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ORIGINAL RESEARCH ARTICLE

Association between prenatal glucocorticoid exposure and adolescent neurodevelopment: An observational follow-up study

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Abstract

Introduction: Prenatal exposure to supraphysiological glucocorticoid (GC) levels may lead to long-lasting developmental changes in numerous biological systems. Our prior study identified an association between prenatal GC prophylaxis and reduced cognitive performance, electrocortical changes, and altered autonomic nervous system (ANS) activity in children aged 8–9 years. This follow-up study aimed to examine whether these findings persisted into adolescence.

Material and Methods: Prospective observational follow-up study involving twenty-one 14- to 15-year-old adolescents born to mothers who received betamethasone for induction of fetal lung maturation in threatened preterm birth, but who were born with a normal weight appropriate for their gestational age (median 37^{+4} gestational weeks). Thirty-five children not exposed to betamethasone served as the reference group (median 37^{+6} gestational weeks). The primary endpoint was cognitive performance, measured by intelligence quotient (IQ). Key secondary endpoints included symptoms of attention-deficit/hyperactivity disorder (ADHD) and metabolic markers. Additionally, we determined electrocortical (electroencephalogram), hypothalamus–pituitary–adrenal axis (HPAA), and ANS activity in response to a standardized stress paradigm.

Results: No statistically significant group difference was observed in global IQ (adjusted mean: betamethasone 103.9 versus references 105.9, mean difference −2.0, 95% confidence interval [CI]: −7.12 to 3.12, $p=0.44$). Similarly, ADHD symptoms, metabolic markers, the overall and stress-induced activity of the HPAA and the ANS did not differ significantly between groups. However, the betamethasone group

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; ANS, autonomic nervous system; BM, betamethasone; CI, confidence interval; GC, glucocorticoid; HOMA-IR, Homeostasis Model Assessment—Insulin Resistance; HPAA, hypothalamus–pituitary–adrenal axis; IQ, intelligence quotient; RIAS, Reynolds Intellectual Assessment Scales; SE, standardized effect size; SEF, spectral edge frequency; SES, socioeconomic status; TSST, Trier Social Stress Test.

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exhibited reduced electrocortical activity in the frontal brain region (spectral edge frequency–adjusted means: 16.0 Hz versus 17.8 Hz, mean difference −1.83 Hz, 95% CI: −3.21 to −0.45, $p = 0.01$).

Conclusions: In 14- to 15-year-old adolescents, prenatal GC exposure was not associated with differences in IQ scores or ANS activity compared to unexposed controls. However, decelerated electrocortical activity in the frontal region potentially reflects disturbances in the maturation of cortical and/or subcortical brain structures. The clinical significance of these changes remains unknown. Given the small sample size, selective participation/loss of follow-up and potential residual confounding, these findings should be interpreted cautiously. Further research is required to replicate these results in larger cohorts before drawing firm clinical conclusions.

KEYWORDS
fetal physiology, fetal programming, glucocorticoids, neurodevelopment, preterm birth

1 | INTRODUCTION

Fetal development is a precisely orchestrated process that follows a chronological sequence of cell differentiation and organogenesis. Glucocorticoids (GC) play a crucial role in this highly regulated process by initiating the switch from tissue accretion to differentiation. Thus, early disturbances in fetal GC homeostasis may result in lasting developmental modifications in various biological systems. These systems include the central nervous system, the autonomic nervous system (ANS), and the endocrine and neuro-endocrine system, specifically the hypothalamus–pituitary–adrenal axis (HPAA).[1,2] Changes induced by GC in the fetal development of these systems are associated with a heightened risk of various cardiovascular, metabolic, and neuropsychiatric diseases later in life.[2,3]

There are two main factors that contribute to abnormal fetal GC homeostasis during human development, both of which result in excessive fetal GC exposure: maternal psychological stress or maternal treatment with synthetic GCs.[4] To study the long-term effects of elevated fetal GC exposure, synthetic GCs are of special interest in human research as they allow to study GC effects in a quasi-experimental setting. Comparatively, maternal psychological stress is often difficult to objectify. Synthetic GCs are often used in obstetric practice to promote fetal lung maturation in cases of potential preterm birth, and most human studies are based on synthetic GCs administered in this indication.[5–9] However, this research approach has a notable limitation as most children in previous cohorts were born prematurely, which could confound the effects of GCs on long-term development.[10] Moreover, human outcome data on prenatal synthetic GC exposure in the long term is limited. For instance, a recent follow-up of a large randomized trial did not reveal an elevated rate of cardiovascular events or an increased prevalence of cardiovascular risk factors 50 years after exposure to GC prophylaxis for neonatal respiratory distress syndrome.[11] However, the study was limited by a low follow-up rate and the absence of in-person assessments.

