Effects of Intrauterine Exposure to Synthetic Glucocorticoids on Fetal, Newborn, and Infant Hypothalamic-Pituitary-Adrenal Axis Function in Humans: A Systematic Review

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Background: Synthetic glucocorticoids are commonly used in reproductive medicine. Fetal organ systems are highly sensitive to changes in the intrauterine environment, including overexposure to glucocorticoids. Structural and functional alterations resulting from such changes may persist throughout life and have been associated with diverse diseases. One system that could be particularly sensitive to fetal glucocorticoid overexposure is the hypothalamic-pituitary-adrenal (hpa) axis. Many human studies have investigated this possibility, but a systematic review to identify consistent, emergent findings is lacking.

Methods: We systematically review 49 human studies, assessing the effects of intrauterine exposure to synthetic glucocorticoids on fetal, neonate, and infant hpa function.

Results: Study quality varied considerably, but the main findings held true after restricting the analyses to higher-quality studies: intrauterine exposure to synthetic glucocorticoids reduces offspring hpa activity under unstimulated conditions after pain but not pharmacological challenge. Although reduced unstimulated hpa function appears to recover within the first 2 wk postpartum, blunted hpa reactivity to pain is likely to persist throughout the first 4 months of life. There is some evidence that the magnitude of the effects is correlated with the total amount of glucocorticoids administered and varies with the time interval between glucocorticoid exposure and hpa assessment.

Conclusions: This systematic review has allowed the demonstration of the way in which intrauterine exposure to various regimens of synthetic glucocorticoids affects various forms of hpa function. As such, it guides future studies in terms of which variables need to be focused on in order to further strengthen the understanding of such therapy, whilst continuing to profit from its clinical benefits. **(*Endocrine Reviews* 30: 753–789, 2009)**

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Abbreviations: CAH, Congenital adrenal hyperplasia; CBG, corticosteroid binding globulin; CDH, congenital diaphragmatic hernia; DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; GR, glucocorticoid receptor; hCRH, human CRH; GBA, glucocorticoid bioactivity; hpa, hypothalamus-pituitary-adrenal; 11β-HSD2, hydroxysteroid (11β) dehydrogenase 2; MR, mineralocorticoid receptor; 17-OHP, 17-hydroxyprogesterone; P-gp, P-glycoprotein; PVNh, paraventricular nucleus of the hypothalamus; RDS, respiratory distress syndrome.

I. Introduction

A. Background

Synthetic glucocorticoids are commonly administered during human fetal development. For example, they successfully reduce morbidity and mortality in preterm infants by accelerating fetal lung maturation (1–3) (see *Section II.A*). However, major concerns have arisen concerning the safety of such therapy because there is evidence from animal and human studies that intrauterine exposure to synthetic glucocorticoids may compromise health and development in affected offspring (4–7). Hence, understanding the impact of synthetic glucocorticoids on fetal organ systems may help to reappraise costs and benefits of different forms of such therapy.

Over recent years, early programming by glucocorticoids has become a topic of particular interest in the scientific community, and the issue has grown to a matter of public concern. The ongoing European Project on Glucocorticoid Programming in Early Life and its Impact on Adult Health (EUPEAH), funded by the European Commission, is one example of large-scale research in this field, investigating in nonhuman primates whether intrauterine overexposure to glucocorticoids is a key trigger of adult disease programming (*e.g.*, Ref. 8).

Synthetic glucocorticoids are agonists of the glucocorticoid receptor [GR (the symbol approved by the Human Genome Organization Nomenclature Committee is NR3C1)] and predominantly act via genomic effects mediated by the GR, a nuclear transcription factor (for detailed description, see Refs. 9 and 10). Because of its marked GR expression, the fetal lung is one of the primary targets of synthetic glucocorticoids administered during fetal development. The effects of synthetic glucocorticoids on the fetal and neonatal lung have been reviewed elsewhere (*e.g.*, Refs. 11 and 12), as has their impact on other organ systems with high GR expression (13, 14), including kidney and brain (*e.g.*, Refs. 15–17). Also, the clinical outcomes that are potentially related to structural and functional changes in the brain after intrauterine exposure to synthetic glucocorticoids, including cognitive, psychological, or behavioral development, have been brought together in previous works (5, 7). In this article, we systematically review the scientific literature on human studies that assessed the effects of intrauterine exposure to synthetic glucocorticoids on the hypothalamus-pituitary-adrenal (hpa) axis.

B. Rationale for this review

Understanding the effects of intrauterine exposure to synthetic glucocorticoids on fetal and infant hpa axis is important for the following reason: fetal organ systems are highly sensitive to changes in the intrauterine environment. Structural and functional alterations that occur as a result of such changes persist throughout life and influence development and health (18). It has been broadly documented in animals that the hpa axis is particularly sensitive to early life factors, among which glucocorticoids play a key role (19). In humans, comparable effects of glucocorticoids on the hpa axis might be clinically relevant because 1) synthetic glucocorticoids are widely used in reproductive medicine; and 2) altered hpa axis activity has been linked to a number of adverse health outcomes and diseases throughout life in humans (20). Much of what we currently know about the effects of intrauterine exposure to synthetic glucocorticoids on hpa axis function comes, due to methodological and ethical constraints, from animal studies (4, 18, 21). To the best of our knowledge, although a considerable number of human studies covering this issue have been published over the last three decades, no concise review has brought these findings together to present a comprehensive picture on the current research in the field. Also, findings from human studies have not been included in recent reviews of animal findings on this topic.

C. Objectives

The objective of this article is to systematically review scientific publications on the effects of intrauterine exposure to synthetic glucocorticoids on offspring hpa function in humans. Our hypothesis was that in humans, intrauterine exposure to synthetic glucocorticoids reduces hpa axis function in the offspring.

Before presenting the methods of the systematic review and the findings from the relevant articles themselves, we will 1) give information on the clinical administration of synthetic glucocorticoids during pregnancy and its historical perspective; 2) summarize the physiological and pharmacological factors regulating fetal drug exposure; and 3) synthesize the current understanding of the functional en-

tity of the fetal hpa axis, which is somewhat different from the postnatal hpa axis or its better-characterized adult counterpart (22).

II. Pharmacological and Physiological Background

A. Clinical treatment with synthetic glucocorticoids during pregnancy and its historical perspective

Glucocorticoids modulate a wide variety of physiological processes. They influence lipid, protein, and carbohydrate metabolism, and regulate cardiovascular, neurobiological, and immunological function. Many of these effects function to maintain homeostasis and mediate coping with and adaptation to stressful situations (23). Given the crucial role of endogenous glucocorticoids in controlling most organ systems, their synthetic analogs are broadly used in clinical practice for a large variety of clinical conditions.

Specifically during fetal development, glucocorticoids stimulate surfactant production and influence structural changes, growth factors, lung fluid metabolism, antioxidant enzymes, and adrenergic receptors in the maturing fetal lung. In late gestation, fetomaternal cortisol secretion increases to complete pulmonary maturation and prepare the fetal lungs for extrauterine life (11, 24). Accordingly, preterm infants are endangered with pulmonary immaturity, which predisposes to respiratory distress syndrome (RDS). Indeed, premature birth is associated with an increased risk of neonatal mortality and morbidity, including RDS. Due to their potency in maturating fetal organ systems, synthetic glucocorticoids are commonly used in cases of high-risk preterm delivery to accelerate fetal lung maturation. Seven to 10% of all pregnancies in North America are under such risk (25). In addition, during gestation, synthetic glucocorticoids are given for several other clinical conditions of the mother or the fetus, including allergies, asthma, and congenital adrenal hyperplasia (CAH).

Gestational glucocorticoid therapy to prevent RDS goes back to the pioneering work of Liggins (26) in the late 1960s, who demonstrated that preterm lambs exposed to glucocorticoids *in utero* have less severe respiratory distress than would be anticipated, suggesting that glucocorticoids hold lung maturational properties. In their subsequent landmark randomized placebo-controlled study, Liggins and Howie (27) confirmed this observation in humans, demonstrating a more than 2-fold decrease in the incidence of RDS and a 5-fold reduction of neonatal death in premature infants exposed to betamethasone *in utero*. Since this initial investigation, numerous randomized trials examining the effects of antenatal glucocorticoid administration in humans have reported positive results. A meta-analysis of 15 randomized trials carried out between 1972 and 1994 showed that the use of antenatal corticosteroids resulted in an overall reduction of complications associated with preterm delivery, such as neonatal RDS, periventricular hemorrhage, necrotizing enterocolitis, and, most importantly, neonatal mortality (28). These results were again confirmed in a recent review that included 21 studies (3). Accordingly, in 1994, the National Institutes of Health (NIH) Consensus Developmental Conference on the Effects of Corticosteroids for Fetal Maturation on Perinatal Outcomes concluded that all fetuses between 24 and 34 wk gestation at risk of preterm delivery should be considered candidates for antenatal treatment with glucocorticoids (25). Recommended treatment consisted of two doses of 12 mg betamethasone given im 24 h apart or four doses of 6 mg dexamethasone given im 12 h apart. However, despite the NIH recommendations, the reported beneficial effects of treatment, and its potential to produce large cost savings in the health care system, obstetricians were slow to incorporate antenatal glucocorticoid use into routine practice. Implementation of treatment has taken 15 yr to rise roughly 10-fold, from 8% in 1985 to 75% in 2000 (29).

In the meantime, antenatal glucocorticoid use in fetuses at risk of preterm delivery has become common practice to prevent postnatal RDS. Treatment protocols, however, remain somewhat inconsistent across institutions and physicians. If women continue pregnancy after a single course of glucocorticoid treatment, then in some cases further courses are subsequently administered. However, due to insufficient scientific data, in 2001 the NIH Consensus Developmental Panel recommended that repeat courses should not be used routinely until insightful findings are available (30).

In sum, after hesitant beginnings almost four decades ago, synthetic glucocorticoids are in the meantime used broadly in obstetric medicine to treat fetuses at risk of preterm birth to prevent RDS.

Synthetic glucocorticoids are further administered to pregnant women due to maternal medical conditions, including autoimmune diseases, allergies, and asthma, or due to fetal needs other than lung maturation, such as CAH. In contrast to preparation for preterm birth, treatment in the latter cases often begins early in pregnancy and lasts throughout gestation.

B. Fetal drug exposure

Different synthetic glucocorticoids are used in obstetric practice. In the studies reviewed, these were in most cases one of the following: betamethasone, dexamethasone, or prednisolone. Synthetic glucocorticoids are marginally different from their endogenous equivalents in chemical

structure. Prednisolone differs from cortisol by a δ-1-dehydro configuration. Dexamethasone and betamethasone both have additional 9-α-fluoro groups, and 16-β- or 16-α-methyl groups, respectively (31). Due to these structural alterations, synthetic analogs of endogenous glucocorticoids exhibit comparatively intensified potency at the GR and reduced activity at the mineralocorticoid receptor [MR (the symbol approved by the HUGO Nomenclature Committee is NR3C2)] (10). Moreover, they vary among themselves in their pharmacokinetic and pharmacodynamic properties. These properties are partly determined by each drug's specific chemical preparation. This is clinically relevant because fetal exposure to synthetic glucocorticoids is generally controlled by the pharmacokinetic and pharmacodynamic characteristics of the specific drug 1) in the mother, including protein binding, the manner and extent of drug absorption, its distribution throughout fluids and tissues, metabolism, and elimination; and 2) in the fetoplacental unit, including the amount of drug that crosses the placenta and the drug's metabolism, distribution, and elimination in the fetoplacental unit (32).

1. Pharmacokinetic properties of synthetic glucocorticoids

a. Plasma and biological half-life. Plasma half-life represents the time that passes until one half of the initial drug concentration has disappeared from the blood (33). Betamethasone half-life in human plasma ranges between 6.5 h (34) and 9 h (35), depending on the formulation given (see below). Reported plasma half-life of dexamethasone averages 4 or 4.6 h, after oral administration of dexamethasone or iv injection of dexamethasone sodium phosphate, respectively (36). The half-life of prednisolone, as administered orally or iv in different preparations, is approximately 3 h (36). Cortisol itself has a plasma half-life of about 1.5 h (33).

The duration of measurable biological activity represented by the biological half-life is longer than plasma half-life, and synthetic glucocorticoids have been divided into short-, medium-, and long-acting substances. Although both betamethasone and dexamethasone have long-acting properties, ranging between 36 and 54 h, prednisolone belongs to the medium-acting category, and cortisol to the short-acting category with biological half-lives of 12–36 h and 8–12 h, respectively (33).

b. Protein binding. The biological activity of glucocorticoids is partly determined by rate and selectivity of protein binding because only the unbound glucocorticoid fraction is biologically active. Across several species including humans, endogenous cortisol binding to corticosteroid binding globulin [CBG (the symbol approved by the HUGO Nomenclature Committee is SERPINA6] ranges between 67 and 87%, whereas a further 7 to 19% of total cortisol is bound to albumin (37), leading to about 95% cortisol being protein-bound in the plasma. Cortisol binding decreases as its concentration increases (38). Prednisolone binds to both proteins (39): with increasing prednisolone concentrations from below 200 to above 700 μg/liter, protein binding decreases nonlinearly from 95 to 60% (40). Conversely, in human plasma, betamethasone and dexamethasone bind predominantly to albumin, which has high capacity but low affinity for ligating, whereas both steroids bind only marginally to CBG (38, 41). Dexamethasone displays higher protein affinity than betamethasone; plasma binding of these synthetic glucocorticoids is 75 and 60%, respectively, and is quite constant across a wide concentration range (36, 38, 42). It is unknown whether different preparations of synthetic glucocorticoids have variable effects on protein binding.

c. Glucocorticoid potency. The primary synthetic glucocorticoids, including betamethasone and dexamethasone, act predominantly via genomic effects mediated by the GR, in accordance with its nuclear transcription factor function (9, 10). The potency of a synthetic glucocorticoid, *i.e.*, a measure of its activity in a biological system, depends on its affinity for its receptor and its efficacy. Genomic glucocorticoid potency of betamethasone was reported to be moderately higher than that of dexamethasone, with both steroids having a 25-fold higher, and prednisolone a 4-fold higher, affinity to the GR than does cortisol. At higher doses, when nonspecific, nongenomic effects that are mediated by steroid-selective membrane receptors come into play, potency of dexamethasone is still 5-fold stronger than potency of prednisolone, whereas potency of betamethasone is lower than that of prednisolone (43). Affinity to the MR was shown to be relatively low in each of the three synthetic preparations, with prednisolone displaying the most pronounced mineralocorticoid potency of the three (10).

d. Phosphate or acetate. Betamethasone is available in two different prodrug formulations: a fast-releasing phosphate ester and a slow-releasing, dual-acting suspension containing phosphate and acetate ester. The two formulations differ in their pharmacokinetic characteristics in human plasma, with the dual formulation being linked to an approximately 30% longer half-life than the phosphate ester (34, 35), raising the possibility of unwanted side effects in the mother and fetus due to longer exposure. Indeed, women treated with the suspension containing phosphate and acetate ester have recently been shown to have an increased incidence of maternal infection (44). In the latter review, however, effects of the dual formulation of beta-

methasone were compared with effects of dexamethasone phosphate. It thus remains unanswered as to whether pure betamethasone phosphate impacts on maternal health in a similar fashion. The different prodrug formulations have been directly compared in a sheep model where the divergence of effects is even more pronounced, with the dual formulation being associated with a more than 3-fold longer half-life than the phosphate ester (45). However, other researchers did not replicate these findings, demonstrating relatively short plasma half-lives of 3 h in both formulations (46).

Interestingly, the dual formulation has been associated with lower peak maternal and fetal betamethasone concentrations, reduced by approximately 50%, when compared with those observed after injection of a similar dose of the phosphate ester. This finding suggests that, due to little betamethasone release from the acetate, the dual formulation will have less effect on maternal and fetal function (46), thereby outweighing the concern about adverse effects on mother and fetus due to longer half-life.

