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Clinical Investigation

Metabolic and Hormonal Effects of Antenatal Betamethasone after 35 Weeks of Gestation

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BACKGROUND: Antenatal corticosteroid therapy recently has been considered for term and near-term infants, in addition to preterm infants, delivered by elective cesarean section, with the aim of preventing an adverse respiratory outcome.
OBJECTIVES: The objective of this study was to investigate hormonal and metabolic effects of antenatal betamethasone when administered to term fetuses.
METHODS: Cord blood levels of cortisol, C-peptide, insulin-like growth factor I and its binding protein 3, and 5 more analytes including glucose were measured in singleton newborns of over 35 weeks of gestational age. In anticipation of a cesarean delivery, the mother was either treated or not treated with 12 mg of intramuscularly administered antenatal betamethasone approximately 24 hours prior to birth. Babies of comparable gestational age, sex, and nutritional status who were not treated antenatally served as controls.
RESULTS: Cord serum cortisol levels of the betamethasone-treated fetuses were suppressed to <10% of that of untreated controls (median levels of 11.6 nmol/L vs. 138.2 nmol/L, respectively), and their C-peptide and glucose levels were significantly higher (2.85 mcg/L vs. 1.19 mcg/L, respectively, $p < 0.0001$; and 62.5 mg/dL vs. 56.0 mg/dL, respectively, $p = 0.01$).
CONCLUSIONS: Prophylactic betamethasone therapy causes immediate hormonal alterations, which might interfere with the metabolic adaptation of the newborn. This issue deserves thorough investigation.

INDEX TERMS: antenatal corticosteroids, betamethasone, hyperinsulinemia, hypoglycemia, neonate

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INTRODUCTION

The administration of corticosteroids antenatally is a standard treatment for pregnancies at risk of birth before 34 weeks of gestation. This treatment has been shown to reduce mortality, respiratory distress syndrome, necrotizing enterocolitis, and intraventricular hemorrhage in preterm infants.[1,2]

Near-term and term neonates delivered by elective cesarean section are at increased risk of respiratory morbidity compared to those by vaginal delivery.[3] Extensive experience with prophylactic corticosteroid therapy for anticipated preterm births prompted the implementation of the same treatment in near-term and term fetuses planned to be delivered by cesarean section at less than 39^{+0} weeks of gestation.[4,5] Contrary to that in preterm newborns, the effectiveness of antenatal corticosteroids in reducing respiratory morbidity in near-term and term infants is controversial. One course of prophylactic corticosteroid therapy has been reported to halve the respiratory morbidity of newborns delivered by planned section at term,[6] but other studies have not documented any benefits of this treatment in near-term and term newborns.[7–9]

Despite the recommendations for antenatal administration of corticosteroids to near-term and term infants, it has been admitted that there is no evidence for the safety of antenatal corticosteroids in babies born after 36 weeks of gestation.[4] This study aimed to investigate possible immediate alterations of the endocrine and metabolic status of term and near-term babies treated antenatally with betamethasone.

MATERIAL AND METHODS

Seventy-six singleton newborns of more than 35 weeks of gestational age (GA), delivered by cesarean section were enrolled in the study. Thirty-two babies received prophylactic corticosteroid treatment antenatally, which included a single dose of 12 mg of betamethasone (6 mg of betamethasone acetate and 6 mg of betamethasone sodium phosphate [Celestone Chronodose; Merck Sharp & Dohme, Kenilworth, NJ) given intramuscularly (IM) to the mother approximately 24 hours prior to birth. Another 44 babies of comparable GA, sex, and nutritional status who were not treated antenatally served as controls. Both the cases and the controls were drawn from the same population of consecutive cesarean deliveries. Prophylactic treatment with betamethasone was decided by the attending obstetrician and was not dictated by clinical indications of any kind. None of the 76 mothers suffered from pregestational or gestational diabetes, and none of them received dextrose solutions intraoperatively.