Key message

In our follow-up study on 14- to 15-years-olds prenatally exposed to glucocorticoid prophylaxis for respiratory distress syndrome, we found that earlier discrepancies in prepubertal IQ scores and autonomic nervous system activity, in comparison to unexposed subjects, do not continue into adolescence.

Our group previously studied the effects of prenatal GC prophylaxis for respiratory distress syndrome on the stress system, cognition, and behavior in children aged 8–9 years.[12] These children were at risk of preterm birth, but were eventually born at or near term with a weight appropriate for their gestational age. We found no significant differences in HPAA activity between children exposed to GC and those unexposed. However, GC-exposed children had a lower intelligence quotient (IQ) score (96.9 vs. 108.0) and presented more core symptoms of attention-deficit/hyperactivity disorder (ADHD). During a stress test, these children also exhibited higher parasympathetic tone in the ANS and elevated electrocortical activity. It remains unclear, however, whether these observed effects continue into adolescence.

The objective of this study is to assess the consistency of previously observed outcomes in a subset of our children's cohort, now aged 14–15 years, with a focus on IQ. We hypothesized that the previously observed IQ discrepancies between children exposed and unexposed to GC would continue into their teenage years. Our hypothesis is based on longitudinal studies of children and adolescents from uncomplicated pregnancies and low-birthweight pregnancies in which IQ shows high temporal stability.[13–15] Additionally, we added an evaluation of insulin resistance to our previous study protocol because insulin resistance is considered a relevant risk factor for cardiovascular and metabolic diseases.[16] While antenatal GC

expose has been correlated with impaired insulin resistance in animal experiments, limited human data are available on this subject.[17]

2 | MATERIAL AND METHODS

2.1 | Research design

We conducted a prospective observational study to evaluate the association between antenatal betamethasone (BM) exposure and cognitive performance, specifically IQ, in children aged 14 to 15 years. We compared a group of children who were exposed (BM group) with those who were not (reference group). The children were born between September 1999 and September 2003 in either Jena University Hospital or the regional hospital in Gera, Germany. We assessed their outcomes in a single day at the Jena University Hospital.

2.2 | Recruitment strategy and study participants

The recruitment strategy replicated the one used in our previous study, thoroughly detailed elsewhere.[12] In brief, children in the BM group were chosen from a known cohort of mothers who had been risk-prone for preterm birth and had joined a randomized controlled trial contrasting two tocolytic treatments.[18] As standard, all trial participants received either one or multiple courses of 2×8 mg BM 24 h apart to promote fetal lung maturation. The children initially at risk of preterm birth but later delivered with normal birthweight after a minimum gestation of 238 days/34^{+0} weeks were identified. The reference group's children were selected from medical birth records and had the same gestational age as the BM group's children. The exclusion criteria, applicable to both groups, included a birthweight under the fifth reference percentile, severe perinatal complications necessitating intensive care, intrauterine exposure to maternal smoking, alcohol, drugs, and long-term GC treatment. The final cohort studied comprised 21 children from the BM group and 35 from the reference group (Figure 1). A total of 17 children from the BM group and 26 children from the reference group had also been previously assessed at the age of 8 to 9 years.[12]

2.3 | Demographic and clinical baseline data

Socioeconomic status (SES) and other demographic variables were collected using a parent questionnaire. SES was calculated as a composite score (range 3–21) based on the highest educational attainment of parents, occupational status reflecting the social prestige of the occupation, and net household income, following the methodology outlined in.[19] Pregnancy and birth data were obtained from hospital records or the German maternity passport. The Zurich Life-Event List was used to quantify the frequency of positive and negative life events during the past 12 months before outcome assessment.[20]