Taken together, the dual betamethasone suspension seems to have a longer half-life, whereas the fast-releasing betamethasone phosphate seems to result in higher peak concentrations. Both pharmacokinetic properties may be linked to unwanted side effects. Whether the type of betamethasone formula used in the reviewed studies has influenced the effects of synthetic glucocorticoids *in utero* on hpa axis needs to be taken into account.

2. Pharmacokinetic properties of synthetic glucocorticoids in the mother and fetus during pregnancy

Most studies have examined the pharmacodynamic and pharmacokinetic properties of synthetic glucocorticoids in nonpregnant women. However, physiological changes that occur during pregnancy, such as changes in the renal, gastrointestinal, cardiovascular, and immune system (47), lead to variations in the processes of absorption, distribution in fluids and tissues, metabolism, and elimination of different kinds of drugs, including synthetic glucocorticoids (32, 47–50). Therefore, findings from nonpregnant women may not be transferable to pregnant women.

In accordance with this assumption, Petersen *et al.* (51) demonstrated that betamethasone clearance and volume of distribution are higher in pregnant than in nonpregnant women, whereas the half-life remained unchanged. Furthermore, pharmacokinetic properties seem to differ between pregnancies, for instance varying with the plurality of birth (35). Accordingly, the half-life of betamethasone in mothers with twin pregnancies was shown to be significantly shorter than in mothers with singleton pregnancies. Interestingly, pharmacokinetic characteristics of betamethasone are different in mother and fetus, as studied in ewes. It is clinically relevant that after injection into maternal muscle tissue, as is common practice in obstetrics, betamethasone half-life is longer in fetal than in maternal circulation, suggesting that fetal drug exposure is more extensive than would be anticipated from maternal pharmacokinetic data (52).

Secondary to plasma volume expansion, maternal serum concentrations of albumin decrease progressively during pregnancy, despite elevated maternal albumin synthesis, resulting in decreased protein-binding capacity (53, 54). This may be clinically important as the unbound, biologically active fraction of synthetic glucocorticoids such as betamethasone or dexamethasone, as they bind to albumin specifically, subsequently increases. Consequently, because only the unbound drug fraction passes the placenta, a greater amount of synthetic glucocorticoids reaches the fetus as pregnancy proceeds. However, fetal albumin concentrations gradually increase during gestation, equaling (55) or even exceeding (56) maternal albumin concentrations at term. Therefore, more glucocorticoid is bound in fetal plasma, thereby partly reducing the drug's biological activity.

It has been argued that enhanced hepatic and renal elimination in the pregnant female might influence the concentration of free drug in the mother and fetus. This protective mechanism has, however, not yet been proven for synthetic glucocorticoids. Moreover, there is some evidence that the fetus itself is capable of metabolizing and eliminating drugs, although most enzymatic processes are immature (32).

3. Transduction from mother to fetus: placental transfer of synthetic glucocorticoids

Drug/substance permeation across the placental barrier is determined by diverse factors including drug properties, placental characteristics, and additional maternal and fetal influencing factors (57).

Placental hydroxysteroid (11β) dehydrogenase 2 [11β-HSD2 (the symbol approved by the HUGO Nomenclature Committee is HSD11B2] represents one key enzyme that selectively regulates the transplacental passage of glucocorticoids (6). Therefore, although endogenous glucocorticoids are highly lipophilic and rapidly cross the placenta, normally the fetus has much lower concentrations than its mother (58, 59). Located in placental syncytiotrophoblast (60), 11β-HSD2 catalyzes the rapid conversion of cortisol and corticosterone to inert 11-keto forms as shown *in vitro* and *in vivo*, forming a potent albeit incomplete barrier (61). However, transplacental passage differs considerably between endogenous and synthetic glucocorticoids (62). After a 1-h incubation, more than 95% of corticosterone, 63% of cortisol, but only 17% of dexamethasone had been metabolized in

mammalian cells transfected with 11β-HSD2 (60). Furthermore, different pharmaceutical preparations of synthetic glucocorticoids have varying 11β-HSD2 metabolism profiles based on their chemical structures. In contrast to endogenous glucocorticoids, 9α-fluorinated glucocorticoids such as betamethasone and dexamethasone show weak oxidase but strong reductase activity with 11β-HSD2, thereby decreasing local inactivation. Conversely, reduction by 11β-HSD2 is increased by the δ-1-dehydro configuration in prednisolone (31). In accordance with this observation, in an early study, Blanford and Murphy (63) found that 67% of cortisol and 52% of prednisolone, but only 2% of dexamethasone and 7% of betamethasone, were converted by 11β-HSD2 to their inactive 11-keto metabolites in human placental tissue *in vitro*. Accordingly, clinical studies demonstrated 10-fold lower prednisolone (64) but only 3-fold lower betamethasone (48, 65) concentrations in fetal compared with maternal plasma when examining cord and maternal blood. In a recent investigation, one group monitored placental metabolism of diverse synthetic glucocorticoids over time, revealing that betamethasone is more rapidly metabolized than dexamethasone and prednisolone in the human placenta, with their 11-keto metabolites being first observed after 60, 120, and 240 min, respectively (66). Comparing rates of inactivation by 11β-HSD2 between these three substances, metabolism of both betamethasone and prednisolone is significantly greater than that of dexamethasone (66). Interestingly, synthetic glucocorticoids have themselves been shown to up-regulate placental 11β-HSD2 activity (67–69) and, hence, to amplify the placental barrier. However, this up-regulation does not seem to significantly affect actions of synthetic glucocorticoids in the fetus (68, 69).

There is evidence that in conjunction with 11β-HSD2, the ATP-binding cassette, sub-family B (MDR/TAP), member 1 [also known as P-glycoprotein (P-gp), the symbol approved by the HUGO Nomenclature Committee is Abcb1] augments the placental glucocorticoid barrier, reducing fetal exposure to elevated levels of glucocorticoids (70). P-gp removes substances, including synthetic glucocorticoids, from cells by ATP-dependent extrusion (71, 72). P-gp is highly expressed in syncitiotrophoblast cells of the placenta (73, 74). It has recently been shown that P-gp activity decreases in the mouse, guinea pig, and human placenta in late gestation, suggesting that the ability of the placenta to exclude synthetic glucocorticoids from the fetus decreases in late gestation (73–78).

Once having entered the fetus, synthetic glucocorticoids can take effect in the fetus, including the fetal brain. In adulthood in the rat, P-gp activity at the level of the blood-brain barrier inhibits betamethasone, dexamethasone, and prednisolone from entering the brain (79–83). However, although present in fetal brain at the end of gestation in rats, levels of P-gp transcripts are low in the fetal blood-brain barrier and increase considerably within the first 60 d postpartum (84). Extrapolating from these findings, the fetal blood-brain barrier would be expected to be considerably more permeable to synthetic glucocorticoids than the blood-brain barrier in postnatal life stages.

In conclusion, there is ready transplacental passage of synthetic glucocorticoids. Once having passed the placental barrier, glucocorticoids may become active in the fetus including the fetal brain, thereby possibly impacting on the developing systems that express GR, for example the hpa axis.

C. Ontogeny of glucocorticoid receptors

The primary synthetic glucocorticoids, including betamethasone and dexamethasone, act via the intracellular GR that binds cortisol with low affinity and synthetic glucocorticoids with high affinity (see *Section II.B.1*). According to the broad physiological functions of endogenous glucocorticoids (see *Section II.A*), which progressively occupy GRs after stress or during the circadian rise (85), GR is expressed in most organ systems, including the brain. In the brain, regions of high GR expression include the amygdala (86), hippocampus (87, 88), and the paraventricular nucleus of the hypothalamus (PVNh) (87, 89). The hypothalamus projects via the median eminence to the pituitary gland, which also expresses high levels of GR (90, 91). Thus, GR expression is high in those regions, namely the amygdala and hippocampus, that regulate CRH synthesis and release in the PVNh, as well as being high in the pituitary, which responds to CRH with increased ACTH release into the circulation. In short, GR is a major modulator of the hpa axis, which is the major source of endogenous glucocorticoid hormones.

Whether synthetic glucocorticoids administered during gestation exert effects on the fetal hpa axis thus depends on the stage of maturation of GR expression in the fetal hpa axis or those regions involved in its regulation, including the hippocampus (92, 93). In mice, from around midgestation until term, the fetal zone of the placenta expresses GR mRNA to a much greater extent than does the decidual zone (94); this suggests that synthetic glucocorticoids may become active at the placental level at an early developmental stage. In the human fetal hippocampus, GR mRNA expression was detected as early as 24 wk gestation and might emerge even before that time; that is, the receptor is present in the fetus at the time when synthetic glucocorticoids are given to the mother to accelerate fetal lung maturation (95). To date, virtually nothing is known about the ontogeny of GR in the fetal PVNh or pituitary in humans.

In sheep that have a pattern of prenatal brain development showing certain similarity to humans and have a high

degree of neurological maturity at birth (96), it has been shown that in mid-late gestation, high levels of GR mRNA are present in the fetal cerebral cortex, hippocampus, and PVNh (97). In the mouse fetus, GR gene expression in hypothalamus and pituitary was detected around midgestation with striking increases in the pituitary toward the end of gestation, when glucocorticoid production rises. In contrast, GR mRNA expression remains low in the mouse fetus adrenals throughout gestation (94). In the common marmoset (*Callithrix jacchus*), GR expression levels in the hippocampus and PVNh of neonates are similar to those of adults. This indicates that brain GR expression is apparently already mature at birth and implies that fetal GR expression will also be considerable, at least in those species that give birth to relatively mature offspring (98).

This early presence of GRs in the fetal brain, combined with the pharmacokinetic evidence that synthetic GR agonists administered to the mother enter the fetus, including the fetal brain, indicate that brain GRs will be substantially activated in the fetus after glucocorticoid treatment. As a result of this, offspring hpa axis function could be acutely or chronically influenced (99).

D. Development of hpa axis

The fetal hpa axis exhibits a number of important differences from the hpa axis at various postnatal life stages, which has been described extensively elsewhere (*e.g.* Ref. 22). The hypothalamus, pituitary, and adrenal gland are each subject to dynamic morphological and/or functional changes as they mature, but do attain adult status and function soon after birth. Furthermore, the placenta is a principal regulator of the fetal hpa axis (for details see below). Thus, it would be more appropriate to use the term "hpa axis of the fetoplacental unit" or "fetal hypothalamic/placental-pituitary-adrenal (hppa) axis." Because the hpa axis is a well-defined concept in literature, we stick to the term "fetal hpa axis" which, however, includes reciprocal effects between the fetal hpa axis and the placenta.

The present knowledge about the developmental, including functional, biology of the human and nonhuman primate fetal hypothalamus, pituitary, and adrenal cortex has been reviewed in detail elsewhere (100–103). Based on these works, in the following section, we summarize function, anatomy, and development of the embryonic and fetal hpa axis throughout gestation.

1. Hpa axis function

Hormones produced by the human fetal adrenocortical system control intrauterine homeostasis and the maturation of fetal organ systems necessary for extrauterine life, including lungs, liver, and gut, and establish the estrogenic milieu of pregnancy. Therefore, adequate development and function of the fetal adrenocortical system and structures involved in its regulation are critical to ensure fetal maturation. Furthermore, the endocrine system itself is subject to diverse morphological and functional changes to prepare for extrauterine survival. After birth, the major physiological role of the adrenocortical system is to synthesize and secrete glucocorticoids for the maintenance of metabolic homeostasis and the stress response and mineralocorticoids for the maintenance of fluid and electrolyte balance (102).

2. Anatomical and functional development of the hpa axis

The human fetal hypothalamus is defined by the seventh week of gestation (101). CRH-positive fibers exist by the 16th week of fetal life (104). An intact portal system for the transport of hypothalamic-releasing factors to the pituitary gland is detectable at 11.5 wk gestation, allowing the establishment of hypothalamic control over pituitary corticotropes around midgestation (105). In the pituitary gland, a rudimentary adenohypophysis can be identified by 6 wk gestation, maturing within the subsequent 8 wk (101). Immunohistochemical studies demonstrate the presence of corticotropes at 7 wk gestation (106) and, in accordance with this observation, ACTH secretion occurs at 8 wk gestation (107). The anlage of the adrenal cortex is detectable from the fourth week of gestation. By 8 wk, the embryonic adrenal cortex is clearly identifiable with its characteristic zonal partitioning (102).

The primate fetal adrenal cortex is mainly composed of three morphologically and functionally different compartments, discerned on the basis of expression of specific steroidogenic enzymes: the fetal, the definitive, and the transitional zone (102). The former comprises a good portion of the fetal adrenal cortex, representing the primary site of growth and steroidogenesis. The fetal zone produces the androgenic C19 steroid dehydroepiandrosterone sulfate (DHEA-S), which is quantitatively the principal steroid product of the primate fetal adrenal gland throughout gestation. The exiguous definitive zone is the likely site of mineralocorticoid production in late gestation, whereas the smaller transitional zone has the capacity to produce glucocorticoids, thus being the precursor of the adult zona fasciculata (102).

Rapid growth of the fetal adrenal cortex, especially the fetal zone, begins after 10 wk gestation and goes on to term; by midgestation, the fetal zone clearly dominates. Between 20 and 30 wk, the size and weight of the fetal adrenals double, achieving a relative size 10 to 20 times that of the adult adrenal, further doubling until term (102). By 30 week gestation, the fetal adrenal cells begin to remodel, taking on a rudimentary appearance of the adult adrenal cortex (108).

Steroidogenic activity starts during early gestation. Low levels of estriol, indicative of DHEA-S production in

the fetal adrenals (109), can first be detected in the maternal circulation after 8 to 10 wk gestation. About 2 to 4 wk later, estriol concentrations in the maternal circulation increase rapidly up to 100-fold. Subsequently, activity of the fetal zone continues to increase during the second and third trimesters, ultimately producing around 200 mg DHEA-S/d at term (102).

Observations of infants with CAH, as well as *in vitro* and *in vivo* studies, indicate that the fetal adrenals begin to produce cortisol between 10 and 20 wk gestation, possibly utilizing progesterone as precursor (102, 110, 111). However, *de novo* synthesis of cortisol from cholesterol is established fairly late in gestation, leading to a remarkable increase of cortisol concentrations in the third trimester (102).

Taken together, hpa axis structures emerge early during pregnancy, grow rapidly, and take on steroidogenic activity by about 8 to 10 wk gestation.

3. Hpa axis regulation

The fetal hpa axis can be stimulated by acute hypoxia, systemic hypotension, hemorrhage, psychological stress, noxious stimuli, and neuropeptides and can be inhibited by glucocorticoids and vagal stimulation (103). As reported in a series of studies by Giannakoulopoulos *et al.* (112, 113), the human fetus can mount hormonal stress responses to invasive stimuli, with rises in β-endorphin, cortisol, and noradrenaline. Interestingly, fetal stress responses were shown to be independent of maternal hormonal reactions to invasive procedures (114).

Ng and co-workers (103) have illustrated the neuroendocrine interaction between the fetal hpa axis and the placenta. In short, ACTH secreted from the fetal pituitary is the principal regulator of the human fetal adrenal cortex. However, fetal adrenal development is further influenced by other elements acting independently or synergistically with ACTH, including growth factors, transcription factors, and placental factors including CRH (102, 115). Placental CRH was shown to stimulate the production of proopiomelanocortin and ACTH in the fetal pituitary (116, 117) and within the placental syncytiotrophoblast cells (118), thereby regulating growth and function of pituitary corticotrope cells and adrenal cortex (102, 103). Because placental and hypothalamic CRH are identical in immunoreactivity, bioactivity, and structure, it is possible that both fetal hypothalamic and placental CRH stimulate fetal pituitary, and in a comparable manner. However, because umbilical venous CRH concentrations were shown to be higher than arterial levels, circulating CRH seems to be largely of placental origin (119).

