a dose-dependent decline in fetal and placental weight (291). The exact action of prenatal glucocorticoids on glucose transport is complex and no doubt modulated by glucocorticoid dose and timing during gestation and may also be species-specific. Although the notion that prenatal glucocorticoid exposure modifies fetal growth via altered nutrient transport is plausible, this hypothesis has not been fully investigated across different gestational times or comparing different species.

B. Amino acid transport

Most amino acids, particularly essential amino acids, are present in much greater concentrations in the fetal than in the maternal circulation (293). Hence, amino acids are actively transported across the syncytiotrophoblast from the mother to the fetus by different amino acid transport systems, within each of which there are several different isoforms. The transport systems are divided into those that transfer neutral, cationic, or anionic amino acids (284). Amino acid availability influences fetal growth in a multitude of ways: amino acids are essential for the formation of all fetal proteins and are potent stimulators for insulin secretion as a primary fetal growth-stimulating hormone (294). Over 20 different amino acid transporters with overlapping specificities are present in the placenta (284, 295, 296). In humans, fetal growth restriction is associated with a downregulation of certain placental amino acid transporters, whereas an upregulation is associated with fetal overgrowth (182, 287). Intrauterine growth restriction has been associated with a decrease in the activity of placental system A, system L, and taurine transport

(297–299). Evidence that the downregulation of system A activity precedes the development of growth restriction suggests that the downregulation of amino acid transporter activity may be part of the cause of growth restriction rather than a consequence (286).

Amino acid transport is regulated by glucocorticoids. In sheep and mice, an elevation in maternal glucocorticoids during pregnancy was associated with altered placental nutrient transfer as well as changes in amino acid transporter gene expression and endocrine signaling pathways (236, 292). Glucocorticoids increased system A activity in human term placental villous explants in culture in association with syncytial regeneration and differentiation (301). Conversely, maternal dexamethasone treatment of mice at midgestation significantly decreased system A-mediated transfer in placentas of male and female fetuses near term (302). Collectively, these data highlight the need to examine further the relationships between altered HPA axis activity and glucocorticoid exposure in mother and/or fetus with changes in placental transporter activity; glucocorticoid-induced changes in amino acid transport might underlie observations of glucocorticoid-induced changes in fetal growth and development.

VIII. Glucocorticoid Exposure and Placental Drug Transporters

Maternal chronic diseases (epilepsy, diabetes, asthma, hypertension, Crohn disease etc,), acute illnesses (infections), or even maternal cancer often require pharmacotherapy during pregnancy (303, 304). Indirect fetal treatment via maternal drug administration has been proposed in some cases of fetal cardiac dysrythmia or to prevent maternofetal transmission of HIV infection (305–307). A very high percentage (64%–96%) of pregnant women take one or more prescription medications, and as much as 5%–10% take medication considered potentially teratogenic (308–310). Under these circumstances, the placenta is the first site of exposure and, not surprisingly, is the first site of barrier protection to the fetus.

A. Structure and function of placental drug transporters

Chemical substances administered to the mother are generally able to pass the placental barrier to some degree, depending on their lipid solubility, molecular size, degree of ionization, and plasma binding (311–313). Placental metabolism of drugs and xenobiotics via phase I and II biotransformation reactions, which have been identified in the human placenta, have not been proven to play an important role in detoxification and therefore considered to be of minor importance in limiting drug passage across the placenta (313–315). As a result, mechanisms must be in place to limit fetal exposure to chemicals. Placental drug efflux transporters are able to limit the transfer of drugs and xenobiotics from the mother to the fetus. This family of proteins consists of ATP binding cassette (ABC) drug efflux proteins in the placenta, including P-glycoprotein (P-gp, multi drug resistance protein 1 encoded by *ABCB1*), multidrug-resistance-associated proteins (MRPs, encoded by *ABCC1–6* and *ABCC10–2*) and breast cancer resistance protein (BCRP, encoded by *ABCG2*) (313). P-gp, a 170-kDa protein with 2 transmembrane domains and 2 nucleotide binding domains, is localized at the microvillous membrane of the syncytiotrophoblast (316). Levels of P-gp protein are highest in early gestation and decrease by 2-fold toward term (317, 318). Multiple binding sites within the P-gp bind a wide range of hydrophilic positively charged antibiotics, chemotherapeutics, opioids, antiemetics, antiretroviral drugs, and synthetic and endogenous hormones such as cortisol, aldosterone, dehydroepiandrosterone, phosphatidylcholine, sphingomyelin, and bilirubin (313, 316). Substrate binding and extrusion from the fetal to the maternal circulation is induced by an ATP-related conformational change of the P-gp protein (319).