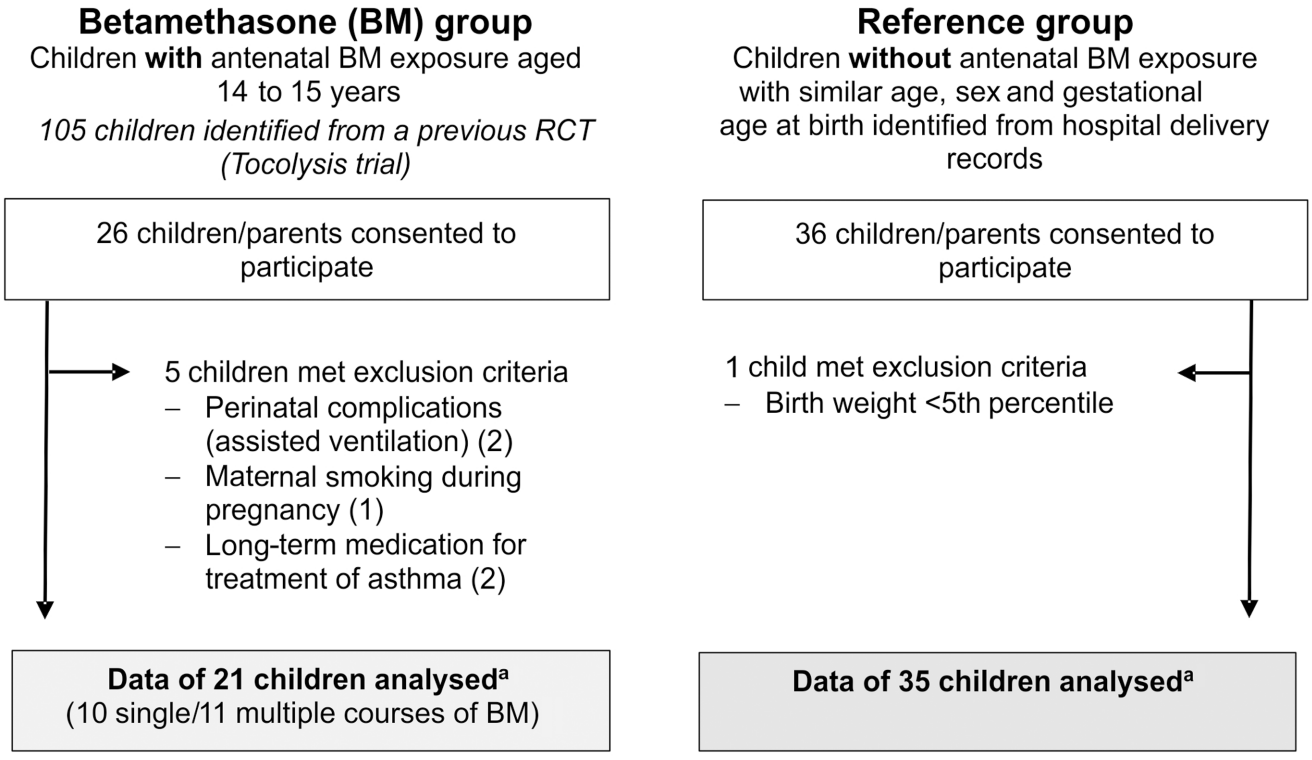

FIGURE 1 Flowchart of the study recruitment process. [a]Seventeen children of the betamethasone group and 26 children of the reference group were also previously assessed at the age of 8–9 years.

2.4 | Outcomes of interest

The primary outcome was cognitive performance, measured by IQ. The key secondary outcomes included (a) core symptoms of ADHD; (b) body mass index (BMI); and (c) the Homeostasis Model Assessment index used to estimate insulin resistance (HOMA-IR). In response to a standardized stress test, the Trier Social Stress Test in a version adapted for children (TSST), we also measured the following secondary outcomes: (d) HPAA activity, which was measured through salivary-free cortisol; (e) ANS activity, which was determined by salivary α-amylase concentration and heart rate variability; and (f) electrocortical activity, which was measured through electroencephalogram (EEG) signals.

2.5 | Summary of study procedure, outcome assessment, and statistical analysis

In-depth methodological details are outlined in Appendix S1. This study generally mirrors our previous research protocol.[12] We evaluated overall cognitive performance (IQ) using the Reynolds Intellectual Assessment Scales (RIAS) IQ test.[21] The RIAS consist of two subtests that measure verbal and non-verbal intelligence and produce a composite IQ score representing children's reasoning, problem-solving, and learning abilities. The FBB-ADHS questionnaire, a German parental ADHD rating scale, was used to identify core ADHD symptoms.[22]

BMI was determined through height and weight measurements [body mass (kg)/height (m^2)]. We calculated the HOMA-IR by multiplying the fasting plasma insulin concentration (mU/L) by the fasting plasma glucose concentration (mmol/L), then dividing by 22.5.

To assess the HPAA and ANS responsiveness to acute stress, children participated in the TSST. This standardized stress test incorporated a preparation period, speech and mental arithmetic assignments in front of an audience, followed by a recovery period.[23] During the TSST, we collected EEG, electrocardiogram, and blood pressure data. In addition, five saliva samples were obtained to measure the concentrations of salivary free cortisol and alpha-amylase. These biomarkers served as proxies for the activity of the HPAA and the ANS, respectively. Heart rate variability frequency indices (mean heart rate, low-frequency [LF] and high-frequency [HF] band power, LF/HF ratio) were used to further approximate ANS activity.[24] Electrocortical activity was determined through power spectral analysis of EEG recordings. The electrocortical activity represents a physiological correlation of cortical and subcortical brain function.[25] The spectral edge frequency (SEF) of the total band power (1.5–30 Hz) was calculated from the gathered EEG recordings and used as a secondary outcome parameter.