Apart from enhancing adrenal secretion of cortisol, which in turn reduces the activity of the axis via negative feedback mechanisms (103, 120), fetal ACTH also stimulates synthesis of DHEA-S (103), which itself is a substrate for placental estrogen synthesis (109). In the baboon at least, placental estrogens were shown to induce the inactivation of cortisol to cortisone in the placenta. This reduces fetal exposure to maternal cortisol and thus decreases inhibition of the fetal hpa axis via negative feedback by maternal cortisol, thereby maintaining the levels of fetal ACTH, cortisol, and DHEA-S synthesis. It has been proposed that this mechanism may underlie the rapid growth of the fetal adrenal cortex around midgestation (121, 122). Furthermore, fetal adrenal glucocorticoids stimulate placental CRH production, thereby establishing a positive feedback circuit in the fetoplacental unit, leading to constantly increasing CRH, ACTH, and cortisol concentrations toward late gestation (103).

Taken together, the fetal neuroendocrine system is subject to complex regulation via negative and positive feedback mechanisms.

4. Adaptation of the hpa axis to extrauterine life

Immediately after birth, the fetal zone of the adrenal cortex degenerates extensively, whereas the zona fasciculata matures within 3 wk (123). Mesiano and Jaffe (102) argue that despite the dramatic remodeling of the adrenal cortex, there is no evidence of adrenocortical insufficiency in term infants during this critical period because adult cortical zones become functional before birth. Indeed, serum levels of cortisol were shown to gradually increase after birth in term infants (124). Even preterm infants born between 24 and 32 wk gestation exhibited a rise in cortisol concentrations within the first day postpartum, presumably due to the stress of delivery. Thereafter, values decreased again up to age 4 wk, but did not fall below concentrations observed around birth (125). However, other investigators showed a rapid decline in ACTH, cortisol, and cortisone precursors, accompanying the decrease of DHEA-S, in term and preterm infants immediately after birth with a nadir approximately 2 months postpartum (100). Further studies are required to clarify this issue.

It is of note that, compared with term infants, premature infants are atypical in that they maintain fetal patterns of hormonal regulation until remodeled adrenals become functional. Not surprisingly, therefore, distinct patterns of hormonal activity, primarily linked to gestational age, birth weight, and state of health, have been reported in premature infants (100, 101, 103).

Reports of the actual patterns of cortisol concentrations in premature infants are rather inconsistent. On the one hand, serum cortisol levels have been shown to be negatively correlated to gestational age, with infants born at shorter gestational ages displaying higher cortisol concentrations within the first year after birth than those born closer to full term (124, 126). It can be hypothesized that

the increased cortisol production rate in preterm infants represents a sustained intrauterine pattern of hormone production that is characterized by a dramatic rise in cortisol production before parturition, whereas comparably lower production rates in infants with higher gestational ages may go along with the adrenal remodeling that takes place around birth. On the other hand, low birth weight infants born between 23 and 32 wk gestation meet criteria for reduced basal endocrine function (127). Preterm infants display significantly increased concentrations of cortisol precursors, including 17-hydroxyprogesterone (17-OHP) and 11-deoxycortisol, compared with term infants, whereas basal cortisol values are similar in the preterm and term groups (128, 129), indicating reduced activity of some steroidogenic enzymes due to adrenal immaturity.

Furthermore, whereas some authors demonstrated fairly adequate adrenocortical responses after pharmacological challenge with CRH in premature infants (130, 131), others observed a decreased adrenal reserve after CRH challenge (127, 132). In sum, the endocrine patterns in preterm and term infants around birth have not yet been fully elucidated and may be subject to a wide range of influencing factors. As noted above, the ontogeny of GR in hypothalamus and pituitary have also not yet been elucidated.

Taken together, hpa axis activity starts early in embryonic life, passing through dynamic changes throughout gestation and the neonatal period up to adult life. Adequate maturation of the hypothalamus, pituitary, adrenal cortex, and systems involved in the regulation of the hpa axis serves appropriate ontogeny. Disturbances of this complex maturational process may thus result in a dysfunctional endocrine system later on, with potential disturbances due to synthetic glucocorticoid activation of GRs in fetal hypothalamus, pituitary, or structures involved in their regulation, particularly in at-risk fetuses, being the focus of this review.

III. Methods of the Systematic Review

We conducted the systematic review according to the guidelines set forth by the Meta-analysis of Observational Studies in Epidemiology (MOOSE) group for reporting of systematic reviews of observational studies (for the MOOSE checklist, see Supplemental Material 1, published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org) (133).

Within this systematic review (see Ref. 134 for definition), we integrated scientific publications describing the effects of intrauterine exposure to synthetic glucocorticoids on fetal, newborn, and infant hpa function in humans. We applied an explicit and reproducible strategy to locate, appraise, and select evidence and give directives for future research. Because we did not review an effect of treatment on pathology, but rather systematically reviewed the evidence for the effect (or lack of effect) of intrauterine exposure to a class of substances (*i.e.*, glucocorticoids) on physiological indicators of a complex physiological system (*i.e.*, hpa axis), this resulted in strongly heterogeneous sources of evidence. Consequently, we did not apply quantitative techniques from meta-analyses, but nonetheless still systematically integrated the evidence available to date.

A. Criteria for including studies in this review

1. Types of study design

All randomized, controlled studies and quasi-experimental studies (allocation of women to study groups on the basis of nonrandomized clinically prescribed treatment) comparing the effects of intrauterine exposure to synthetic glucocorticoids *vs.* placebo or *vs.* no treatment on hpa function were considered for inclusion in this review. Furthermore, studies comparing the effects on hpa function of different glucocorticoid doses or different time intervals between treatment and birth or treatment and sample collection were taken into account. Other study designs (*i.e.*, case reports and studies assessing the effects of intrauterine glucocorticoids on hpa activity within other primary research questions) were also included in this review, so as to present as complete a picture as possible of the research conducted in this field. Case reports were kept where external reference values were applied to analyze the effects of intrauterine glucocorticoid exposure. As recommended, we used broad inclusion criteria and then performed analyses relating design features to outcome (133). This is the first systematic review on this subject in humans to date.

2. Participants and type of exposure

We included all investigations of the offspring of women who were treated during pregnancy with any kind of synthetic glucocorticoid due to any medical condition and regardless of other comorbidity. We aimed at studying the offspring, regardless of gestational age at birth, birth weight, and health status, at fetal, neonatal, infant, or adult life stage. Hence, no restrictions were placed.

3. Outcome measures

Outcome measures of interest were those considered to be directly indicative of hpa axis activity and comprised: ACTH, cortisol, the cortisol precursors 11-deoxycortisol and 17-OHP, the cortisol metabolite cortisone, the quan-

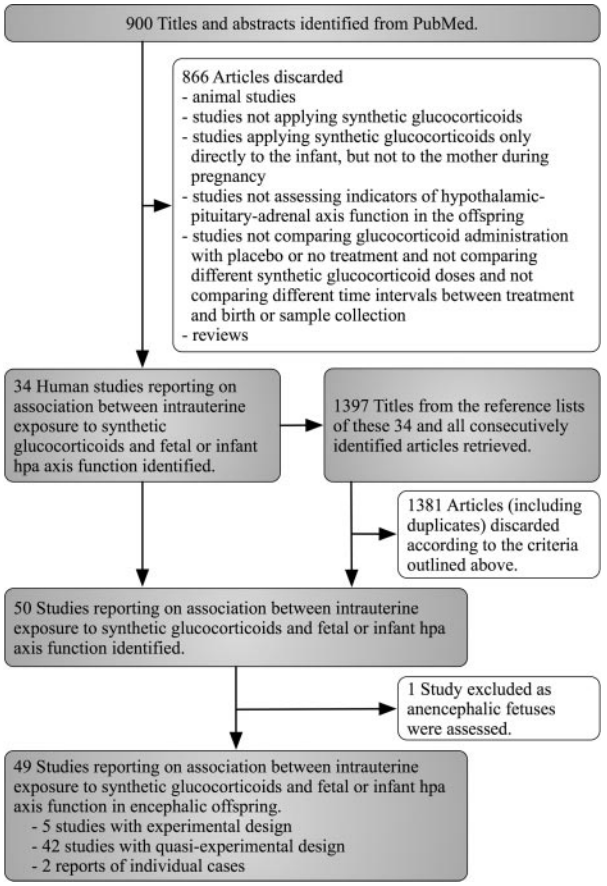

FIG 1. Flowchart of the literature search for studies on the association between intrauterine exposure to synthetic glucocorticoids and fetal, newborn, and infant hpa axis function.

titatively principal steroid hormone of the fetal adrenal gland dehydroepiandrosterone (DHEA), its sulfated form DHEA-S, and placental CRH mRNA. These substances were measured in amniotic fluid, (cord) blood, saliva, or placental tissue.

B. Search strategy for identification of studies

We searched PubMed from the U.S. National Library of Medicine via the web-based search and retrieval system provided by the National Center for Biotechnology Information, from January 1965 to July 2007, using the web-browser Firefox. We placed no restrictions on language. A detailed description of the search strategy is provided in Supplemental Material 2.

The articles identified by this search in PubMed were assessed according to the criteria of consideration for this review outlined above. We also contacted several authors for unpublished and unrecognized studies on the topic. There was no further contact with authors. Furthermore, we hand-searched bibliographies of identified original publications and evaluated them according to the criteria. The flowchart of the literature search for studies on the association between intrauterine exposure to synthetic glucocorticoids and fetal, newborn, and infant hpa function is provided in Fig. 1.

C. Methods of the review and study characteristics

1. Selection and coding of the data

Two investigators (M.T. and G.M.) reviewed all articles independently. If there was ambiguity in selection or coding, the two investigators discussed this issue and resolved disagreements until they reached consensus. We identified 50 published studies from the last 32 yr that met our inclusion criteria. One of the 50 studies assessed anencephalic fetuses (135). Because anencephalic fetuses do not have an intact hpa system and rarely survive the first 4 wk after birth (136), we decided to exclude this study from the review. Hence, 49 studies were finally included in the analyses.

The extracted data included the results regarding the effect of synthetic glucocorticoids on the hpa axis, as well as study design, number of subjects, type of glucocorticoid administered, route of administration, details on preparation, number of administrations and dose, treatment indication, gestational age at birth, and birth weight. The extracted data are presented in tables as Supplemental Materials 3, 4, and 5.

2. Assessment of heterogeneity

Heterogeneity of studies was assessed with regard to relevant study characteristics, including design, type of glucocorticoid, treatment indication, measure of hpa activity, data collection method, control of potential confounders, and sample size. In sum, there was substantial heterogeneity of the studies in several key aspects. Of the 49 studies, five used experimental and 42 used quasi-experimental designs, whereas two authors reported on individual cases exclusively. Apart from the case reports, the mean number of subjects was 108 (median, 61; range, 6 to 710). The main glucocorticoid agents studied were betamethasone (in 31 studies) and dexamethasone (in 17 studies). These were primarily administered in cases at risk of preterm delivery to induce fetal lung maturation using an im route of administration. However, other glucocorticoid agents (*e.g.*, prednisolone) and other treatment indications (*e.g.*, asthma) were also studied (see Supplemental Materials 3, 4, and 5).

3. Description of method to integrate the study results

As previously suggested, in case of substantial heterogeneity across studies, pooled estimates should not be published (137). Although several procedures exist that allow for quantitative synthesis despite heterogeneity, we refrained from applying them because in our case their caveats (138) would have outweighed the advantage of pro-

ducing summary scores with questionable explanatory power. Instead, we separately summarized the results according to: 1) several aspects and ontogenetic time points of hpa function (namely basal function in fetus, basal function in neonate/infant/adult, hpa reaction to pharmacological/physiological challenge, and hpa reaction to psychological challenge); 2) amount of glucocorticoid administered; and 3) time interval between treatment and sample collection. To address the problems of potential bias due to restrictive study inclusion as well as heterogeneity, we used broad inclusion criteria for studies but then grouped the findings according to study and outcome features.

4. Assessment of study quality, including control for confounding

We individually assessed relevant methodological aspects in separate study quality indicators and explored their influence on the results (139). The quality indicators included randomization, comparability of treatment and control group or adequate control for confounding, homogeneity of type of treatment/treatment indication, and sample size. Randomization quality was scored as: no randomization (0), partial randomization (1), or complete randomization (2). Comparability of treatment and control group or adequate control for confounding (*e.g.*, inclusion as covariate), with respect to indication, gestational age at birth (evaluated as comparable, if difference of group means <1 wk), and birth weight (evaluated as comparable, if difference of group means <100 g), was scored as: comparable/controlled in one or no parameter (0), comparable/controlled in two parameters (1), or comparable/controlled in all three parameters (2). Homogeneity of type of treatment and treatment indication was scored as: both nonhomogenous (0), only one homogenous (1), or both homogenous (2). Sample size was scored as number of subjects with exposure to glucocorticoids: fewer than 25 (0), 25–50 (1), or more than 50 (2). We took sample size as a study quality criterion into account, instead of calculating *post hoc* power analyses because the latter procedure has been strongly criticized (140, 141), and, moreover, the determination of appropriate and comparable effect sizes would have been mostly unfeasible due to large heterogeneity of design and statistical analyses, and, in some of the earlier publications, lack of report of detailed statistics. The results of the quality scores are depicted in Table 1. The studies differed substantially in their quality according to all quality indicators. We therefore stratified the findings according to indicators of study quality (see Ref. 133). This allowed us to take into account the study characteristics that may have influenced the study results, especially study design, heterogeneity within studies, and sample size.

IV. Results

The description and integration of study results are divided into five sections: 1) the effects of synthetic glucocorticoids on basal hpa function in the fetus; 2) the effects of synthetic glucocorticoids on basal neonate, infant, and adult hpa function—we define "basal hpa function" as hpa function without systematic pharmacological or psychological stimulation as opposed to pharmacologically or psychologically stimulated hpa function (142); 3) the effects of synthetic glucocorticoids on neonate and infant hpa function after pharmacological or physiological challenge; 4) the effects of synthetic glucocorticoids on neonate and infant hpa function after psychological challenge; and 5) the relationships between the amount of glucocorticoid administered, or the time interval between treatment and sample collection on the one hand, and basal and reactive hpa function in the fetus, neonate, and infant on the other hand. Supplemental Materials 3, 4, and 5 summarize the major findings of the studies.

A. Effects of intrauterine exposure to synthetic glucocorticoids on basal hpa function

1. Effects in the fetus

A number of studies assessed basal hpa function in the fetoplacental unit by measuring markers of fetal hpa activity in cord blood and amniotic fluid during gestation and at birth. Compared with unexposed healthy fetuses, cortisol concentrations are significantly lower in otherwise healthy fetuses exposed to synthetic glucocorticoids *in utero*, with values decreasing to only 10% of the controls (59, 65, 68, 143–155). Likewise, fetuses with congenital diaphragmatic hernia (CDH) exposed to synthetic glucocorticoids *in utero* exhibit significantly lower plasma cortisol concentrations than both healthy fetuses and CDH fetuses not exposed to betamethasone *in utero* (156). In fetuses of asthmatic mothers who refrain from taking synthetic glucocorticoids during pregnancy, cortisol concentrations are significantly increased compared with fetuses of healthy controls; in fetuses of treated asthmatic mothers, a dose-dependent restoration of cortisol levels down to control values occurs with increasing glucocorticoid doses (157), suggesting that synthetic glucocorticoids suppress the hpa axis in the fetus. Similarly, placental CRH mRNA is slightly increased in asthmatic parturients not treated with synthetic glucocorticoids compared with controls, whereas treated asthmatic parturients exhibit normal levels irrespective of the treatment dose (157).

Similar to cortisol, ACTH (150, 156), DHEA (156), and DHEA-S (143, 152) concentrations are reduced in treated fetuses.