The second placental drug is a 72-kDa protein; BCRP is also localized on the microvillous membrane of the syncytiotrophoblast but also localized to fetal blood vessels of the villous core (320). BCRP facilitates efflux of a wide range of substrates with many overlapping substrates with P-gp. Each chemical, however, has different affinities for the transporters: antiviral medication, antibiotics, calcium channel blockers, carcinogens, and avinoids as well as endogenous substrates including porphyrins, estrones, estrogens (17β-estradiol), and glucuronide conjugates (321–323). It remains unclear which role BCRP plays in regulating the passage of these substrates into the fetal circulation (324). However, it has been suggested from experimental data that placental BCRP plays an important role in fetal protection and detoxification during pregnancy (325).

The family of MRPs consists of a large subfamily of ABC transporters (ABCC1–13) that transport a wide spectrum of organic anion drugs that overlap with P-gp and BCRP (324, 326, 327). Different placental locations have been described; MRP1 was found at the basal membrane of the syncytiotrophoblast and fetal vessel endothelium (328, 329), whereas MRP2 is localized to the apical membrane of the syncytiotrophoblast (330).

The regulation of drug transfer from mother to fetus with respect to the natural occurring placental maturational changes across gestation is not well understood. Many of the potentially teratogenic drugs that are BCRP

and P-gp substrates would freely cross the placental barrier without drug efflux transporters (331). Synthetic glucocorticoids such as betamethasone and dexamethasone are substrates for P-gp and may also have regulatory effects on placental P-gp and MRP function (316, 324, 332). It has been shown in cell lines that dexamethasone increases MRP1 mRNA expression (324, 333). Dexamethasone treatment in the mouse model resulted in a dose-dependent inhibition of BRCP function (332). Iqbal and colleagues (324) postulated that neonatal outcomes with lower birth weight and impaired neurological and behavioral function after maternal glucocorticoid exposure may in part be due to glucocorticoid-induced alterations in drug transporter function and thus their altered regulation of endogenous substrates in the fetal circulation. Especially on the background of additional drug treatment of pregnant women threatened by preterm labor, the protection of the fetus via placental drug efflux transporters may be impaired with immediate and possible long-term health consequences for the fetus.

IX. Placental Sensing Mechanisms

The adaptive ability of the fetus to respond and modulate its development in response to external stimuli must have evolved under selective pressure to benefit a population for which the ability of the population to sense and integrate environmental signals that have been historically most predictive of the future environment can be used to further the success of that population. Fetal, maternal, and placental cues that impact signaling pathways integral in placental growth, development and function as well as the ability of the placenta to compensate for changes in the prenatal environment are critical to the survival of both the mother and the fetus and as a result, the population. The placenta by its very nature, therefore, must act as a sensor to numerous endogenous and exogenous molecules. Nutrient sensing, not surprisingly, is integral in placental modulation of fetal growth and development (184, 334).