We aimed to enroll 35 children per group, which would have 80% power to detect a standardized mean difference of 0.68, as observed in the cognitive performance (IQ) component of our initial study. We used linear models for single outcomes and mixed linear models for outcomes repeatedly measured. We determined the HPAA, ANS, and electrocortical activity during the entire TSST including baseline and recovery period (overall activity model) and the baseline adjusted stress-response during the acute stress phase (baseline-adjusted model). Results were reported as unadjusted and predefined covariate adjusted estimates (difference of means, ratio of geometric means) with corresponding 95% confidence intervals. In the baseline-adjusted stress response model, a common baseline mean was additionally calculated, indicating whether the average outcome measurement increased or decreased in each group. A two-sided significance level of 0.05 was set. Analyses of secondary outcomes were exploratory, so we did not adjust the significance level for multiplicity as we aimed at searching for adverse signals.[26] Post hoc, we investigated a dose–response relationship for IQ and the two RIAS subscales. Furthermore, a sensitivity analysis was conducted for the primary outcome IQ, which only included children who were previously assessed at the age of 8 to 9 years.

3 | RESULTS

3.1 | Demographic and clinical baseline data and exposure characteristics

The study groups were evenly matched in terms of most demographic, pregnancy, and birth-related variables (Table 1). However, children in the BM group had undergone more adverse life events in the year before enrollment compared to the reference group. Furthermore, their parents had a lower SES. As per our recruitment strategy, all children in the BM group underwent tocolytic treatment compared to two children in the reference group. Ten children were exposed to a single course of BM and 11 children to multiple courses of BM (range: 2 to 5).

3.2 | Outcomes

3.2.1 | Cognitive performance (primary outcome) and behavior (secondary outcome)

Our study found no statistical difference in global IQ between the BM group and the reference group. On average, IQ scores in the BM group were 102.5 (standard deviation [SD] 9.63) compared to 106.4 (SD 9.57) in the reference group and therefore 3.95 points lower (95% confidence interval [CI]: −9.26 to 1.36, $p = 0.14$). However, when adjusted for SES and sex, the gap was lessened to 2.0 points (95% CI: −7.12 to 3.12, $p = 0.44$) (Table 2). Similarly, for non-verbal IQ, the BM group scored 2.06 points less (95% CI: −7.03 to −2.92), and for verbal IQ, they scored 3.56 points less (95% CI: −8.77 to 1.67) compared to the reference group, after accounting for SES and sex. The BM group's non-verbal and verbal IQs were 102.2 and 103.4, respectively, while those of the reference group were 104.3 and 107.0. A sensitivity analysis was conducted, which only considered children who had earlier assessments at ages 8 to 9 years. The results were

TABLE 1 Cohort and exposure characteristics.

	BM ($n=21$)	Refs ($n=35$)	p Value
Demographic and socioeconomic data			
Child			
Age (year), mean (SD)	14.7 (0.60)	14.3 (0.64)	0.06
Female gender, *n* (%)	13 (62)	19 (54)	0.78
Living together with parents, *n* (%)	15 (71)	26 (74)	1.00
Only child, *n* (%)	1 (5)	5 (15)	0.39
More than one unpleasant life event (ZLEL), *n* (%)	16 (76)	16 (46)	0.03
Type of school currently attending, *n* (%)			0.41
High school (Gymnasium)	14 (67)	19 (54)	
Secondary school or below	7 (33)	16 (46)	
Parents			
Maternal age [year], mean (SD)	43.8 (5.6)	43.6 (4.8)	0.87
Paternal age [year], mean (SD)	46.5 (6.6)	46.8 (6.3)	0.83
Maternal BMI [kg/m^2], mean (SD)	24.7 (2.9)	25.0 (3.7)	0.75
Paternal BMI [kg/m^2], mean (SD)	27.4 (5.3)	27.4 (4.6)	0.97
Socioeconomic status, mean (SD)	13.7 (4.9)	15.9 (4.3)	0.08
Pregnancy related data			
Hyperemesis during pregnancy, *n* (%)	0 (0)	2 (6)	0.52
Abortus imminens, *n* (%)	3 (14)	2 (6)	0.35
Other bleeding during pregnancy, *n* (%)	1 (5)	0 (0)	0.38
Preeclampsia, *n* (%)	0 (0)	1 (3)	1.00
Tocolytic treatment, *n* (%)	21 (100)	2 (6)	<0.01
Maternal age at birth [year], mean (SD)	29.6 (5.5)	29.8 (4.6)	0.85
Birth related data			
Cesarean section, *n* (%)	4 (19)	6 (17)	1.00
Gestational age at birth [weeks], median (IQR)	37^{+4} (36^{+2}–38^{+6})	37^{+6} (36^{+4}–38^{+4})	0.85
Gestational age at birth, *n* (%)			0.20
<35 weeks	2 (10)	0 (0)	
35 to <37 weeks	7 (33)	11 (31)	
≥37 weeks	12 (57)	24 (69)	
Birthweight [g], mean (SD)	3083 (543)	3104 (456)	0.88
Birth length [cm], mean (SD)	49.2 (3.7)	49.7 (2.4)	0.62
Head circumference [cm], mean (SD)	33.9 (2.0)	34.2 (1.2)	0.59
APGAR 10, median (IQR)	9 (9–10)	9 (9–10)	0.36
Neonatal monitoring, *n* (%)	4 (19)	6 (17)	1.00
Exposure characteristics			
Single course of 2×8 mg BM 24 h apart, *n* (%)	10 (48)		
Multiple courses of 2×8 mg BM 24 h apart, *n* (%)	11 (52)		
Number of BM courses, median (range)	2 (1–5)		
Start of BM (gestational week), median (IQR)	32 (31–33)		