TABLE 1. Quality indicators of studies that assessed in individuals exposed to synthetic glucocorticoids *in utero* the hpa axis function under basal conditions, after pharmacological challenge, and after pain-related stimulation

	Study quality indicators			
First author, year (Ref.)	Randomization[a]	Comparability of treatment and control groups[b]	Homogeneity of type of treatment and treatment indication[c]	Sample size[d]
Basal conditions				
Arnold, 1998 (164)	0	0	0	0
Ballard, 1975 (65)	1	1	1	1
Balllard, 1980 (143)	1	0	2	2
Banks, 1999 (200)	0	0	1	2
Dalziel, 2005 (184)	2	2	2	2
Dorr, 1986 (144)	0	0	2	0
Dorr, 1993 (158)	0	0	2	1
Gatelais, 2004 (198)	0	0	2	2
Gennser, 1976 (145)	0	1	2	1
Kajantie, 2003 (68)	0	1	2	2
Kajantie, 2004 (147)	0	1	2	2
Karlsson, 2000 (166)	0	1	2	2
Kauppila, 1978 (148)	0	0	2	1
Kavelaars, 1999 (149)	0	0	1	1
Manabe, 2005 (159)	0	0	0	0
Marinoni, 1998 (150)	0	1	2	0
Murphy, 2002 (157)	0	1	1	2
Murphy, 2003 (69)	0	1	1	2
Ng, 1997 (131)	0	1	1	0
Ng, 2001 (199)	0	0	2	2
Nordenstrom, 2001 (181)	0	0	0	2
Nykänen, 2007 (160)	0	0	1	1
Ohrlander, 1975 (151)	1	1	2	0
Osathanondh, 1977 (59)	2	0	2	0
Paddock, 2004 (156)	0	0	2	0
Parker, 1996 (152)	0	0	2	2
Shulman, 2001 (177)	0	0	0	2
Sippell, 1980 (153)	0	0	2	0
Sybulski, 1977 (154)	0	0	2	1
Terrone, 1997 (169)	0	2	2	2
Wittekind, 1993 (168)	0	0	2	0
After pharmacological challenge				
Battin, 2007 (170)	1	2	2	2
Bradley, 1994 (165)	0	0	0	0
Kairalla, 1992 (146)	0	0	2	0
Ng, 1997 (174)	0	0	2	1
Ng, 1999 (172)	0	0	2	0
Ng, 2002 (167)	0	1	2	2
Noguchi, 1978 (185)	0	1	1	0
Ohrlander, 1977 (175)	0	0	0	1
Sandesh Kiran, 2007 (176)	0	2	2	2
Teramo, 1980 (155)	2	0	2	0
Terrone, 1999 (186)	0	0	1	0
Wilson, 1988 (178)	0	0	1	0
After pain-related stimulation				
Ashwood, 2006 (186)	2	1	2	1
Davis, 2004 (171)	0	0	2	0
Davis, 2006 (188)	0	1	2	0
Glover, 2005 (180)	0	0	0	1
Miller, 2004 (179)	0	0	1	0

[a] Randomization scores: no randomization, 0; partial randomization, 1; complete randomization, 2.

[b] Scores for comparability of treatment and control group or adequate control for confounding [regarding treatment indication, gestational age at birth (evaluated as comparable, if difference of group means $<$1 wk); birth weight (evaluated as comparable, if difference of group means $<$100 g)]: comparable/controlled in one or no parameter, 0; comparable/controlled in two parameters, 1; comparable/controlled in all three parameters, 2; not reported is coded as not comparable.

[c] Homogeneity of type of treatment and treatment indication scores: both nonhomogenous, 0; only one homogenous, 1; both homogenous, 2; not reported is coded as not homogenous.

[d] Sample size scores: number of offspring with exposure to glucocorticoids $<$ 25, 0; 25–50, 1; $>$50, 2.

Levels of the cortisol metabolite cortisone are reduced by 50% in umbilical venous blood of fetuses exposed to synthetic glucocorticoids (144, 153). Furthermore, the cortisol precursor 11-deoxycortisol is reduced in cord blood (144). However, in midpregnancy amniotic fluid, both cortisone and 11-deoxycortisol concentrations are similar in treated and untreated CAH pregnancies as well as in treated and untreated pregnancies without pathological outcomes (158). Results for amniotic fluid and cord blood 17-OHP, the precursor of 11-deoxycortisol, are inconsistent; 17-OHP is either decreased (153) or unchanged (144) in cord blood and is nonsignificantly reduced in amniotic fluid (144, 153, 158).

In sum, 18 of 22 studies demonstrated, in terms of at least one marker, that basal hpa function as measured in amniotic fluid and cord blood is reduced in fetuses exposed to synthetic glucocorticoids *in utero*, whereas no study observed the opposite effect. However, four groups report no significant effects of synthetic glucocorticoids on fetal hpa axis (69, 158–160).

Restricting the synthesis to studies with either randomized study assignment (study quality indicator "randomization" ≥ 1) or comparability between exposed and controls (study quality indicator "comparability" = 2) revealed that all five identified studies (59, 65, 143, 151, 155) demonstrate that basal hpa function is reduced in fetuses exposed to synthetic glucocorticoids *in utero*.

Restricting the synthesis to studies with homogeneity of type of treatment and treatment indication (study quality indicator "homogeneity" = 2) revealed that 15 of the 16 identified studies (59, 68, 143–148, 150–156) did, but one (158) did not, demonstrate that basal hpa function is reduced in fetuses exposed to synthetic glucocorticoids *in utero*.

Restricting the synthesis to studies with large sample size (study quality indicator "sample size" = 2) revealed that five of the six identified studies (68, 143, 147, 152, 157) did, but one (69) did not, demonstrate that basal hpa function is reduced in fetuses exposed to synthetic glucocorticoids *in utero*.

In sum, sensitivity analysis revealed that the finding of a reduced basal hpa function in glucocorticoid-exposed fetuses is strongly consistent in studies with higher quality.

Secretagogues and metabolites of fetal hpa axis, including cortisol, ACTH, DHEA, DHEA-S, 11-deoxycortisol, 17-OHP, and cortisone, have been shown to be suppressed in amniotic fluid and cord blood of glucocorticoid-exposed fetuses. This observation indicates either an inhibition of the entire fetal hpa axis or an inhibition at a higher hpa axis level rather than a reduced function of particular zones or enzyme systems in the adrenal cortex, although not all groups have confirmed suppression of cortisol, 11-deoxycortisol, 17-OHP, and cortisone. Discrepant findings between studies that observed hpa axis suppression and those that did not may be rooted in varying study quality and characteristics, including treatment indication, agent, or dose, as will be discussed in *Sections IV.C*, *IV.D*, and *V.B*. Interestingly, concentrations of cortisone and 11-deoxycortisol are reduced in cord blood, whereas they are unchanged in midpregnancy amniotic fluid, suggesting that cortisone and 11-deoxycortisol reduction as a consequence of exposure to synthetic glucocorticoids does not occur as early as midgestation and that it takes some time until synthetic glucocorticoids take effect on fetal hpa axis. It should be noted that most authors have studied cortisol as "the" marker of fetal hpa axis function, although DHEA is the major adrenal steroid hormone produced in the fetus that is under pituitary control.

The slightly decreased placental CRH mRNA in treated compared with untreated asthma patients may suggest that synthetic glucocorticoids, besides suppressing fetal hpa axis, directly impact on placental CRH production. Against the background of placental CRH being involved in a positive feedback circuit with the fetal hpa axis (see *Section II.D*), the observed reduced placental CRH mRNA in treated compared with untreated asthmatic parturients may also be either a direct cause or a consequence of reduced fetal hpa axis activity.

There are some methodological differences between studies, with some reporting on mixed cord blood and others analyzing arterial and/or venous blood separately. However, glucocorticoid concentrations in arterial and venous cord blood have been shown to be largely equal (144, 145), indicating that the umbilical vein, although carrying blood of placental origin, also accurately predicts hormonal activity in the fetus. Furthermore, it cannot be excluded that endocrine markers, as measured in cord blood or amniotic fluid, are of maternal origin at least in part. However, there is evidence that cortisol concentrations in amniotic fluid are closely associated with the level of cortisol in fetal plasma (145). Finally, stress during birth itself has recently been shown to alter cortisol concentrations in cord blood (161–163). Therefore, it cannot be excluded that in the studies reviewed, indicators of hpa function in cord blood have been influenced by stress of delivery and do not represent the fetal environment exclusively. To further evaluate the impact of intrauterine glucocorticoid exposure on the endocrine system, hpa function needs to be determined in a setting that is independent from delivery.

2. Effects in the neonate and infant

We identified 21 studies that have investigated the impact of intrauterine exposure to synthetic glucocorticoids on basal hpa function at some age between the first day

and 1 yr postpartum. Most of these studies focused on the first postnatal days and weeks. One third of the studies report that in treated infants, plasma cortisol concentrations are reduced relative to controls during the first postnatal days and weeks (153, 164–168). In one report, this phenomenon is at least evident in some but not all of the neonates studied (169). In addition, some of these same studies observed that cortisol concentrations subsequently increase to control levels by 2 wk postpartum at the latest (153, 166–168). Slightly more than 60% of the studies report unchanged basal cortisol concentrations during the first days and weeks postpartum (144, 146, 160, 170–178) or during the first 4 months of life (179) after intrauterine exposure to synthetic glucocorticoids. Recently, one group has reported a significant positive association between intrauterine exposure to synthetic glucocorticoids and basal cortisol concentrations at 4 months of age (180).

Other secretagogues of hpa axis, namely ACTH (167, 168, 170), 17-OHP (144, 153, 181), and 11-deoxycortisol (170) have been shown to be in the normal range, at least within the first week postpartum, in infants exposed to synthetic glucocorticoids *in utero*.

It is of note that one study assessed the effects of intrauterine glucocorticoids on hpa function separately in preterm and term infants each with a respective control group (148). Interestingly, the effects are different in preterm and term infants. In preterm neonates, glucocorticoid exposure is associated with increased cortisol concentrations on postnatal d 6. In term neonates, intrauterine exposure to glucocorticoids is associated with significantly lower cortisol concentrations at 30 and 60 min after delivery relative to controls, indicating a decreased stressor response to birth. The observation that gestational age influences the effects of intrauterine exposure to synthetic glucocorticoids on hpa function is discussed in detail in *Section V.B*.

In sum, one third of the studies show that intrauterine exposure to synthetic glucocorticoids results in reduced cortisol concentrations in the neonate and infant, one study demonstrates increased basal cortisol levels, and more than half of the studies report no effect.

Restricting the synthesis to studies with either randomized study assignment or comparability between exposed and controls revealed that one (169) of these studies found some indication that intrauterine exposure to synthetic glucocorticoids results in reduced cortisol concentrations in the neonate and infant during the first days and weeks postpartum, whereas two studies did not (170, 176).

Restricting the synthesis to studies with homogeneity of type of treatment and treatment indication revealed that five of 13 identified studies (153, 166–169) report that intrauterine exposure to synthetic glucocorticoids results in reduced cortisol concentrations in the neonate and infant during the first days postpartum, whereas seven do not observe significant effects (144, 146, 170–172, 174, 176), and one study (148) shows mixed results.

Restricting the synthesis to studies with large sample size revealed that three (166, 167, 169) studies show some indication that intrauterine exposure to synthetic glucocorticoids results in reduced hpa activity in the neonate and infant during the first days postpartum, whereas four studies do not (170, 176, 177, 181).

In sum, sensitivity analysis revealed a picture similar to that of the synthesis including all available studies. More than half of the results from studies with higher quality show that intrauterine exposure to synthetic glucocorticoids is not associated with changes in hpa activity in the neonate and infant, whereas roughly one third of the studies demonstrate reduced basal cortisol levels, but no study shows increased basal values.

a. Pulsatile cortisol secretion. One study specifically investigated the pulsatile characteristics of cortisol secretion in infants antenatally exposed to glucocorticoids (164). The median amplitude of cortisol secretory bursts, as measured at 15-min intervals for 6 h, is significantly lower in treated infants, whereas the number of secretory bursts, interpulse interval, cortisol secretory burst half-duration, mass of cortisol secreted per burst, production rate, and elimination half-life are comparable to unexposed subjects (164). In studies in healthy adults, it is well documented that cortisol and ACTH secretion are regulated mainly by amplitude rather than other characteristics of the secretory bursts (182, 183), indicating that the observed reduction of cortisol amplitude secretory bursts is important. However, results should be interpreted with caution, according to study quality indicators.

b. Time course of recovery of basal adrenal activity. To assess the development of basal hpa activity within the first postnatal weeks in infants prenatally exposed to glucocorticoids, a number of studies have measured endocrine markers repeatedly at different points in time. Some of these started with assessment in cord blood at birth, whereas others began in the first postnatal hours or days.

Remarkably, the exact timing of adrenal recovery varies considerably between studies. One group reports decreased glucocorticoid concentrations at birth, followed by normalization in cortisol and 11-deoxycortisol levels after 2 h. During the further postnatal course, the levels of the treated group are not different from control levels (144). Moreover, 17-OHP values are within normal range by 2 h postpartum, although they are reduced in cord

blood (153). Further results show that betamethasone-induced suppression of adrenal function, as evidenced in the umbilical vein immediately after birth, is no longer demonstrable at 24 h of age (155), at d 5 (146), or at d 14 postpartum (153). Other authors measured reduced cortisol concentrations in prenatally treated infants at 12 h (166) or 1 wk (167, 168) after birth, reaching normal levels again at 2 d and 2 wk postpartum, respectively. Finally, one group showed that cortisone levels recover 9 d postpartum (153).

Taken together, there are two main findings: 1) the overall pattern of time course of basal hpa function is roughly comparable across studies, consisting mostly of a short period of reduced activity, which is then followed by a recovery to normal levels; and 2) most of the studies found restored hpa function by 2 wk postpartum.

However, the question remains as to whether basal hpa axis activity remains normal beyond the first 2 wk of life because infants were followed beyond that time in four studies only (168, 177, 179, 180). In three of these four studies, normal adrenal function was observed beyond 2 wk of life, indicating that recovery may be completed by that time (168, 177, 179). Restoration may be achieved through the dramatic remodeling of the adrenal cortex within the first days postpartum (see *Section II.D*), which probably compensates for early dysregulation. In the fourth study, specifically, a significant positive association between prenatal glucocorticoid exposure and basal cortisol concentrations at 4 months of age was reported (180). The latter finding provides isolated evidence for a switch from reduced hpa function in the first postnatal weeks to an overactive endocrine system later on. This issue is discussed in detail in *Section V.E*.

Mild, temporary suppression of cortisol concentrations by antenatal glucocorticoid exposure does not appear to co-occur with altered levels of 11-deoxycortisol (170), 17-OHP (144, 153, 181) or ACTH (167, 168, 170) concentrations within the first week postpartum. These results are different from the findings in cord blood described above. Taken together, these observations indicate that pituitary function and certain steps in adrenal steroidogenesis might recover earlier than cortisol synthesis.

The following investigation gives reason to hypothesize that intrauterine glucocorticoid exposure leads to fairly complex endocrine dynamics that cannot be reduced to a scheme delineating a simple time course of suppression of fetal and newborn hpa axis that is followed by sustained recovery. Rather, these complex endocrine patterns seem to persist beyond the first weeks of life. Miller *et al.* (179) reported on a specific endocrine pattern that they detected only in infants exposed to prednisolone *in utero*, but not in controls. They demonstrated a trend toward an inverse relationship between basal cortisol concentrations at 2 months and basal cortisol levels at 4 months, those with increased basal cortisol concentrations at 2 months having decreased basal cortisol concentrations at 4 months and vice versa. This phenomenon describes an unstable endocrine system that is not characterized by either strict hypo- or hyperactivity as a basic principle and gives reason to reconsider rigid, universal disorder models of hypo- or hyperfunction. This issue should be addressed further.