During the first postnatal hours, the nutritional status of the babies was thoroughly evaluated using the Clinical Assessment of Nutritional Status (CANS)[10] score and allowed for 1 modification.[11] Furthermore, 4 anthropometric indices of the babies were measured and recorded (i.e., birth weight [BW], birth length, mid-arm circumference, and chest circumference), and the ponderal index was calculated. Babies with at least 2 of the 4 indices at or below the 25th centile for GA were regarded as intrauterine growth restricted (IUGR), and those with all 4 indices above the 25th centile for GA as non-IUGR babies. This use of combined anthropometric indices has been proven to be a better approach for discriminating between IUGR and non-IUGR babies than using BW or any other index in isolation.[12]

Immediately after delivery of the placenta cord, vein blood was drawn, chilled to 4°C, centrifuged as soon as possible, and stored at −84°C. All samples were concurrently assayed. Cord serum cortisol was assayed by a competitive immunoassay using direct chemiluminescent technology (Advia Centaur, Siemens Healthcare, Tarrytown, NY). C-peptide levels were measured by immunoassay (Modular Analytics product no. E170; Roche Diagnostics, Singapore). C-peptide was chosen over insulin as C-peptide is unaffected by several blood processing conditions, contrary to insulin, which is easily degraded (e.g., in the presence of even barely visible hemolysis).[13,14] Cord serum insulin-like growth factor I (IGF-I) and its binding protein 3 (IGFBP-3) were assayed by enzyme-labeled chemiluminescent immunometric assays (Immulite 2000; Siemens Healthcare Diagnostics). Cord serum albumin, total cholesterol, glucose, total protein, and triglyceride concentrations were measured with an automated chemistry analyzer (Architect c8000; Abbott Laboratories, Abbott Park, IL). The conversion of C-peptide, cholesterol, glucose, and triglyceride levels to international units is effected by their multiplication by the conversion factors 0.333, 0.0259, 0.0555, and 0.0113, respectively (i.e., mcg/L × 0.333 = nmol/L, mg/dL × 0.0259 = mmol/L, mg/dL × 0.0555 = mmol/L, and mg/dL × 0.0113 = mmol/L, respectively).

Ethics

The Ethical Committee of the Elena Venizelou General and Maternity Hospital granted ethical permission for the study. Informed consent was obtained from the mothers for drawing cord blood and for the enrollment of their babies in the study.

Statistical Analysis

The power calculations revealed that the total sample size needed to detect a reduction of the cortisol cord blood levels in the treated fetuses to one-forth or one-third with an error of 5% and 80% power was 70 (35 treated and 35 non-treated) and 40 (20 treated and 20 non-treated), respectively. The concentration of 160 nmol/L was taken as mean cord blood cortisol level of untreated subjects.

Continuous variables were compared by the Mann-Whitney *U* test for independent samples and categorical data by the chi-square test. Limit of significance was regarded as a p value of <0.05. Statistical analysis was carried out using MedCalc version 12.6.1.0 software (Ostend, Belgium).

RESULTS

None of the babies, whether treated or not, experienced respiratory morbidity postnatally. The profiles of the betamethasone-treated and non-treated babies are shown in Table 1. The male:female ratio was 10:22 in the treated group

Table 1. Characteristics of Betamethasone-treated and Non-treated Fetuses

Characteristic	Treated (n=32)		Controls (n=44)		p Value
	Median	95% CI	Median	95% CI	
Birth weight (g)	2647	2440-2983	2845	2729-3180	0.09
Birth weight centiles	18	3-37	26	15-39	0.32
Gestational age (weeks)	37.5	36.0-38.0	38.0	37.0-39.0	0.40
CANS score	27	26-28	27	27-28	0.40
Placental weight (g)	522	482-612	519	491-598	0.82

CANS, Clinical Assessment of Nutritional Status

and 16:28 in the control group (p = 0.98). The median GA and BW of the treated babies were a little lower than those of the non-treated babies, but the differences were not significant. In addition to nutritional status of the treated and the control babies, the proportion of non-IUGR to IUGR babies did not differ significantly between the groups (14 of 18 vs. 22 of 22, respectively, p = 0.88).