Note: Missing data (BM/reference group): Living together with parents (0/1), only child (0/1), paternal age (1/2), maternal body mass index (2/1), paternal body mass index (3/9).

Abbreviations: BM, betamethasone; BMI, body mass index; IQR, interquartile range (25th–75th percentile); *n*, number of subjects; Refs, non BM-exposed reference group; SD, standard deviation; ZLEL, Zurich Life-Event List of unpleasant life events during the last 12 month before examination.

similar, with no statistically significant differences in IQ observed among the groups.

In our exploratory post hoc analysis, we failed to identify a dose–response relationship between BM exposure and cognitive performance. The model-based average IQ, adjusted for sex and SES, was 105.9 for children in the reference group, 101.4 for children in the BM group exposed to a single BM treatment course, and 106.1 for those exposed to multiple BM treatment courses.

There were no statistically significant differences between the groups on the core symptoms of ADHD (Table 2).

3.2.2 | BMI and insulin resistance (secondary outcomes)

Our study found no statistically significant group differences in BMI and HOMA-IR reflecting insulin resistance (Table 2).

TABLE 2 Primary and secondary outcome(s) representing cognitive performance, behavior and metabolic markers.

	Estimated means		BM versus reference group		
	BM	Refs	DIF (95% CI)	SDIF	*p* Value
Primary outcome					
Cognitive performance (IQ)					
Unadjusted	102.5	106.4	−3.95 (−9.26 to 1.36)	0.41	0.14
Adjusted[a]	103.9	105.9	−2.00 (−7.12 to 3.12)	0.22	0.44
Secondary outcomes					
ADHD symptoms (DISYPS)					
Unadjusted	101.0	102.4	−1.48 (−7.75 to 4.79)	0.13	0.64
Adjusted[a]	101.1	102.5	−1.37 (−7.96 to 5.22)	0.12	0.68
Body mass index [kg/m^2][b]					
Unadjusted	21.7	20.2	1.53 (−0.60 to 3.65)	0.41	0.15
Adjusted[c]	21.3	20.3	1.01 (−1.04 to 3.07)	0.30	0.33
HOMA-IR[d]					
Unadjusted	2.6	2.3	0.30 (−0.36 to 0.95)	0.26	0.37
Adjusted[a]	2.5	2.3	0.14 (−0.51 to 0.79)	0.12	0.68

Note: Arithmetic means and their differences were estimated by linear models. For better comparison, differences were standardized similar to Cohen's *d*.

Abbreviations: BM, betamethasone; CI, confidence interval; DIF, difference of means; SDIF, standardized difference of means; IQ, intelligence quotient; ADHD, attention-deficit/hyperactivity disorder; DISYPS, Diagnostic System for Psychiatric Disorders in Children and Adolescents; HOMA-IR, Homeostasis Model Assessment index; Refs, non BM-exposed reference group.

[a]Adjusted for socioeconomic status and sex.

[b]2 BM, 1 reference subjects excluded due to missing data for maternal BMI.

[c]Adjusted for socioeconomic status, sex, and maternal body mass index.

[d]1 BM, 3 reference subjects excluded due to missing data for fasting blood glucose.

3.2.3 | HPAA, ANS, and electrocortical activity during the TSST (secondary outcomes)

We found no statistically significant group difference in HPAA and ANS outcome parameters in our overall activity analysis comprising all phases of the TSST (Table 3). However, the electrocortical activity in the frontal brain regions was lower in children of the BM group compared to those in the reference group (SEF, 16.1 Hz vs. 17.9 Hz, $p = 0.01$). This difference remained statistically significant after making adjustments for confounding variables: Adjusted SEF 16.0 Hz versus 17.8 Hz, mean difference −1.83 Hz, 95% CI: −3.21 to −0.45 Hz, $p = 0.01$. No statistically significant group differences were found for the electrocortical activity in other brain regions.