In sum, a number of studies indicate that in glucocorticoid-treated infants, recovery of cortisol suppression, the physiological basis of which may be a reduced amplitude of cortisol secretory bursts, is completed by 2 wk postpartum, whereas there is inconclusive evidence for a chronic effect on hpa axis dysregulation beyond that time.

3. Effects in the adult

To date, only a single study with high quality followed individuals that were exposed to synthetic glucocorticoids *in utero* into adulthood (184). At age 30, mean early morning cortisol concentrations show a tendency to be increased by 7% in the betamethasone group when compared with the placebo group. This average increase was abolished after adjusting for sex, trial type, birth weight, gestational age at birth, body mass index, and oral contraceptive use, although it can be speculated that this adjustment eliminated not only confounding but also mediating factors. In line with the findings by Glover *et al.* (180) described above, this finding tentatively suggests a switch from reduced hpa function to increased activity of the endocrine stress system in the long term.

B. Effects of intrauterine exposure to synthetic glucocorticoids on hpa reactivity

Reactivity of neonatal and infant hpa axis has been evaluated by means of pharmacological challenge (146, 155, 165, 167, 170, 172, 174–176, 178, 185, 186) after physiological burden (143), as well as after painful stressors (171, 179, 180, 187, 188). Basal hpa activity does not reflect hpa reactivity to stimulation (142). Therefore, in the following sections, we separately present the results on the associations of intrauterine exposure to synthetic glucocorticoids and hpa reactivity.

1. Pharmacological challenge

Eleven studies evaluated the axis' ability to react to one of the following pharmacological challenges after being exposed to synthetic glucocorticoids *in utero*: ACTH or ACTH agonists (155, 165, 175, 176, 178, 185, 186), metyrapone (146, 170), or human CRH (hCRH) (167, 172, 174).

a. ACTH challenge. Seven studies tested adrenal function in newborns exposed to synthetic glucocorticoids *in utero* by stimulation via ACTH or ACTH agonists within the first 2 months of life. Infants prenatally exposed to betamethasone show an increase in serum cortisol concentrations after stimulation by ACTH within the first 3 d of life, indicating only, given that there was no control group included in these studies, that they are actually able to mount an adrenocortical response to ACTH (165, 186). Cortisol responses to ACTH administration are not significantly different in glucocorticoid-treated and untreated infants on the first and third days postpartum, although the average increment in cortisol concentration is somewhat lower in the glucocorticoid treatment group (155, 176). Likewise, four glucocorticoid-treated infants studied within another primary research question had cortisol and DHEA-S responses comparable to those of untreated infants after ACTH stimulation 1 and 4 wk postpartum (185). Finally, intrauterine exposure to synthetic glucocorticoids has no effect on ACTH stimulated cortisol increases in a highly selective sample of newborns who were also postnatally treated with dexamethasone due to bronchopulmonary dysplasia, when studied up to 6 wk after birth (178).

In sum, within the first 6 wk postpartum there is no evidence for altered adrenocortical reactivity to a standard dose of ACTH after exposure to synthetic glucocorticoids *in utero*.

Terrone *et al.* (186) and Teramo *et al.* (155) used similar test protocols, administering synthetic ACTH at a standard dose of 0.25 mg/1.73 m^2 and drawing poststress samples after 120 min. Using different protocols with ACTH doses of 0.015 mg/kg and sample collection as soon as 60 min after injection (175) or ACTH doses of 0.1 μg/kg (176), neither of the latter groups found differences in cortisol responses between treated and untreated infants either.

Terrone *et al.* (186) considered a mean cortisol increase of 7.62 μg/100 ml to be indicative of normal adrenal function in infants prenatally exposed to steroids. However, the latter investigation did not include a control group. Interestingly, cortisol rises in treated and untreated infants reported by other groups range between 20 and 50 μg/100 ml (155, 165), indicating that adrenal function in the former investigation might have been slightly suppressed.

Studies consistently demonstrating a normal cortisol response after the ACTH stimulation test in infants exposed to synthetic glucocorticoids *in utero* are to some extent counterintuitive given the observations of suppressed basal hpa function after intrauterine exposure to synthetic glucocorticoids (see *Section IV.A*). Taking both findings together, it may be speculated that the level of hpa axis inhibition leading to low basal cortisol values is most likely located at the level of the hypothalamus or pituitary gland rather than at the adrenocortical level.

b. Metyrapone challenge. To study the integrity of the pituitary and the adrenocortex, 2 d after birth metyrapone was administered to infants exposed to single or repeated betamethasone courses *in utero*, and blood ACTH, cortisol, and 11-deoxycortisol concentrations were measured 3 h thereafter (170). Although cortisol levels fall in response to metyrapone, this response is not different between the single and repeated course groups. Furthermore, groups do not differ in ACTH responses and 11-deoxycortisol concentrations measured 3 h after metyrapone administration. Another author performed a metyrapone test on a set of betamethasone-treated triplets on d 5 after birth (146). Only one of the infants shows the predicted cortisol decrease from pre-metyrapone levels, indicating that inhibition of the enzyme 11-β hydroxylase that converts 11-deoxycortisol to cortisol was not complete. However, plasma 11-deoxycortisol levels rise 10- to 20-fold to expected values of around 3 μg/dl in all infants after oral stimulation with metyrapone.

Results from the study using metyrapone stimulation 1) confirm findings from studies using the ACTH test, both indicating an intact adrenocortical activity in infants exposed to synthetic glucocorticoids *in utero*; and 2) demonstrate an uncompromised pituitary reserve and feedback because the metyrapone test has been shown to be a reliable index of secondary adrenal insufficiency (189). To confirm observations of these reports, more controlled studies using the metyrapone test and involving a larger sample size are needed.

c. CRH challenge. In a series of studies, Ng *et al.* (167, 172, 174) used the hCRH stimulation test to assess pituitary and adrenal function on d 7 and 14 after birth. In their first study in 1997 they found no suppressed ACTH or cortisol concentrations in preterm, very low birth weight infants prenatally exposed to one, two, or more doses of dexamethasone, either at baseline or at 15, 30, and 60 min after hCRH stimulation (174). In a later study, the same group reported lower baseline and 15 and 60 min post-hCRH stimulation levels of cortisol in infants prenatally exposed to one or two, but not in infants exposed to more than two, dexamethasone doses, when compared with controls at d 7 (167). However, net cortisol increases were comparable in all groups. Furthermore, no significant differences in baseline and post-hCRH stimulation ACTH concentrations were observed among the groups on d 7 and 14 (167).

To address the question of whether multiple courses of antenatal dexamethasone would suppress hpa activity, Ng *et al.* (172) performed another prospective study investigating the effect of eight or more doses of dexamethasone. The number of doses ranged between eight and eighteen, with a mean of 11.6 doses. In the absence of an ideal control group, results were compared with previous findings from untreated infants who had their hpa function assessed in a study applying a comparable protocol (174). The serum cortisol concentrations 60 min after hCRH stimulation on day 7, specifically, are significantly lower in the treatment group than in the nontreatment group, indicating a mildly reduced adrenal reactivity. In sum, in one of three studies, intrauterine exposure to dexamethasone is associated with a mildly suppressed cortisol response after stimulation by hCRH on d 7 postpartum, and this effect disappears by 2 wk after birth. None of the studies observes a reduced ACTH response after stimulation by hCRH in treated infants.

Similar to recovery of basal hpa function (see *Section IV.A.2*), the mildly suppressed cortisol response after stimulation by hCRH is no longer observed after 2 wk postpartum (167), supporting the hypothesis of temporally restricted cortisol suppression in infants exposed to synthetic glucocorticoids *in utero*.

The finding of a reduced cortisol response in infants prenatally exposed to a mean number of 11.6 dexamethasone doses (172) is different from that reported by the same authors in 2002 when they demonstrated overall suppressive effects before as well as after hCRH administration only in infants exposed to less than three doses of dexamethasone *in utero* (167). The influence of the amount of synthetic glucocorticoids administered is discussed in *Section IV.C.*

There is only weak evidence for suppressed cortisol response after hCRH administration in dexamethasone-exposed infants. Moreover, no significant influence of dexamethasone exposure on post-hCRH ACTH concentrations has been reported.

It is of note that a single group, using comparable study protocols, conducted all studies applying the hCRH test. More studies are needed to test the reproducibility of these findings.

In conclusion, pharmacological stimulation does not reveal suppression of hpa function, except for weak evidence of mild inhibition of the cortisol response after hCRH administration 7 d postpartum.

Restricting the synthesis to studies with either randomized study assignment or comparability between exposed and controls revealed that all three identified studies (155, 170, 176) show no association between intrauterine exposure to synthetic glucocorticoids and hpa reactivity to pharmacological stimulation.

Restricting the synthesis to studies with homogeneity of type of treatment and treatment indication revealed that five (146, 155, 170, 174, 176) of the seven identified studies show no association between intrauterine exposure to synthetic glucocorticoids and hpa reactivity to pharmacological stimulation, whereas two studies report indications of a slightly reduced hpa reactivity to hCRH (167, 172).

Restricting the synthesis to studies with large sample size revealed that two of the identified studies (170, 176) show no association between intrauterine exposure to synthetic glucocorticoids and pituitary-adrenal reactivity to pharmacological stimulation, whereas one study (167) reports indication of slightly reduced hpa reactivity to hCRH.

In sum, sensitivity analysis revealed a picture similar to that of the synthesis of all available studies: in studies with higher quality, pharmacological stimulation does not reveal suppression of hpa function except for weak evidence of mild inhibition of the cortisol response after hCRH administration.

2. Respiratory stress

To evaluate the responsiveness of hpa axis to physiological stressors in infants prenatally treated with betamethasone, one study with high quality determined serum cortisol levels in infants who were stressed by either intrapartum asphyxia or RDS, both clinically highly relevant stressors (143). Among 27 betamethasone-treated premature infants with significantly lower baseline cortisol concentrations at birth compared with untreated infants, cortisol increased in all infants except one in response to the stress of disease. Increases observed within the first 2 h in asphyxiated and within the first 2 d in RDS infants are not significantly different between treated and untreated infants, suggesting an intact endocrine reactivity to physiological stress in the form of respiratory disturbances in infants exposed to synthetic glucocorticoids *in utero*.

3. Pain and discomfort

Five very recent studies examined hpa reactivity after moderate pain by pinprick or after discomfort triggered by a medical examination or intervention in newborns exposed to glucocorticoid medication *in utero* (171, 179, 180, 187, 188). More precisely, the authors used blood sampling by heel prick (171, 187, 188), a physical exam (171), or nasopharyngeal suction via endotracheal tube (187) within the first week of life; blood sampling by heel prick 4 to 6 wk after birth (188); or immunization at 2 and 4 (179) or at 4 and 12 (180) months of age as stressors.

In two studies, Davis *et al.* (171, 188) demonstrate that a single course of betamethasone, as opposed to no prenatal glucocorticoid treatment, results in a blunted salivary cortisol response to the heel prick in newborns within the first postnatal week. Moreover, on d 3 after birth, the salivary cortisol response to either the heel prick or nasopharyngeal suction is lower in infants exposed to repeat courses of glucocorticoids compared with those treated with a single course (187). The suppressive effect of prenatal glucocorticoid treatment on the hpa axis response to pain-related stress can still be observed 4 to 6 wk after birth (188). Furthermore, antenatal glucocorticoid treatment has been linked to decreased hpa axis reactivity to vaccination at 4 months postpartum (180). This association is no longer apparent 12 months postpartum (180). In contrast to the aforementioned findings, Miller *et al.* (179) report that the hpa axis responses to vaccination at 2 and 4 months after birth are comparable in glucocorticoid-exposed and unexposed infants.

In sum, four of five studies reported reduced pain-related hpa axis reactivity in neonates and infants exposed to glucocorticoids *in utero*. Results are, however, inconsistent as to the sustainability of this reduction, with uncompromised hpa function being apparent between 2 and 12 months postpartum.

Restricting the synthesis to studies with either randomized study assignment or comparability between exposed and controls revealed that the remaining study (187) shows reduced pain-related hpa axis reactivity in offspring exposed to glucocorticoids *in utero*.

Restricting the synthesis to studies with homogeneity of type of treatment and treatment indication revealed that all three identified studies (171, 187, 188) show reduced pain-related hpa axis reactivity in offspring exposed to glucocorticoids *in utero*.

None of the identified studies had a large sample size.

In sum, sensitivity analysis, with regard to randomization and homogeneity, revealed a consistent picture: studies with higher quality show reduced pain-related hpa axis reactivity in offspring exposed to glucocorticoids *in utero*.

a. Association with basal values and time course of suppression and recovery. The observation that the hpa axis response to pain-related stress is decreased in treated infants within the first postnatal week is in line with those studies that reported reduced basal hpa function shortly after birth (see *Section IV.A.1*), documenting a reduction of hpa axis activity that is not restricted to basal function but also appears after painful stimulation immediately after birth.

The ability to mount a cortisol response to pain-related stress is impaired even beyond the first postnatal days and can still be observed 4 months after birth. As described above (*Section IV.A.1*), at this stage of development it appears that basal hpa axis activity has returned to control levels. Taken together, the evidence suggests that there are two time frames of endocrine hypofunction. Within the first 2 wk of life, hpa axis activity can be suppressed in terms of basal levels as well as in response to painful challenge. Thereafter, reduction of endocrine function seems to be restricted to painful experiences up to 4 months of age. Interestingly, a persisting inhibition of endocrine reactivity to pain-related stress along with normalized basal values, as has been detected in glucocorticoid-treated newborns in the study by Davis *et al.* (171), has previously been demonstrated in remitted depressed patients (190). One might hypothesize that this endocrine pattern represents a (lasting) vulnerability for a dysregulated stress reactivity that may be linked to pathology, as has been shown in the depressed patients. The pattern might go back to an abiding depleted capacity of the adrenals, which especially takes effect under an increased hormonal challenge. Alternatively, this pattern might hint to an increased feedback sensitivity, which becomes especially pronounced in challenging situations.

Findings from several studies provide evidence for the age of recovery from glucocorticoid-induced suppression of hpa axis reactivity to pain-related stress. Glover *et al.* (180) demonstrate uncompromised hpa reactivity to a painful stressor in infants antenatally exposed to glucocorticoids when they are 12 months of age. Another group reports appropriate hpa axis responses to pain-related stress in treated infants as early as 2 and 4 months after birth (179). These findings indicate that endocrine reactivity to a painful challenge recovers during the first year of life but occurs after recovery of basal hpa function. The exact timing of recovery and the factors that influence this process both need further study because the recovery and its timing may be important in terms of disease vulnerability.

There is a putative limitation to the comparison of findings from studies focusing on basal *vs.* reactive hpa function. Although the latter measured cortisol concentrations exclusively in saliva, the former predominantly assessed hormone levels in blood. However, high correlations between salivary and serum/plasma concentrations of cortisol have been reported in different populations, including newborns and children (191).

Taken together, there is initial evidence that blunted basal hpa function and blunted hpa axis reactivity to pain-related stress decouple after the first 2 wk of life, with the former recovering and the latter exhibiting a persisting suppression up to age 4 months.

b. Behavior and heart rate. Interestingly, although endocrine reactivity to the heel prick is blunted in infants prenatally treated with glucocorticoids when compared with controls, the increase of behavioral distress after the challenge is comparable between the two groups, and heart rate response is actually elevated in the betamethasone group during the first postnatal week as well as 4 to 6 wk postpartum (171, 188). This indicates that the treated newborns are able to 1) perceive the situation as stressful, and 2) react to the stressful situation in terms of arousal, suggesting that antenatal glucocorticoid exposure selectively inhibits hpa function but not other aspects of the stress response. In conclusion, intrauterine exposure to synthetic glucocorticoids seems to selectively inhibit hpa axis reactivity but not other components of the stress response.

c. Evaluation of stressors. The methods used to assess stimulated hpa activity provide a valid indication of the axis' normal biological capacity in response to physiologically relevant pain-related stressors such as heel prick and vaccination. These procedures have recently been shown to result in significant activation of the hpa axis in newborns (*e.g.*, Refs. 192–194). However, neither prenatally glucocorticoid-exposed nor prenatally untreated infants display cortisol increases in response to a physical exam (171). The authors argue that infants may habituate to multiple exams on a daily basis. In support of this interpretation, all infants additionally displayed smaller behavioral responses to the physical exam than to the heel prick.