Table 2 shows the hormonal and metabolic profiles of the treated fetuses and the controls. Fetuses treated antenatally with a single dose of 12 mg of betamethasone had remarkably lower cortisol levels, averaging 8% of the levels measured in the non-treated controls. Cortisol levels ranged from 5.5 to 47.8 nmol/L in the treated fetuses and from 93.7 to 464.7 nmol/L in the non-treated fetuses.

The median C-peptide level in treated fetuses was more than double that of the C-peptide levels in the non-treated ones, a highly significant difference. Fetal IGF-I levels were lower in those treated with betamethasone. Because IGF-I levels are lower in IUGR fetuses, the IGF-I levels of the treated and non-treated IUGR babies were compared. The IGF-I levels of the treated IUGR babies were significantly lower than those of non-treated babies; the median IGF-I level of the non-treated IUGR babies was 54.7 mcg/L (95% confidence interval [CI]: 45.2-58.1, n=22) versus 33.3 mcg/L (95% CI 26.7-44.9, n=18) of the treated (p = 0.02). The GA, BW, centiles of BW, CANS scores, and the placenta weight of the 2 IUGR groups did not differ significantly, and had corresponding p values of 0.99, 0.07, 0.10, 0.33, and 0.38, respectively. By contrast, the IGF-I levels of the non-IUGR treated fetuses were not significantly different from those of untreated fetuses (45.7 mcg/L vs. 49.1 mcg/L, p = 0.50).

Cord serum glucose levels of the betamethasone-treated newborns were significantly higher than those of controls. The cholesterol levels of the betamethasone-treated cases were higher, albeit with marginal statistical significance. Total protein, albumin, and triglyceride levels did not differ significantly between treated and non-treated neonates.

DISCUSSION

Immediately after birth and the subsequent abrupt interruption of the maternal glucose supply, the newborn must commence endogenous glucose production in order to maintain euglycemia. This is accomplished by the initiation of gluconeogenesis and the increase in activity of phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting enzyme of this pathway. A key role in these adaptive processes is attributed to the fall of neonatal insulin and the rise of glucagon levels soon after birth.[15] In vitro studies have shown that the transcription of the hepatic PEPCK gene is stimulated by glucocorticoids. This effect is outweighed by a subsequent treatment with insulin, which inhibits PEPCK gene transcription even in the absence of glucocorticoid pretreatment.[16]

The recommended corticosteroid regimens for antenatal prophylactic treatment of preterm babies include either 2 IM doses of 12 mg of betamethasone given 24 hours apart or 4 IM doses of 6 mg of dexamethasone given 12 hours apart.[17] The betamethasone dose administered to the mothers of term and near-term fetuses in our study was half the recommended amount (i.e., a single dose of 12 mg betamethasone given approximately 24 hours before the cesarean section). Even so, cord blood cortisol levels of the betamethasone-treated fetuses were greatly suppressed. Likewise, cord serum C-peptide levels of the treated fetuses were

Table 2. Hormonal and Metabolic Profile of Betamethasone-treated and Control Fetuses

Factor	Treated (n=32)		Controls (n=44)		p Value
	Median	95% CI	Median	95% CI	
Cortisol (nmol/L)	11.6	8.8-20.5	138.2	115.6-161.3	<0.0001
C-peptide (mcg/L)	2.85	2.44-3.47	1.19	1.09-1.29	<0.0001
IGF-I (mcg/L)	36.1	32.2-48.5	52.7	45.7-56.4	0.01
IGFBP-3 (mg/L)	1.15	0.98-1.30	1.20	1.11-1.30	0.34
Albumin (g/L)	31	30-32	30	29-32	0.49
Cholesterol (mg/dL)	69.5	61.0-78.0	57.5	48.0-62.0	0.05
Glucose (mg/dL)	62.5	59.0-64.0	56.0	56.0-60.0	0.01
Protein total (g/L)	48	45-51	49	44-50	0.95
Triglycerides (mg/dL)	19.5	15.0-26.0	17.5	14.1-23.0	0.72