We found no statistically significant group difference for HPAA, most ANS, and all electrocortical outcome parameters in our baseline-adjusted analysis of the acute stress response (Table 4). Even though children in the BM group revealed a smaller increase in salivary alpha-amylase compared to the reference group (42.2 U/mL vs. 71.8 U/mL, $p = 0.04$), the ratio of the geometric means was no longer statistically significant after taking into account the predetermined covariates SES and sex.

4 | DISCUSSION

The present study examined the association between prenatal exposure to BM, used to promote fetal lung maturation in threatened preterm birth, and cognitive performance in a group of term or near term born children at the age of 14–15 years. Contrary to our initial hypothesis, we found no statistically significant difference in IQ between the BM and the reference group, as demonstrated in our cohort 6 years earlier.[12] This result was somewhat surprising given that cognitive abilities, including IQ scores, are generally stable from childhood through adolescence to adulthood, as multiple longitudinal studies,[14,15] including those involving low-birthweight children, have established.[13] Biologically, considering the high plasticity of the brain, our findings might suggest that brain function compromised by GC either improves over time or that GC induced a developmental delay 6 years earlier, which has now been rectified. A longitudinal study of children born with very low birthweight previously demonstrated the brain's principal ability for a developmental catch up: reading deficits at the age of 9 improved and were no longer detectable at the age of 15.[27] Consistent with our findings, follow-up studies of randomized controlled trials have not identified any link between antenatal GC prophylaxis for respiratory distress syndrome

TABLE 3 Neuroendocrinological, autonomous, and electrocortical outcome parameters covering all phases of the Trier Social Stress Test (overall activity model).

	Estimated means		BM versus reference group		
Outcome measures	**BM**	**Refs.**	**DIF or ratio (95% CI)**	**SDIF**	***p* Value**
HPAA					
Saliva cortisol (nmol/L)					
Unadjusted	2.37[a]	2.26[a]	1.05[b] (0.76 to 1.44)	0.08[c]	0.78
Adjusted[d]	2.31[a]	2.25[a]	1.03[b] (0.74 to 1.42)	0.05[c]	0.88
Autonomic nervous system					
Saliva α-amylase (U/mL)					
Unadjusted	49.2[a]	46.4[a]	1.06[b] (0.46 to 2.43)	0.04[c]	0.89
Adjusted[d]	54.8[a]	43.3[a]	1.27[b] (0.55 to 2.90)	0.16[c]	0.57
Mean heart rate (1/min)					
Unadjusted	89.0	91.0	−1.91 (−8.72 to 4.90)	0.16	0.58
Adjusted[d]	88.7	90.8	−2.10 (−9.17 to 4.98)	0.17	0.55
LF band power (ms^2/Hz)					
Unadjusted	1118[a]	1018[a]	1.10[b] (0.73 to 1.65)	0.13[c]	0.65
Adjusted[d]	1207[a]	1028[a]	1.17[b] (0.78 to 1.76)	0.23[c]	0.43
HF band power (ms^2/Hz)					
Unadjusted	611[a]	541[a]	1.13[b] (0.64 to 2.00)	0.12[c]	0.67
Adjusted[d]	636[a]	544[a]	1.17[b] (0.64 to 2.12)	0.15[c]	0.60
LF/HF					
Unadjusted	1.83[a]	1.88[a]	0.97[b] (0.71 to 1.33)	0.05[c]	0.85
Adjusted[d]	1.90[a]	1.89[a]	1.00[b] (0.73 to 1.39)	0.01[c]	0.98
Electrocortical activity					
SEF frontal (Hz)					
Unadjusted	16.1	17.9	−1.80 (−3.13 to −0.48)	0.76	0.01
Adjusted[e]	16.0	17.8	−1.83 (−3.21 to −0.45)	0.76	0.01
SEF temporal (Hz)					
Unadjusted	21.2	20.9	0.37 (−1.63 to 2.36)	0.10	0.71
Adjusted[e]	21.0	20.8	0.12 (−1.87 to 2.11)	0.03	0.91
SEF parietal (Hz)					
Unadjusted	17.2	17.3	−0.16 (−1.50 to 1.18)	0.07	0.81
Adjusted[e]	17.0	17.2	−0.25 (−1.54 to 1.03)	0.11	0.70
SEF occipital (Hz)					
Unadjusted	18.7	18.2	0.55 (−1.28 to 2.39)	0.17	0.55
Adjusted[e]	18.5	18.1	0.42 (−1.37 to 2.21)	0.13	0.64

Note: Excluded subjects due to missing data (BM/Refs.): Cortisol (1/0), α-amylase (1/0), Autonomous nervous system (0/1), Electrocortical activity (0/1). Excluded subjects due to implausible data (BM/control): α-amylase (0/1). Estimated by mixed linear models: Arithmetic means and their difference or geometric means ([a]) and their ratio ([b]) after transforming back results of logarithmic scaled variables to the original scale. For better comparison, differences were standardized similar to Cohen's *d*. [c]Standardized differences based on logarithmic scale. [d]Adjusted for socioeconomic status and sex. [e]Adjusted for side of electroencephalogram electrodes, socioeconomic status, and sex.