Finally, some characteristics of the immunization and heel-prick paradigms should be noted. 1) Vaccination may lead to hpa activation by either the painful pinprick or the immune challenge. Immune challenge, such as tetanus vaccination, usually results in maximum cortisol increases about 4 to 5 h after injection (195). However, in both studies that used the immunization paradigm, saliva samples were collected as soon as 20 min after vaccination and exhibited increased cortisol levels (179, 180). Therefore, the painful pinprick seems to have elicited the hpa axis response. 2) The immunization and the heel-prick paradigms are not fully comparable. On the one hand, the squeezing of the heel instead of the sharp lancing itself is the most stressful event during the heel-prick procedure (196). Consequently, one might hypothesize that the vaccination is not as stressful as the heel-prick procedure because squeezing is not required. On the other hand, routine immunization injections have been described as the most common painful medical procedure in childhood (197). However, the stress-induced rises from baseline in cortisol, amounting to approximately 60 to 100%, are roughly comparable in the untreated newborns in the vaccination and heel-prick paradigms (171, 179, 188). The inconsistent results of the studies that used vaccination as a pain-related stressor (179, 180) may therefore not be rooted in the paradigm chosen, but in the different study characteristics as described in *Section V.B*. In sum, the two main pain-related stress paradigms used, that is heel prick and vaccination, elicit comparable endocrine responses.

4. Pain vs. pharmacological challenge

When comparing results from studies using pharmacological challenge *vs.* painful stimulation, it is striking that in infants exposed to synthetic glucocorticoids *in utero* compared with controls, pharmacological stimulation revealed at best only mildly suppressed cortisol reactivity 1 wk postpartum, whereas painful stimulation revealed sustained inhibition of cortisol reactivity up to 4 months of age. One reason for this finding may be that the applied pharmacological tests are not able to reveal processes that take place above the hypothalamic level. Therefore, hpa axis changes detected under painful, but not under pharmacological, stimulation may reflect reduced input to the hypothalamus from higher levels (*e.g.*, limbic system, cortical structures).

C. Influence of glucocorticoid dose on hpa function

Comparing the studies presented in the previous sections, there is quite large variation in numbers of antenatal treatment courses and total doses of glucocorticoids used (see Supplemental Materials 3, 4, and 5). Some of the studies assessed whether number of antenatal glucocorticoid treatment courses or total dose of the administered substance influenced basal hpa function or hpa axis reactivity in the fetus or newborn.

There is evidence for an inverse association between number of antenatal glucocorticoid treatment courses or total dose of the substance administered and hpa activity at baseline or after pharmacological and painful stimulation in the fetus and newborn. That is, exposure to a relatively large amount of synthetic glucocorticoids *in utero* is associated with relatively low basal or reactive hpa function (68, 147, 157, 172, 187, 198). However, some groups did not observe a negative association between number of antenatal glucocorticoid treatment courses or total dose of the substance administered and specific secretagogues of fetal and newborn hpa function at particular time points at baseline or after stimulation by hCRH, ACTH, and metyrapone within the first weeks postpartum (166, 167, 169, 170, 174, 180, 186, 199). In a recent study, Ng *et al.* (167) even report that cortisol concentrations, as measured after 1 wk postpartum at baseline and after hCRH stimulation, are only lower in infants exposed to one or two (but not in infants exposed to more than two) glucocorticoid doses compared with unexposed individ-

uals, leading to the conclusion that low glucocorticoid doses may have a different impact on hpa function compared with high glucocorticoid doses.

Taken together, approximately 50% of all studies looking at the effects of the amount of substance administered on basal or reactive hpa function find that high levels of synthetic glucocorticoids are more likely than low levels to have a suppressive effect on basal hpa activity and endocrine stress reactivity in the fetus and newborn. There is evidence that it is the total amount of synthetic glucocorticoids administered rather than the number of antenatal glucocorticoid courses that underlies this association (143). The influence the amount of synthetic glucocorticoids exerts on fetal and infant hpa axis may partially explain discrepant results of the reviewed studies presented in the previous sections.

Restricting the synthesis to studies with either randomized study assignment or comparability between exposed and controls revealed that one (187) of the three identified studies shows that high levels of synthetic glucocorticoids are more likely than low levels to have a suppressive effect on basal hpa activity and endocrine stress reactivity, in the fetus and newborn, while the other two studies (169, 170) do not.

Restricting the synthesis to studies with homogeneity of type of treatment and treatment indication revealed that five (68, 147, 172, 187, 198) of the 11 identified studies show that high levels of synthetic glucocorticoids are more likely than low levels to have a suppressive effect on basal hpa activity and endocrine stress reactivity in the fetus and newborn, whereas the other six studies (166, 167, 169, 170, 174, 199) do not report such an association.

Restricting the synthesis to studies with large sample size revealed that five (68, 147, 157, 187, 198) of the 10 identified studies show that high levels of synthetic glucocorticoids are more likely than low levels to have a suppressive effect on basal hpa activity and endocrine stress reactivity in the fetus and newborn, whereas the other five studies (166, 167, 169, 170, 199) do not report such an association.

In sum, sensitivity analysis revealed that studies with higher quality are less likely to show that high levels of synthetic glucocorticoids compared with low levels have a stronger suppressive effect on basal hpa activity and endocrine stress reactivity in the fetus and newborn.

D. Influence of time between glucocorticoid treatment and assessment of hpa function

The time interval that has elapsed between intrauterine exposure to synthetic glucocorticoids and birth or hpa axis assessment may influence the observed effects of synthetic glucocorticoids on hpa axis. Some authors have gone further into this issue. It is of note that they have defined the time intervals inconsistently, starting either with the first or last dose of glucocorticoid treatment. Therefore, comparisons between studies should be drawn with caution.

In cord blood, ACTH, DHEA-S, and cortisol concentrations are suppressed in infants delivered within 24 h of, and up to 1 wk after, antenatal glucocorticoid administration, mostly compared with longer time intervals (59, 68, 125, 143, 145, 150, 152, 154, 155, 160, 166). The concentration of CRH in cord blood is significantly lower in those samples collected within 24 h of betamethasone administration compared with controls. In contrast, CRH concentrations are significantly higher relative to controls in cord blood obtained more than 24 h and 1 wk after the last treatment dose (150). This increase of CRH concentrations might be rooted in a decreased negative feedback regulation of the hpa axis due to low circulating cortisol concentrations.

In amniotic fluid obtained during pregnancy or at birth, the effects of the time between treatment and birth or sample collection on the concentration of cortisol are similar to those observed in cord blood (59, 145, 150, 154). Interestingly, in contrast to findings in cord blood, ACTH concentrations in amniotic fluid are not significantly different from control values through 48 h after the last dose of treatment, but there is a significant decrease in ACTH levels in samples collected within and after 1 wk from last betamethasone injection (150). The different patterns of ACTH production in cord blood and amniotic fluid suggest that the ACTH in amniotic fluid may be produced at a different site than ACTH in cord blood. Finally, there is no significant effect of betamethasone on CRH levels in amniotic fluid up to 1 wk after treatment (150). This finding is in contrast to the sequence of CRH decrease and increase within 1 wk after last treatment dose in cord blood. However, CRH concentrations in amniotic fluid collected more than 1 wk after betamethasone treatment are significantly higher than control values (150). This suggests that a certain time is needed before CRH changes in blood are seen in amniotic fluid. Again, the different patterns of CRH levels in cord blood and amniotic fluid raise the question of whether CRH in amniotic fluid may be from a different source than CRH in cord blood.

In the neonate, some studies assessed whether the effects of intrauterine exposure to synthetic glucocorticoids on basal hpa function depend on the time interval between treatment and birth/sample collection. Results from these studies are equivocal. More than one third of these investigations report that the longer the time interval between treatment and birth/sample collection, the greater the suppression of 17-OHP, cortisol, or ACTH relative to controls (125, 172, 198). These observations contrast with the aforementioned results from cord blood and amniotic fluid. This leads to the question of whether the relation-

ship, time interval between treatment and sample collection *vs.* the suppressive effect on hpa function, may follow a U-shape: a relatively long time interval is linked to relatively low suppression of hpa activity *in utero*, but relatively strong suppression of hpa activity in the first postnatal week. This hypothesis implies that the effects of the time interval between treatment and birth/sample collection on pre- and postnatal hpa axis may be mediated by different mechanisms. However, it also needs to be remembered that there are studies in the neonate that report that the time interval between treatment and birth/sample collection is either positively associated with (199) or without influence (160, 170, 171, 181) on basal hpa activity.

The time interval between antenatal glucocorticoid administration and delivery/sample collection does not impact on the extent of the effect of synthetic glucocorticoids on hpa reactivity to ACTH or metyrapone (170, 175, 186). Similarly, three investigations that evaluated the effect of the time interval between treatment and delivery or sample collection on hpa axis, without specifying whether they looked at basal or reactive measures, report nonsignificant results (171, 172, 187).

Interestingly, the influence of the time that has elapsed between treatment and sample collection has been shown to depend on the dose administered (167, 187, 200). At age 2 h in infants delivered 1–72 h after treatment, plasma cortisol concentrations are lower after three or more courses than after one or two courses of antenatal glucocorticoids. Cortisol suppression persists for neonates delivered 73–240 h after the last of multiple doses (200). Finally, the time interval between the last dexamethasone dose and delivery is positively associated with serum cortisol concentrations 15 and 60 min after CRH stimulation at 1 wk, but not 2 wk, after birth in infants who received more than two doses of antenatal glucocorticoids (167).

In sum, relatively short time intervals between treatment and birth/sample collection primarily lead to relatively high suppression of fetal basal hpa function but relatively low suppression of neonatal basal hpa function. The time lag between glucocorticoid administration and delivery/sample collection does not seem to have an influence on hpa axis reactivity within the first weeks of life. Discrepant findings between the studies reviewed may partially originate in the different time intervals between treatment and sample collection that have been used.

Restricting the synthesis to studies with either randomized study assignment or comparability between exposed and controls revealed that two (143, 155) of the four identified studies that addressed the effect of the time interval provide evidence for an effect of the time interval between treatment and birth or sample collection on hpa function, whereas two studies do not (170, 187).

Restricting the synthesis to studies with homogeneity of type of treatment and treatment indication revealed that 12 (68, 143, 145, 150, 152, 154, 155, 166, 167, 172, 198, 199) of the 15 identified studies that addressed the effect of the time interval provide evidence for an effect of the time interval between treatment and birth or sample collection on hpa function, whereas three studies do not (170, 171, 187).

Restricting the synthesis to studies with large sample size revealed that eight (68, 125, 143, 152, 166, 167, 198, 199) of the 11 identified studies that addressed the effect of time interval provide evidence for an effect of the time intervals between treatment and birth or sample collection on hpa function, whereas three studies do not (170, 181, 187).

In sum, sensitivity analysis provides evidence that the time interval between treatment and birth or sample collection influences hpa function, which is in line with the picture provided by the synthesis including all available studies.

E. Summary of the sensitivity analyses

The sensitivity analyses revealed that the integrated findings from higher quality studies are mostly in line with the picture presented by the total number of available studies. Most notably, the higher quality studies even provided slightly stronger evidence for effects of intrauterine exposure to glucocorticoids on hpa axis in the offspring. The only exception was the dose effect, which was less pronounced in the higher quality studies compared with the total number of studies.

V. Discussion

A. Main results of the studies

This review of human studies has revealed that intrauterine exposure to synthetic glucocorticoids reduces fetal and, in some cases, newborn and infant hpa activity under basal conditions, and, more consistently, after pain-related stress. These effects may be more marked with a higher total amount of glucocorticoids administered and vary with the time between glucocorticoid exposure and hpa axis assessment. Although reduced basal hpa function seems to recover within the first 2 wk postpartum, there is preliminary evidence that blunted hpa axis reactivity to pain-related stress persists throughout the first 4 months of life.

A part of the identified studies showed weaknesses with respect to different aspects of study quality. However, our

conclusions are based on a large number of studies, and the sensitivity analyses revealed that the findings from higher quality studies are in line with the picture presented by the total number of available studies. In light of the clinical relevance of prenatal glucocorticoid treatment and hpa activity, future high-quality research is urgently warranted.

B. Overall study limitations and alternative explanations for observed results

Some overall methodological issues complicate the interpretation of the reviewed studies, as discussed in the following sections.

1. Study design, including confounding

Due to ethical constraints, in most studies pregnant women could not be assigned randomly to the glucocorticoid treatment or no-treatment groups. In fact, the majority of studies employed quasi-experimental study designs, allocating women on the basis of the clinically prescribed treatment—that is only those women whose attending obstetrician decided to start glucocorticoid treatment due to clinical indication were considered as candidates for the glucocorticoid treatment group, whereas those without treatment indication and, hence, no glucocorticoid treatment were considered as candidates for the control group ("confounding by indication"). Thus, it cannot be excluded that the endocrine effects observed in the fetus or newborn are a consequence of the clinical indication (or other factors linked to it) rather than the antenatal glucocorticoid.

One of the main reasons for glucocorticoid treatment during pregnancy is prophylactic preparation for preterm birth; accordingly, in most of the reviewed studies, glucocorticoid treatment has been indicated by risk of preterm delivery. One of the factors linked to this treatment indication is the length of gestation, which in the majority of cases is shorter in glucocorticoid-treated fetuses than in untreated fetuses. Consequently, in some studies reviewed, treatment groups were not comparable regarding gestational age. Such group differences are problematic because fetal and newborn hpa activity is subject to dynamic changes and the adrenal cortex dramatically remodels around birth (see *Section II.D*). Thus, it cannot be excluded that results have partly been influenced by different gestational ages in treated and untreated fetuses. One method to deal with this problem has been applied by Davis *et al.* (188): their glucocorticoid group covered subjects born between 28 and 30 wk gestation, whereas due to scarcity of infants of equal gestational age without prenatal glucocorticoid treatment their control group consisted of infants born between 33 and 34 wk gestation. To account for this limitation, group comparisons were only performed between glucocorticoid-treated infants as they reached postconceptional ages of 33 to 34 wk and control infants at birth. However, different postnatal ages still complicate the interpretation of the findings.

Gestational age has indeed been shown to influence the effects that intrauterine exposure to synthetic glucocorticoids exerts on hpa function (166, 187). Umbilical cortisol concentrations have been reported to be reduced only in treated preterm, but not in treated term, infants compared with term controls (148), raising the question of whether endocrine suppression observed after prenatal glucocorticoid exposure is primarily due to immaturity rather than to the treatment *per se*. However, recently, another group observed the opposite effect: that is, fetal hpa suppression after intrauterine glucocorticoid exposure was more pronounced in fetuses born at 32 to 36 wk gestation than in fetuses born at 26 to 32 wk gestation, indicating that glucocorticoid exposure also takes effect in more mature fetuses (149). Moreover, infants who were born between wk 30 and 33 had a median 17-OHP value that was lower in the prenatally treated group within the first postnatal week; conversely, in infants born before wk 30, the median 17-OHP value was higher in the treated group (181). These observations indicate that it is not prematurity, but rather the glucocorticoid treatment that leads to the reduction of hpa activity. In support of this interpretation, other investigations have demonstrated hpa-suppressing properties of intrauterine synthetic glucocorticoids when comparing infants with largely comparable gestational ages (65, 152, 169, 175, 185).