A number of different potential placental nutrient sensors have been described in the literature; the most important ones aside from the mammalian target of rapamycin (mTOR), are the amino acid response signal transduction pathway (335), AMP-activated protein kinase (AMPK) as the global cellular energy sensor (336), and glycogen synthase kinase-3 (337). Experimental studies have explored various nutritional models and their impact on these integral placental nutrient sensors. In rats, maternal protein restriction activated the amino acid response signal transduction pathway (338). Under the assumption that a low-protein diet does not result in extensive placental energy deprivation, placental AMPK activity was not altered in rats (339). In sheep, however, maternal caloric restriction increased AMPK phosphorylation but was decreased in placenta of obese sheep (340, 341). Glycogen synthase kinase-3 functions as a glucose sensor and is inhibited by insulin and IGF-1 and has been shown to be upregulated in IUGR fetuses (337, 342, 343). It has recently been proposed that placental nutrient sensing is grounded in the serine/threonine protein kinase (mTOR (287). This has been been extensively reviewed elsewhere (334). Two different complexes with different functions exist, mTOR complex 1 (mTORC1) with the accessory protein raptor and mTORC2 working with the protein rictor (344, 345). mTORC2 is not directly involved in nutrient sensing (346). mTORC1 is thought to represent a key intrinsic placental nutrient-sensing mechanism regulating amino acid transporter activity (182, 344). mTORC1 regulates cell growth by modifying protein translation according to nutrient and growth factor availability, and in this way, it is thought to function as a nutrient sensor. mTORC1 integrates various extracellular signals; nutrients (particularly amino acids) and growth factor levels are major upstream regulators of mTORC1 (347). Therefore, mTOR as an integrator of extracellular signals may be an adaptive mechanism within the placenta to modulate development based upon environmental cues. In this regard, placental nutrient sensing can influence signaling pathways relating to growth, resulting in a small fetus when the maternal supply line and food are scarce, whereas in a maternal environment with nutritional excess, the result may be fetal overgrowth (287).

Consistent with this notion, mTOR is highly expressed in the syncytiotrophoblast and is regulated by amino acids and glucose as well as growth factors, and its expression is downregulated in placentas of growth-restricted fetuses with inhibited insulin/IGF-1 signaling (343, 348–351). The primary function of the syncytiotrophoblast is nutrient transport, and it therefore determines placental and fetal growth. Inhibition of mTOR signaling by rapamycin, and a subsequent decrease in phosphorylated downstream signaling molecules eukaryotic initiation factor 4E-binding protein-1 and p70 ribosomal S6 kinase, resulted in a significant decrease in the activity of system A, system L, and taurine amino acid transporters in cultured primary trophoblast cells, without affecting the protein expression of any of the transporters (352). This is highly suggestive of placental mTOR having a role in regulating fetal growth (348). In animal models, a reduced placental mTOR activity has been found in hyperthermia-induced growth-restricted sheep, maternal low-protein diet in rats, and maternal caloric restriction in baboons (334, 339, 353). In

human pregnancies, placental mTORC1 activity is low in growth-restricted fetuses but high in pregnancies complicated by maternal obesity (334, 343, 348). This presents an interesting endocrine link between maternal obesity, placental function, and fetal growth. It has been recently shown that maternal adiponectin inhibits placental mTORC1 and was associated with IUGR; because adiponectin levels are low in obesity, this presents the possibility that in cases of maternal obesity, maternal circulating adiponectin may act on placental factors to modulate fetal growth (334, 354).

A connection between the mTOR pathway and glucocorticoid signaling has been established in cell lines (355), but the effect of glucocorticoids on placental mTOR signaling is not known. Elevated endogenous levels of glucocorticoid in the rat have been shown to suppress mTOR signaling in skeletal muscle (356). A recent study on human term placentas in women with self-reported high levels of environment-related stressful conditions during pregnancy showed a significant downregulation of Dishevelled, Egl-10, and Pleckstrin domain-domain-containing and mTOR-interacting protein (DEPTOR) as a modulator of mTOR signaling (357). There are currently no data regarding antenatal glucocorticoid exposure and placental mTOR signaling. Given the importance of antenatal administration of synthetic glucocorticoids in the management of preterm delivery, it is essential that future studies interrogate these pathways.