Abbreviations: BM, betamethasone; CI, confidence interval; DIF, difference; HF, high frequency; HPAA, hypothalamus–pituitary–adrenal axis; LF, low frequency, SDIF, standardized difference; SEF, spectral edge frequency.

and cognitive performance in individuals aged 20–31 years.[6,28] However, our results may be affected by some selection bias. Children in the BM group who were lost to follow-up showed lower IQ scores than those who also participated in the current study (91.6 vs. 99.9).[12] In contrast, in the reference group, this difference was in the opposite direction (111.2 vs. 106.6). Thus, varied participation rates, especially among children with IQ scores at the extreme ends, could have produced an overestimated group difference in IQ at the 8–9 year follow-up or masked a group difference in the present study. Selective attrition related to IQ is a common occurrence

TABLE 4 Baseline-adjusted neuroendocrinological, autonomous and electrocortical outcome parameters covering the active phases of the Trier Social Stress Test (stress response model).

	Estimated means			BM versus reference group		
Outcome measures	**Baseline**	**BM**	**Refs**	**DIF or ratio (95% CI)**	**SDIF**	***p* Value**
HPAA						
Saliva cortisol (nmol/L)	1.68[a]					
Baseline adjusted		2.28[a]	2.55[a]	0.89[b] (0.64 to 1.24)	0.21[c]	0.49
Further adjusted[d]		2.18[a]	2.58[a]	0.85[b] (0.61 to 1.18)	0.31[c]	0.32
Autonomic nervous system						
Saliva α-amylase (U/mL)	38.2[a]					
Baseline adjusted		42.2[a]	71.8[a]	0.59[b] (0.35 to 0.98)	0.61[c]	0.04
Further adjusted[d]		43.8[a]	71.1[a]	0.62[b] (0.36 to 1.05)	0.54[c]	0.07
Mean heart rate (1/min)	80.7					
Baseline adjusted		97.0	96.1	0.94 (−6.35 to 8.22)	0.07	0.80
Further adjusted[d]		96.9	95.3	1.57 (−5.81 to 8.95)	0.12	0.67
LF band power (ms^2/Hz)	1033[a]					
Baseline adjusted		892[a]	977[a]	0.91[b] (0.65 to 1.28)	0.15[c]	0.59
Further adjusted[d]		939[a]	975[a]	0.96[b] (0.68 to 1.37)	0.06[c]	0.83
HF band power (ms^2/Hz)	590[a]					
Baseline adjusted		486[a]	564[a]	0.86[b] (0.53 to 1.39)	0.18[c]	0.53
Further adjusted[d]		500[a]	585[a]	0.85[b] (0.53 to 1.39)	0.19[c]	0.52
LF/HF	1.75[a]					
Baseline adjusted		1.81[a]	1.75[a]	1.03[b] (0.75 to 1.41)	0.06[c]	0.84
Further adjusted[d]		1.85[a]	1.70[a]	1.09[b] (0.79 to 1.50)	0.15[c]	0.61
Electrocortical activity						
SEF frontal (Hz)	16.7					
Baseline adjusted		17.0	18.0	−0.98 (−2.23 to 0.27)	0.47	0.12
Further adjusted[e]		17.1	17.9	−0.80 (−2.09 to 0.50)	0.36	0.22
SEF temporal (Hz)	18.6					
Baseline adjusted		21.5	22.0	−0.46 (−2.27 to 1.34)	0.14	0.61
Further adjusted[e]		21.5	22.0	−0.53 (−2.42 to 1.36)	0.16	0.57
SEF parietal (Hz)	16.9					
Baseline adjusted		17.3	17.4	−0.13 (−0.93 to 0.67)	0.09	0.74
Further adjusted[e]		17.3	17.4	−0.06 (−0.88 to 0.77)	0.04	0.89
SEF occipital (Hz)	17.7					
Baseline adjusted		18.7	18.5	0.23 (−0.86 to 1.31)	0.12	0.68
Further adjusted[e]		18.7	18.5	0.21 (−0.93 to 1.34)	0.10	0.72