Some investigations were indeed based on experimental study designs (151, 155, 170, 187). Moreover, the methodological limitations in the studies that used quasi-experimental study designs have in part been overcome by applying various approaches: some investigations 1) included only uncomplicated pregnancies (59); 2) assigned fetuses endangered of preterm delivery to all study groups (*e.g.*, Refs. 145, 150, and 176); 3) used control groups consisting of women with glucocorticoid treatment indication but no treatment (69, 156–158); or 4) looked at the effects of the amount of substance administered or the time interval between treatment and birth or sample collection on hpa function (see *Sections IV.C and IV.D*). Overall, findings from these studies support the interpretation that endocrine effects observed in the fetuses and infants are due to the glucocorticoid treatment itself.

Due to practical constraints, some studies were not even able to fully tap the potential of the quasi-experimental study design. In these cases, concise conclusions are further hindered by the absence of appropriate control groups: only a few authors used external controls, whereas others included infants treated remotely, or even fully

passed on a control group, as for instance in case reports. Finally, for practical reasons, most of the studies did not use placebo-controlled study designs. In such cases, it cannot be excluded that the treatment procedure, rather than the substance itself, led to changes in fetal or newborn hpa function, although this concern seems to be rather implausible, especially when considering the lasting effects.

To overcome limitations of the (quasi-experimental) study design, future studies should apply placebo-controlled experimental study designs, if possible. Researchers may, due to the ethical constraints that have been mentioned above, vary number of treatment courses rather than glucocorticoid treatment itself, as long as no clear evidence exists that favors a defined number of courses. There are already large clinical trials [*e.g.*, Maternal Infant and Reproductive Health Research Unit, Multiple Courses of Antenatal Corticosteroids for Preterm Birth Study (MACS) (201)] ongoing that implement such an experimental study design. If experimental trials are not feasible, then, as an alternative, appropriate control groups consisting of subjects with gestational glucocorticoid treatment indication but no treatment should be included, if available. Finally, effects of varying doses and time intervals between treatment and hpa assessment should be assessed.

2. Sample size

The sample size varied considerably between studies. Some of the authors reported relatively small sample sizes (see Supplemental Materials 3, 4, and 5). Findings from these investigations should be interpreted with caution because their statistical uncertainty may be rather high due to the small sample size.

3. Long-term follow-up

Infant hpa function has predominantly been assessed immediately after birth and up to the first 2 months postpartum. However, little is known about the long-term consequences of intrauterine exposure to synthetic glucocorticoids on endocrine function in humans. Any long-term effects might well play a role in the long-term health consequences that have been observed in treated individuals and in the onset of other stress-related diseases, as discussed in the next section.

More follow-up studies in treated individuals are strongly needed to evaluate hpa function after antenatal exposure to synthetic glucocorticoids at various stages of development.

4. Sex differences

Only one group (69) categorically studied the effects of antenatal glucocorticoid therapy on cortisol concentrations in cord blood in males and females, whereas most of the other authors did not even specify the sex of the study probands. Murphy *et al.* (69) found comparable endocrine patterns in male and female newborns. However, males and females may be differentially affected by antenatal glucocorticoid treatment, given the recent observation in pigs that elevated maternal cortisol levels during mid and late gestation impact on the offspring's hpa axis in a sex-specific manner (202). Moreover, placental 11β-HSD2 activity after antenatal glucocorticoid treatment is influenced in a sex-specific manner in humans (69), as described in the next section. Finally, emerging evidence indicates that programming effects of an adverse intrauterine environment in general are highly sex-specific (21). Therefore, there is a strong need for investigations comparing the effects of antenatal glucocorticoid treatment in males and females.

5. Glucocorticoid preparation

The antenatal glucocorticoids are administered in different preparations (*e.g.*, a dual formulation containing betamethasone acetate and phosphate or pure betamethasone phosphate) that differ in their pharmacokinetic properties (see *Section II.B.1*) and may thus differentially affect fetal and infant hpa (re-)activity. However, we did not find evidence for hpa axis differences depending on the preparation used, although incomplete documentation of preparation in a number of articles hindered comparisons.

6. Heterogeneity of studies

Finally, investigations differed with regard to other important study characteristics and aspects of study quality, such as treatment indications, glucocorticoid agents, preparations, intrastudy heterogeneity of these factors, route of drug administration, analytes of hpa axis chosen, time of hpa axis assessment, and the compartment in which the analyte was measured (see Supplemental Materials 3, 4, and 5). Moreover, we cannot exclude further potential sources of heterogeneity on which information is lacking [*e.g.*, whether basal hpa function in newborns was assessed under fasting conditions, the stage of perinatal remodeling of the adrenocortex, diurnal variability of hpa activity (203)]. These differences have limiting as well as supporting properties. On the one hand, they hamper comparisons between studies because the effects of these differences may override the rather subtle effects of the glucocorticoid treatment itself. On the other hand, all of these factors serve to improve the studies' ecological validity, broaden the insight into the complexity of factors that modify the impact of antenatal glucocorticoid treatment on hpa function in the fetus and newborn, and offer a more specific analysis of contributing parameters. Taken together, large, randomized, placebo-controlled trials

with concealment of study allocation on short- and long-term consequences of antenatal exposure to synthetic glucocorticoids on male *vs.* female hpa activity are strongly needed in humans. If feasible, the ecological validity should be broadened by including a wide range of treatment regimes and outcome measures in homogenous subgroups.

7. Bias and generalizability

Because we applied broad inclusion criteria, we minimized the risk of bias due to selective exclusion of studies. Still, publication bias represents a threat to the validity of our findings, especially because we included observational studies into our systematic review. Indeed, findings from observational studies are at a higher risk of publication bias than randomized trials (204). However, we reduced some of the potential of publication bias by taking study design into account as quality criterion. Similarly, we accounted for effects of sample size. Sample size has been shown to be negatively associated with publication bias (205, 206).

With regard to generalizability, in the majority of studies, glucocorticoids were administered to pregnant women with certain medical conditions (*i.e.*, risk for preterm delivery, risk for CAH, CDH, asthma). Therefore, generalizability of the results to offspring of women who received glucocorticoids due to other reasons may be limited.

C. Mechanisms of hpa axis suppression

A number of studies have assessed the physiological effects of intrauterine exposure to synthetic glucocorticoids at diverse levels of the fetal hpa axis-placental unit and related these to the putative mechanisms via which this can lead to the reduction of the fetal and newborn hpa activity. Some groups looked at the impact of intrauterine exposure to synthetic glucocorticoids on the activity of placental 11β-HSD2, the major enzyme that limits the amount of glucocorticoids that pass from mother to fetus. Others have asked whether changes in hpa axis activity were short-term effects of synthetic glucocorticoids while they were present in blood or lasting effects after they had been cleared. A third approach has been to focus on the alterations of fetal and newborn hpa axis structures and secretagogues that have been shown to occur after antenatal glucocorticoid treatment.

1. Placental 11β-HSD2

The enzyme 11β-HSD2 inactivates maternal glucocorticoids at the placental level, thereby reducing the amount of cortisol that passes from mother to fetus. Because synthetic glucocorticoids have been shown to increase placental 11β-HSD2 activity, maternal glucocorticoids may be even more strongly prevented from transplacental passage in glucocorticoid-treated pregnancies (see *Section II.B.3*). The observed lower cortisol concentrations in cord blood after prenatal glucocorticoid treatment (see *Section IV.A.1*) may thus be due to reduced cortisol concentrations of maternal origin. However, due to undersupply with maternal glucocorticoids, the fetal hpa axis might be anticipated to up-regulate rather than down-regulate its activity in the medium term. Therefore, reduced transplacental passage of maternal glucocorticoids due to an amplified placental barrier after administration of synthetic glucocorticoids does not seem to be an obvious candidate mechanism underlying the medium-term hypocortisolism.

2. Short-term vs. long-term effects of synthetic glucocorticoids on hpa axis

Once having passed the placental barrier, synthetic glucocorticoids would be anticipated to have two opposing effects in the fetus.

First, due to their own glucocorticoid properties, they might generate a transient hypercortisolemic state in the fetus until they have been cleared.

To determine how long synthetic glucocorticoids are biologically active, one investigation looked at the circulating glucocorticoid bioactivity (GBA), made up of synthetic and endogenous glucocorticoids, in cord blood. The authors demonstrate that infants born within 12 h after the last betamethasone dose display an average 4-fold higher GBA than infants with more than 7 d since the last dose before birth or without treatment (147). In the former infants, 85% of the total GBA is attributable to synthetic glucocorticoids. When 24 to 48 h elapse between the last glucocorticoid dose and birth, in cord blood GBA is primarily attributable to endogenous cortisol, indicating that exogenous glucocorticoids no longer exert acute effects on the fetus.

Secondly, in contrast to generating a hypercortisolemic state in the fetus, synthetic glucocorticoids might, by inhibiting the fetal hpa axis, induce a hypocortisolemic state in the fetus.

The question arises as to whether these oppositional effects of synthetic glucocorticoids, putatively inducing either a hyper- or hypocortisolemic state, have comparable potency and occur simultaneously, thereby balancing each other out and resulting in a net neutral effect on endocrine state, or whether one of them outweighs the other.

To directly compare synthetic and endogenous glucocorticoid levels, Ballard *et al.* (143) looked at the GBA in relation to the detectable concentrations of betamethasone in cord blood and simultaneously measured both betamethasone concentrations and GBA at different time lags from treatment. Mean betamethasone levels are max-

imal during the first 48 h, after which they start to decrease and show an inverse relationship with the levels of endogenous cortisol. Betamethasone is not detected in any sample obtained beyond 60 h of the last dose. Consequently, unbound GBA is maximal 1 h after treatment and falls below untreated levels by 60 h after treatment, when betamethasone has been cleared. These findings indicate that recovery from hpa hypofunction takes longer than clearance of betamethasone, thus persisting beyond exposure to synthetic glucocorticoids.

There are two main conclusions to be drawn from these observations. First, the reduction of fetal and newborn hpa activity may be a direct consequence of the inhibiting effects of the synthetic glucocorticoids administered to the mother while they are present in blood. Evidence for this hypothesis comes from a number of investigations that studied the effects of synthetic glucocorticoids soon after their administration, before they could have been cleared from blood (143, 147, 150, 152, 154, 155). However, a considerable number of studies measured hpa reactivity at least 72 h after birth at the earliest, when synthetic glucocorticoids would be expected to have been cleared from blood according to the findings mentioned above from the groups of Kajantie *et al.* (147) and Ballard *et al.* (143). Indeed, these studies reported lasting adrenal suppression beyond the clearance of the synthetic glucocorticoids (152, 167, 171, 172, 180, 187, 188, 199), leading to the second of the main conclusions, *i.e.*, that hpa activity becomes independent from exogenous influence, whereas changes in structure and function of hpa axis or brain regions involved in the regulation of hpa axis develop that keep the axis' activity low.

3. Alterations in hpa axis

Different alterations in hpa axis structures may underlie the reduced hpa activity observed in fetuses and infants prenatally treated with glucocorticoids. According to findings in patients with diverse stress-related disorders, potential changes may be 1) reduced biosynthesis or release of hpa axis secretagogues or placental CRH as one main stimulator of the fetal endocrine stress system, resulting in decreased activation of their particular target receptors; 2) hypersecretion of CRH or ACTH with an overcompensatory down-regulation of their respective target receptors and a subsequently decreased stimulation at the adrenal level; 3) an increased negative feedback sensitivity to glucocorticoids at pituitary and hypothalamic level or a decreased positive feedback sensitivity at placental site; 4) a decreased availability of free cortisol; or 5) morphological changes at either level of the hpa axis or the placenta (207, 208).

There is preliminary evidence for some of these mechanisms underlying hypocortisolemic patterns in human newborns exposed to synthetic glucocorticoids *in utero*. 1) In support of a primary adrenal insufficiency, two authors found decreased basal cortisol concentrations, along with normal levels of ACTH, in infants prenatally exposed to synthetic glucocorticoids within the first week postpartum (167, 168). Findings of decreases in both cortisol and ACTH concentrations, however, rather point to a secondary adrenal insufficiency (150, 156). 2) Other studies suggest an adaptive CRH- or ACTH-receptor down-regulation when reporting decreased cortisol responses in the CRH stimulation test (172). 3) Moreover, an increased unbound glucocorticoid bioactivity after betamethasone treatment (147) may probably lead to increased GR binding the at pituitary or hypothalamic level, resulting in a subsequently increased negative feedback that could explain the reduced hpa activity. However, the reported 10- to 20-fold rise in plasma 11-deoxycortisol after metyrapone administration in infants exposed to betamethasone *in utero* hints to unchanged feedback sensitivity at the pituitary and hypothalamic level (146). To our knowledge, as yet no study has looked at whether infants displaying a hypocortisolemic phenotype have a compensatory increased sensitivity to glucocorticoids in target tissues other than the hpa axis itself. This issue would be interesting because glucocorticoid sensitivity has been shown to vary tissue specifically (209), and the clinical risk of the observed reduced hpa activity would appear to be closely linked to the capability of the remaining amount of endogenous glucocorticoids to take effect in their target tissues. If glucocorticoid sensitivity of other tissues and cell types increases in a compensatory manner, no adverse consequences will be anticipated. 4) Finally, one study found significantly decreased CBG capacity in cord blood in those samples obtained within 24 h of glucocorticoid treatment and, irrespective of the time between glucocorticoid treatment and assessment, unchanged CBG capacity in cord blood, as well as reduced CBG capacity in amniotic fluid (210). These findings suggest an increased rather than a decreased availability of free plasma cortisol, which obviously cannot explain the phenomenon of reduced hpa activity in infants treated with synthetic glucocorticoids *in utero*.

In sum, there is evidence that different alterations of hpa axis, including primary or secondary adrenal insufficiency or (compensatory) down-regulation of receptors at higher hpa axis levels, may underlie the observed hypocortisolemic phenotype in infants exposed to synthetic glucocorticoids *in utero*. There is, however, only inconclusive evidence for an increased negative feedback sensitivity of the hpa axis to glucocorticoids or no hint for changes in CBG capacity.

4. Alterations at higher brain sites

At least in animals, moderate amounts of betamethasone, dexamethasone, and prednisolone penetrate the brain poorly because of P-gp activity at the level of the blood-brain barrier (79–83). Synthetic glucocorticoids may, therefore, exert their effects on hpa axis predominantly at the pituitary level, where they are retained in high amounts, because they are not bound to CBG (82, 211). Nonetheless, it is likely that small amounts of synthetic glucocorticoids are able to pass the blood-brain barrier. Furthermore, the fetal blood-brain barrier is still more permeable for synthetic glucocorticoids than is the postnatal blood-brain barrier (see *Section II.B.3*). In sum, small amounts of synthetic glucocorticoids may well pass the fetal blood-brain barrier and act on central GR. This bypass, which becomes relevant if a sufficient amount of synthetic glucocorticoid is administered, might underlie the observed changes in cognitive, psychological, and motor function after exposure to synthetic glucocorticoids *in utero* (see *Section V.F* and Refs. 5 and 7).

In animal studies, functional changes in the hpa axis after intrauterine exposure to synthetic glucocorticoids have been demonstrated to be associated with changes at higher brain levels, including alterations in diverse brain structures involved in hpa axis regulation, particularly the hippocampus, changes in glucocorticoid receptor expression, and variations in other neuroendocrine and neurotransmitter systems (4, 5, 21), rather than being associated with primary biological variations of hpa axis structures. For example, it has been shown in the guinea pig that hippocampal GR and MR mRNA expressions are influenced sex-specifically by intrauterine exposure to synthetic glucocorticoids (212, 213). However, in humans, GR and MR expression levels are comparable in prenatally treated or untreated newborns that died within 4 d after delivery (95). Because the sample size in the latter study was rather small, more studies are needed to learn about the effects of synthetic glucocorticoids on fetal GR and MR expression in humans.