X. Transgenerational Effects and Epigenetic Gene Regulation

The impact of early-life glucocorticoid exposure on long-term HPA axis function is not simply limited to the immediate offspring of a challenged pregnancy but may also be transmitted through the maternal and paternal lines affecting subsequent generations, resulting in what is termed transgenerational effects (5, 358). One explanation is that fetal exposure to glucocorticoids induces alterations in the epigenetic regulation of gene expression, which are transferred to the next generation with subsequent changes in the physiology of the next generation without these individuals having been exposed themselves. In animal models, maternal stress during pregnancy (changes in diet, glucocorticoid exposure, and behavioral stress) induces physiological changes in the second generation, including increased body length, insulin resistance, modification in HPA axis and function and cardiovascular function, and anxiety or depression-like behavior (359–365). A large number of key genes involved in the regulation of the HPA axis appear to be epigenetically regulated (*GR*, *CRH*, *POMC*, *11βHSD2*, and *ERα*), and there are implications that more may exist (82, 366–370). Studies investigating the transgenerational impacts of early-life adversity are very limited. Data from the Dutch Hunger Winter studies demonstrated that pregnant women who were exposed in the first trimester of pregnancy to extreme nutrient deprivation gave birth to neonates with normal birth weight but with transgenerational effects observed in the second generation (371, 372). Changes in grandparental nutrition in humans have been linked in epidemiological studies to a greater disease risk in the second generation (373, 374). Third-generation children from parents who were exposed to the Jewish Holocaust (1940–1945) showed higher levels of fear, neurotic behavior, aggression, and social withdrawal at 4 to 13 years of age as compared with controls (375). In an elegant study from Long and colleagues (26), the multigenerational metabolic effects of clinically relevant doses of dexamethasone on F1 and F2 female offspring demonstrated decreased fetal and postnatal growth associated with long-term changes in pancreatic β-cell response to a glucose tolerance test (26). The fundamental mechanism behind those effects remains largely unexplored (358, 360, 376, 377), although 3 possible pathways have been recently suggested (358): 1) alterations in the maternal endocrine and cardiovascular adaptation to pregnancy, 2) alterations in maternal behavior after birth, and 3) transgenerational transmission of modifications in the epigenome.

Epigenetic variations may represent one of the most important mechanisms linking environmental changes during pregnancy to specific pregnancy outcomes (378). One could argue that the placenta is the most exposed organ to intrauterine environmental factors providing a footprint or memory of the prenatal insult, and therefore, investigation of placental epigenetic profiles could provide a unique window into the triggers of fetal programming (379). Despite the fact that the placenta is strictly a prenatal organ and is discarded at birth, one could use identifiable epigenetic clues within this organ that could shed light on the prenatal environment within which the fetus develops to make inferences regarding postnatal disease risk.

Epigenetic regulation of both imprinted and nonimprinted genes is important in placental development, and its disturbance can lead to abnormal placental development and function with possible consequences for the fetus and disease susceptibility in later life (380). Indeed, specific patterns of placental DNA methylation have been recently identified that could differentially classify intrauterine growth restriction and small for gestational age (SGA) placentas from appropriate for gestational age placentas (381). Because of their inherent plasticity, epige-

netic mechanisms are susceptible to environmental influences; this susceptibility is thought to be greatest during early development. As such, this process is emerging as an important regulator of the changes in gene expression undergone by the placenta during early-life adversity. Importantly, epigenetic marks may be reprogrammed in response to both stochastic and environmental stimuli, such as changes in diet and stress in the in utero environment (382, 383). Recent experimental data highlight the fact that these epigenetic modulators may be sex-specific. Gabory et al (383) demonstrated sex-specific epigenetic and transcriptomic functional differences in the mouse placenta when imposing differential maternal diets during pregnancy. In mice, a maternal high-fat diet resulted in differences in male and female fetuses not only in the number and variation of genes but also in the functions and networks involved. In a similar model, female placentas appear to have higher levels of genome-wide methylation compared with male placentas under control conditions, but when exposed to a high-fat diet during pregnancy, methylation is suppressed in female placentas (384).

Whether exposure to elevated levels of glucocorticoids mediates placental gene expression through epigenetic mechanisms is still unclear, although studies investigating the relationship between placental epigenetic gene regulation and neonatal outcome have begun to shed light on this potential regulatory pathway. Multivariate linear regression revealed a significant association between differential methylation of the placental *GR* gene and large for gestational age status (385). Furthermore, whereas placental 11βHSD2 methylation was greatest in infants with the lowest birth weights, it was associated with reduced scores of early neurobehavioral outcome in neonates (386). These data are among the first to associate fetal growth with epigenetic alterations in a gene that is central to HPA axis development. Because these alterations were associated with infant development, these data strengthen the notion that placental epigenetics is a critical potential modulator of long-term health and disease risk (387).