IGF-I, insulin-like growth factor I; IGFBP-3, insulin-like growth factor binding protein 3

significantly higher than those of the untreated fetuses (Table 2). C-peptide levels of treated fetuses exhibited only a narrow overlap with those of the untreated ones and were of the same magnitude or even higher than the C-peptide levels reported in infants of diabetic mothers.[18,19] In addition to C-peptide levels, the glucose cord serum levels of the treated fetuses were also significantly higher. The high fetal glucose and C-peptide levels could be attributed to maternal hyperglycemia, a previously documented side effect of antenatal glucocorticoid treatment.[20] It is therefore evident that fetuses subjected to betamethasone treatment are born hyperinsulinemic, given that C-peptide is secreted in equimolar concentrations with insulin. This observation poses several questions which deserve elucidation; for example, what are the patterns of hormonal and metabolic adaptations of the term newborns exposed to synthetic corticosteroids prior to birth, and is gluconeogenesis promptly activated or not after birth? Moreover, it is unclear whether these newborns are prone to hypoglycemia due to hyperinsulinemia per se, in conjunction with the limited amount of milk that especially those establishing breast feeding take. In preterm infants, the hyperinsulinemia has been reported to persist in the intrauterine environment for approximately 24 hours[21]; however, there are a paucity of data for the time course of the postnatal normalization of insulin levels. In a recent cohort study involving newborns of more than 34 weeks GA, an almost 2-fold increased risk for hypoglycemia (blood glucose levels of <45 mg/dL or <2.5 mmol/L) was detected in those exposed to betamethasone up to 1 week prior to delivery.[9] Despite extended experience with synthetic corticosteroid treatment of preterm fetuses, possible persistence of hyperinsulinemia for some time after birth may well have escaped attention as preterm infants are routinely offered parenteral dextrose solutions.[22] Moreover, they are regularly screened for hypoglycemia, and those in need are treated without further investigation of its underlying mechanism, unless protracted. Undoubtedly the issue deserves further investigation. For the time being, it may be worthwhile for term and near-term babies subjected to prophylactic betamethasone treatment to undergo blood glucose monitoring, as in other conditions associated with neonatal hyperinsulinemia, like maternal diabetes mellitus and perinatal asphyxia.

A reduction of the basal function of the hypothalamic-pituitary-adrenal axis (HPA) of fetuses exposed to synthetic corticosteroids in utero has been already demonstrated, with fetal cortisol values decreasing to 10% of that of controls.[23] Individual reports have suggested that this reduction occurs only in treated preterm newborns (not in term newborns), thus attributing the adrenal suppression to immaturity rather than to the treatment per se.[23] Our study adds to the evidence that term fetuses exhibit an immediate adrenal suppression, even by receiving half the recommended betamethasone treatment. In addition to the basal function of the HPA axis, suppressed cortisol response to painful stress has been reported in betamethasone-treated infants, which persisted for at least 4 to 6 weeks after

birth.[24] Moreover, research evidence provides ample reason for concern about the programming effects of the antenatal corticosteroids even in term fetuses.[25,26]

An additional untoward effect, especially of repeated courses of antenatal corticosteroids, is the restriction of fetal growth.[27,28] The underlying mechanism is so far unclear, but the IGF system is possibly implicated. The IGF-I levels of fetuses exposed to synthetic corticosteroids have been reported to be reduced,[29] an observation also supported by this study (Table 2). Our finding of lower IGF-I levels only in the treated IUGR but not in the treated non-IUGR newborns might suggest that IUGR fetuses are more vulnerable to this corticosteroid effect. Undoubtedly this finding needs further confirmation.

The current limited application of the recommendation for antenatal corticosteroid treatment of term fetuses delivered by cesarean section favors a thorough investigation of its safety in addition to a reevaluation of the recommended corticosteroid regimens.[30] Considering antenatal corticosteroids as an a priori safe "antidote," able to counterbalance the untoward effects of cesarean delivery at term, may affect adversely otherwise healthy term babies.

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Abbreviations BW, birth weight; CANS, Clinical Assessment of Nutritional Status; GA, gestational age; HPA, hypothalamic-pituitary-adrenal axis; IGF-I, insulin-like growth factor I; IGFBP-3, insulin-like growth factor binding protein 3; IUGR, intrauterine growth restriction; PEPCK, phosphoenolpyruvate carboxykinase

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