Taken together, despite increasing placental 11β-HSD2 activity after exposure to synthetic glucocorticoids *in utero*, the latter easily pass the placental barrier. While inducing a short-term hypercortisolemic state in the fetus due to their glucocorticoid properties, synthetic glucocorticoids concomitantly reduce hpa function. Furthermore, synthetic glucocorticoids are capable of altering the endocrine stress system, such that it operates independently from the acute influence of synthetic glucocorticoids in the intermediate term. The hpa reduction might be due to various biological mechanisms. Moreover, it cannot be finally excluded that changes in hpa function after intrauterine exposure to glucocorticoids are secondary to their therapeutic effects. For example, one may speculate that administration of glucocorticoids to pregnant women reduced respiratory stress and hypoxia in the offspring, thereby decreasing fetal hpa function. Interestingly, there was no association between intrauterine exposure to glucocorticoids and hpa reactivity to respiratory stress (see *Section IV.B.2*), the reduction of which is, however, the major clinically desired effect of maternal glucocorticoid treatment during pregnancy on the offspring. More studies are needed to look further at the mechanisms that underlie reduced hpa function after intrauterine exposure to synthetic glucocorticoids in humans. These future studies should assess biosynthesis, secretion, and biological activity of diverse hpa axis secretagogues and the number and function of their respective receptors, including, if feasible, postmortem analyses of receptor programming and brain morphology.

D. Integrative model of altered hpa function due to intrauterine exposure to synthetic glucocorticoids

The findings from studies across different assessment paradigms that have been presented in *Section IV* provide the basis for the following model. Intrauterine exposure to synthetic glucocorticoids leads 1) to reduced fetal hpa axis activity at different hpa axis levels, as measured in cord blood and amniotic fluid; 2) to a subsequent recovery of basal hpa axis activity at the level of the pituitary, as indicated by normalized ACTH concentrations within the first week postpartum, but with continuing reduced adrenocortical activity; 3) to a subsequent recovery of basal hpa axis activity at the level of the adrenocortex, as indicated by normalized (or even increased) basal concentrations of indicators of cortisol synthesis or secretion by the end of the second week postpartum; and 4) to ongoing reduced hpa axis reactivity during the first months of life that probably originates from changes at suprapituitary levels, as is suggested by studies applying pharmacological and psychological challenge.

E. Clinical implications: adverse health consequences of reduced hpa activity during infancy

Suppressed fetal and infant hpa function may, due to alterations in tissue exposure to endogenous glucocorticoids, result in an altered rate of development and/or in acute or delayed health impairment. This question has, to our knowledge, not been comprehensively addressed to date. Nonetheless, the functional physiology of endogenous glucocorticoids and several experimental and clinical observations indicate that compromised endocrine activity at an early stage of life will be of clinical relevance.

1. Physiological evidence

Overall, endogenous glucocorticoids regulate the function of almost all organ systems. For instance, they regulate lipid, protein, and carbohydrate metabolism, and impact on cardiovascular, neurobiological, and immunological function. Most importantly, they maintain bodily homeostasis in stressful situations (23). Against this physiological background, it appears that reduced hpa function in infants exposed to synthetic glucocorticoids *in utero* may indeed compromise a wide variety of bodily functions.

2. Experimental evidence

There is experimental evidence that 3-month-old infants who show a cortisol decrease during a learning task have significantly impaired memory relative to infants who show a cortisol increase, indicating that appropriate cortisol reactivity is required during learning to guarantee adequate advancement (214). It can be hypothesized that reduced hpa axis reactivity in infants treated with synthetic glucocorticoids *in utero* is not restricted to painful stressors (see *Section IV.B.3*) but also occurs during cognitive tasks, thereby impairing learning and memory. To our knowledge, this issue has not been studied to date.

3. Clinical evidence

The clinical pictures of adrenal insufficiency or hypofunction may allow for assessment of the medical relevance of early life hpa hypofunction due to intrauterine exposure to synthetic glucocorticoids. Glucocorticoid deficiency due to apparent adrenal hypofunction occurs in diverse clinical conditions, such as primary and secondary adrenal insufficiency (215, 216) and CAH (217), at diverse life stages. These conditions comprise a large number of symptoms, including fatigue, gastric pain, nausea, and myalgia, indicating that adrenal insufficiency is indeed capable of compromising health. Adrenal insufficiency is usually characterized by 1) long duration of illness and 2) pronounced intensity of glucocorticoid suppression. By contrast, 1) the reduction of baseline hpa function after exposure to synthetic glucocorticoids *in utero* is probably transient, and 2) the magnitude of inhibition of hpa function is rather subclinical. Taken together, clinical models of adrenal insufficiency may not be informative about the putative role of early endogenous glucocorticoid deficiency in pathogenesis.

4. Developmental evidence

In a series of studies, atopic diseases have been linked to attenuated cortisol stress responses in children aged 7 to 14 yr (218, 219) and adults (220). Interestingly, elevated hpa reactivity has been observed in infants with positive parental atopic heritage and increased IgE concentrations in cord blood 3 d postpartum, suggesting that increased endocrine stress reactivity is linked to atopic disposition in neonates (192). In their recent study, the authors speculated that with the onset or chronification of the disease, hpa axis might switch from a hyperactive state to a hyporeactive state (192). Fries *et al.* (207) recently discussed this developmental model in detail, arguing that a switch in hpa activity might be due to overadjustment of the endocrine system. According to their model, it can be hypothesized that newborns exposed to glucocorticoids *in utero* similarly overadjust endocrine activity; in contrast to the patients with atopic diseases, individuals exposed to synthetic glucocorticoids *in utero* may start with suppressed hpa function and switch to increased activity later on. First evidence for this hypothesis comes from two groups that studied hpa function in 4-month-old infants and 30-yr-old adults, demonstrating a positive association between intrauterine glucocorticoid exposure and basal (morning) cortisol concentrations (180, 184).

If the hypothesis that hpa axis switches from a hypo- to a hyperactive state at some point in life holds true, it is of note that not only decreased, but also excessive and sustained cortisol secretion has long been linked to a wide range of stress-related diseases, primarily depression, hypertension, osteoporosis, metabolic syndrome, cardiovascular disease, gastrointestinal disease, and suppression of reproductive, growth, and immune function (20, 221–223).

This association between hypercortisolism and pathology has recently been observed in children as well, suggesting that the relationship is not just a function of adult pathology. A subset of small for gestational age children with high serum cortisol/cortisone ratio have poor catch-up growth within the first 7 yr of life, high serum total and low-density lipoprotein cholesterol, a high homeostasis model assessment insulin resistance index, and early pubertal stage (224).

In sum, there is first evidence for hpa axis switching from a hyper- to a hypoactive state due to overadjustment. Accordingly, one might hypothesize that reduced hpa function in infants exposed to synthetic glucocorticoids *in utero* would conversely switch to a hyperactive state at a later time. If this hypothesis is true, affected infants may be vulnerable to stress-related diseases that are associated with hypercortisolemic phenotypes, including depression and metabolic and cardiovascular diseases.

5. Evidence from postnatal glucocorticoid exposure

The question of whether a blunted endocrine system early in life impacts on development can be indirectly answered from a number of investigations studying the long-term consequences of early postnatal glucocorticoid therapy due to respiratory distress because this treatment has

repeatedly been linked to suppression of hpa function (*e.g.*, Refs. 225–230). Taken together, infants neonatally treated with dexamethasone for respiratory distress exhibit poorer behavioral and cognitive performance and show compromised neuromotor and psychomotor development up to school age (*e.g.*, Refs. 231–234). However, to date the question remains unanswered as to whether the observed suppression of hpa function in infants exposed to synthetic glucocorticoids after birth mediates the reported developmental outcomes of the treatment. Moreover, pre- and postnatal glucocorticoid treatments may not be comparable because postnatal therapy has different treatment indications that might themselves influence hpa axis function and usually lasts longer than prenatal therapy. Hence, stronger effects would be anticipated under postnatal treatment.

Taken together, there is first experimental and clinical evidence that reduced hpa function early in life may impair development and health in the short term and in the long term. However, more studies are needed to assess the clinical implications of altered tissue exposure to endogenous glucocorticoids in fetuses and newborns due to antenatal treatment with synthetic glucocorticoids.

F. Hpa axis dysregulation as a mechanism linking intrauterine environment to compromised health

Exposure to synthetic glucocorticoids *in utero* has been linked to impaired fetal growth (235) and modulated fetal immune function (149), indicators of compromised cognitive (236, 237), neurological (238), and psychological function (239) and increased blood pressure (240) into adolescence (5, 7). Given that 1) exposure to synthetic glucocorticoids *in utero* impacts on health and development in humans, as has been indicated by these studies; 2) exposure to synthetic glucocorticoids *in utero* suppresses fetal and infant hpa function in humans, as is one major finding of this review; and 3) reduced hpa function during infancy is associated with developmental and health impairment in humans (149, 207, 208, 241, 242), the question arises as to whether suppressed fetal and infant hpa function after exposure to synthetic glucocorticoids *in utero* may represent a mechanism that links exposure to synthetic glucocorticoids in the intrauterine environment to the observed adverse developmental and health consequences. To our knowledge, no animal study and no human study has comprehensively addressed this issue as yet. Therefore, we cannot conclusively evaluate whether infant hpa axis dysregulation mediates adverse health consequences of antenatal exposure to synthetic glucocorticoids, but there is emerging evidence that this may be the case.

Alternatively, developmental and health impairments associated with intrauterine exposure to synthetic glucocorticoids may be mediated by brain regions with GR expression that are not involved in regulation of the hpa axis or by other organ systems that express GR. In this case, changes of the hpa axis may only be an indicator of these adverse central or peripheral changes but not themselves linked to the observed adversities in individuals exposed to synthetic glucocorticoids *in utero*. Figure 2 illustrates the pathways via which intrauterine exposure to synthetic glucocorticoids may influence development and health.

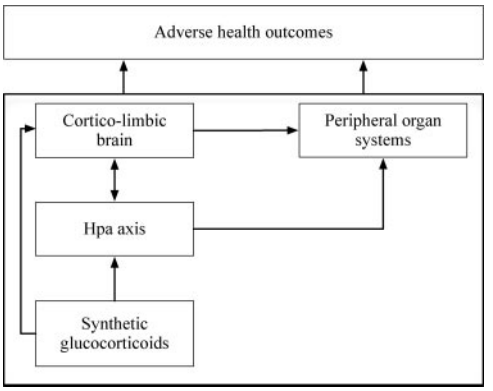

FIG 2. A model to illustrate the possible pathways via which intrauterine exposure to synthetic glucocorticoids can lead to short- and long-term alterations of hpa function and health impairment. It should be noted that putative glucocorticoid effects on hpa axis could be the cause or a concomitant biomarker of the relationship between treatment and health outcome.

G. Relevance of the findings for understanding effects of psychosocial stress during pregnancy

It is challenging to decide whether intrauterine exposure to synthetic glucocorticoids and intrauterine exposure to stress in various forms, including psychosocial stress of the mother, effectively result in similar endocrinological patterns shortly after birth. Only a few human investigations measured physiological indices of hpa axis functioning in offspring after maternal stress during pregnancy. In one investigation, prenatal maternal stress has been shown to be associated with cortisol concentrations in the offspring at school age (243). Children whose mothers reported more fear of having a handicapped child exhibit higher cortisol levels than children of mothers who had reported less fear. This finding would well fit to our above-described hypothesis that reduced hpa axis activity early in life will switch to a hyperactive state in later life due to overadjustment. In contrast to this hypothesis, newborns of mothers with antepartum and postpartum depressive symptoms already show increased cortisol concentrations shortly after birth (244).

However, the latter finding needs to be interpreted with caution because even healthy individuals having a first-degree relative with an affective illness and, therefore, be-

ing at high risk for a depressive disorder have been shown to exhibit a hypercortisolemic phenotype (245), suggesting a genetic rather than a stress-induced alteration of hpa activity in offspring of depressed mothers. More studies evaluating whether antenatal synthetic glucocorticoids are a valid model of maternal gestational stress of different forms are needed in humans.

H. Future studies

As has been discussed in detail in *Section V.B*, future human studies on intrauterine programming of hpa function by synthetic glucocorticoids are needed. Specifically, there is a strong need for 1) large randomized, placebo-controlled trials with concealment of allocation on the effects of exposure to synthetic glucocorticoids *in utero* on the offspring's hpa function; 2) long-term follow-up of endocrine function after exposure to synthetic glucocorticoids *in utero*; 3) investigations addressing sex differences in susceptibility of hpa axis to intrauterine glucocorticoid exposure; 4) investigations assessing the effects of intrauterine glucocorticoid exposure on the diurnal variability of hpa activity, as shown in animal studies (246); 5) trials that further address the mechanisms of reduced hpa function after intrauterine exposure to synthetic glucocorticoids by assessing biosynthesis, secretion, and biological activity of diverse hpa axis secretagogues and the ontogeny, number, and function of their respective receptors, including, if feasible, postmortem analyses of programming and brain morphology; 6) further studies looking at the clinical implications of fetal and infant hpa axis suppression after exposure to synthetic glucocorticoids *in utero*; and 7) evaluating whether exposure to synthetic glucocorticoids *in utero* constitutes a valid model of intrauterine exposure to maternal stress.

Furthermore, extrapolation from animals to humans may help guiding and interpreting human studies and can provide support for the human evidence. Most importantly, animal studies have the advantage of being experimentally controlled and allow scrutinizing the mechanisms underlying the observed effects of glucocorticoids on hpa axis (*e.g.*, see *Section V.C*).

In the offspring of rodents, early exposure to synthetic glucocorticoids blunts both hpa basal activity and stressor reactivity during the neonatal period and in later life (*e.g.*, Refs. 247–249). However, in a larger number of studies, early exposure to synthetic glucocorticoids has been linked to hpa hyperactivity in various species, including nonhuman primates, and at different life stages (*e.g.*, Refs. 250–254). Taken together, data from animal studies indicate that lifelong hpa axis dysregulation, rather than either static hypoactivity or hyperactivity of hpa axis, is a common consequence of early exposure to synthetic glucocorticoids (4, 5, 7, 18, 21, 255). A more detailed comparison between the human and animal literature is urgently needed. This comparison should especially take into account the differences between species such as rats, rabbits, and mice, where the hpa axis primarily matures postnatally, and species such as primates, sheep, and guinea pigs, where hpa axis maturation primarily occurs prenatally (96).

VI. Summary and Conclusions

Exposure to synthetic glucocorticoids *in utero* reduces fetal and, in some cases, newborn and infant hpa activity under basal conditions, and, more consistently, after pain-related stress. These effects may depend on the total amount of glucocorticoids administered and the time that elapses between glucocorticoid exposure and hpa axis assessment. Although baseline hpa function seems to recover within the first 2 wk postpartum, there is initial evidence that blunted hpa axis reactivity to pain-related stress persists throughout the first 4 months. It remains unclear, however, whether exposure to synthetic glucocorticoids *in utero* profoundly influences hpa function beyond the first months postpartum into adult life. Emerging evidence indicates that early dysregulation of hpa axis is adverse, possibly leading to compromised development and health in the short term. It is as yet unclear as to whether long-term health disturbances are to be expected. Specific pharmacokinetic properties of synthetic glucocorticoids and their impact on placental 11β-HSD2 activity both determine the amount of substance that passes from mother to fetus. The mechanisms of glucocorticoid-induced changes in hpa axis function are complex, including possible alterations at subcortical and cortical levels of the brain. Finally, it remains to be answered whether the endocrine effects of synthetic glucocorticoids *in utero* shed light on the impact of maternal gestational stress on fetal and newborn endocrine function. Early dysregulation of hpa axis after intrauterine exposure to glucocorticoids may be a mechanism leading to compromised development and health. More randomized human follow-up studies are needed to better understand the short- and long-term effects of antenatal exposure to synthetic or endogenous glucocorticoids on the offspring's hpa axis and potential short- and long-term consequences on health and development.

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