Recent data suggest that these epigenetic adaptations may be modulated through maternal dietary intake of methyl donors. In a study to investigate the possibility that maternal diet modulates placental and fetal cortisol-regulating genes, Jiang et al (388) found that maternal supplementation of choline (930 vs 480 mg/d) over 12 weeks yielded higher average CpG methylation in placental tissue of the *CRH* promoter region and lower transcript abundance. In cord leukocytes, higher maternal choline intake resulted in lower average promoter methylation of *CRH*. These data provide compelling evidence that maternal choline intake during the third trimester of pregnancy can modify global and site-specific epigenetic marks in fetal-derived tissues, epigenetic marks that may have long-lasting functional effects and may be suggestive of an effect of maternal choline intake on programming of the placenta and developing fetal HPA axis (388).

Other models have shown similar modulation of epigenetic gene regulation. In experimental studies, prenatal alcohol consumption reduced placental DNA methylation (389), whereas in human placentas, it slightly elevated *LINE-1* (long interspersed nuclear element-1) methylation (381). Maternal smoking resulted in placental DNA methylation with a dominant effect among oxidative stress pathways (390). Adaptive responses to smoking were demonstrated in placental tissue through an up-regulation of a placental protein (cytochrome P450, family 1, subfamily A, polypeptide 1) that catabolizes nicotine into less harmful metabolites, thereby protecting the fetus from effects of the nicotine (391). Besides understanding the implications of altered placental size, vascular abnormalities, and placental endocrine aberrations in growth-restricted fetuses, analysis of placental epigenetic gene regulation could provide a link between variations in placental function, fetal growth, and long-term disease risk. In this regard, human methylation array analysis has identified methylation loci that could be used to predict SGA or IUGR pregnancies (392). The association between placental DNA methylation and neonatal outcomes has been reviewed elsewhere (379). In SGA infants, elevated wingless-type mouse mammary tumor virus integration site family member 2 (WNT2) and 11βHSD2 were found in the placenta (387, 393). In fetuses that are large for gestational age, the GR promoter region *NR3C1* showed higher methylation levels compared with SGA and control infants (385). Methylation changes in *11βHSD2* and *NR3C1* have recently been associated with changes in infant neurobehavior and may thus provide a potential mechanism underlying the link between the placenta, fetal growth and development, and long-term disease risk (386, 387, 394).

XI. Conclusions

Glucocorticoids can alter placental growth and development and influence the development of fetal blood vessels, the placental exchange surface area, and the abundance of nutrient transporters. Glucocorticoids influence placental hormone production, modulating the allocation of early-life nutrients. Those developing organ systems that possess high levels of the GR and 11βHSD may be most vulnerable, because both circulating and intra-tissue concentrations of cortisol can have long-term effects on glucocorticoid-sensitive genes. Therefore, cross-talk between the

placenta and the developing fetal HPA axis represents an important regulator of the overall effects of glucocorticoids on long-term health and disease risk. Because increased levels of cortisol are necessary for normal fetal growth and development as pregnancy advances, it is essential to comprehensively understand the interplay between the placenta and the developing fetal HPA axis. The time period during pregnancy when antenatal glucocorticoids are given has become more and more important in the consideration of antenatal glucocorticoid practice. Organ systems show different time-dependent sensitive windows of plasticity during pregnancy and therefore may be more or less vulnerable to glucocorticoid treatment in the presence or absence of rapid organ growth.

With this knowledge, we can then better understand the effects of inappropriate exposure to high levels of either endogenous or exogenous glucocorticoids in the fetus and the potential impact on its future health.

An enhanced appreciation of placenta-mediated signaling pathways in determining long-term health in offspring will be crucial in the efforts to design interventional strategies in population at risk of disease.

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