Note: Excluded subjects due to missing data (BM/Refs.): Cortisol (1/3), α-amylase (1/3), Autonomous nervous system (0/1), Electrocortical activity (0/1). Excluded subjects due to implausible data (BM/control): α-amylase (0/1). Estimated by linear (α-amylase) or mixed linear models: Baseline adjusted arithmetic means and their difference or geometric means ([a]) and their ratio ([b]) after transforming back results of logarithmic scaled variables to the original scale. For better comparison, differences were standardized similar to Cohen's *d*. Standardized differences ([c]) based on logarithmic scale. [d]Further adjusted for socioeconomic status and sex. [e]Further adjusted for side of electroencephalogram electrodes, socioeconomic status and sex.

Abbreviations: BM, betamethasone; CI, confidence interval; DIF, difference; HF, high frequency; HPAA, hypothalamus–pituitary–adrenal axis; LF, low frequency; Refs, non BM-exposed reference group; SDIF, standardized difference; SEF, spectral edge frequency.

in longitudinal studies, with those having lower IQs more prone to drop out than their higher IQ counterparts.[29] The reason for selective dropout among children with higher IQ in the reference group, however, remains unclear.

Regarding our secondary outcomes, we found that children in the BM group exhibited slower electrocortical activity in the frontal brain regions, as evidenced by a lower SEF in these areas. The SEF represents an effective estimate of the frequency content

of the EEG power spectrum generated by thalamo-cortical and cortico-cortical networks.[25,30] Changes in SEF were also noted in children exposed to BM during our earlier assessment, even though the pattern of changes differed between the two time-points.[12] Unlike cognitive performance, the SEF is unlikely to be affected by self-selection bias. Thus, it is reasonable to suggest that BM is associated with maturational changes of cortical and/or subcortical brain structures. Similarly, an MRI study that included infants exposed to antenatal GC prophylaxis showed significant differences in cortical brain maturation in comparison to controls not exposed to GC.[31] Slower frequencies of the frontal EEG have been regularly related to adolescent ADHD[32,33] and to a lower cognitive performance in children[34] and adolescents.[35] However, the debate if EEG frequencies and intelligence are positively linked is still ongoing.[36]

Exposure to intrauterine BM did not show any statistically significant association with insulin resistance or BMI as primary markers of the metabolic syndrome, despite the fact that the BMI of BM-exposed children was 1 kg/m^2 higher than in the reference group. In animals, antenatal exposure to synthetic GCs has been regularly linked to markers of the metabolic syndrome such as enhanced fat deposition and decreased insulin sensitivity.[17] Similarly, a long-term follow-up of a large clinical trial reported that adult subjects who were antenatally exposed to BM displayed a heightened insulin response to an oral glucose test compared to a placebo group.[6] This trial also indicated a possible dose-dependent effect. In contrast, exposure to repeated courses of antenatal BM compared with a single course of BM did not increase cardiometabolic risk factors in early school-aged children.[37] It is possible that changes in cardiometabolic risk factors induced by GC, including alterations in glucose metabolism, may not be detectable at early ages but may develop over time.

In agreement with our previous assessment 6 years earlier, but in contrast to the findings of Alexander et al.'s follow-up studies of children and adolescents who were antenatally exposed to GC prophylaxis with BM,[9,38] HPAA activity during the TSST was not associated with prenatal BM-exposure. As previously discussed,[12] this difference may be attributed to the different dosing regimens of BM used in our cohort compared to Alexander's cohort. We further did not detect any significant increase in ADHD core symptoms after antenatal GC exposure, in contrast to our previous assessment. This result aligns with the general observation from large birth cohorts reporting that ADHD symptoms in children and adolescence decrease over time.[39,40]

There are certain limitations to our study.[12] We cannot rule out the possibility that the reduced sample size and statistical power in this study led to a type II error. However, a small difference in IQ scores within the observed range may not necessarily reflect meaningful differences in cognitive ability or real-world functioning. Moreover, selection bias could arise from both selective participation and loss of follow-up.[41] Due to loss of follow-up, the current sample does not fully match the 8- to 9-year-old sample which was susceptible to selective participation bias. However, 43 of 56 children (77%) participated in both examinations. Our sensitivity analysis of the primary outcome IQ revealed similar results when the analysis was restricted to this subgroup. Maternal psychosocial stress associated with preterm birth may have modified our outcomes.[1] Future studies may add a separate control group with comparable maternal stress elicited for example by adverse life events to overcome this issue. Additionally, our study included approximately one-third of adolescents born late preterm, a factor that has been independently linked to adverse neurocognitive outcomes. While control subjects were similar by gestational age, it cannot be ruled out that specific pre-conditions which prevented late preterm controls from BM exposure may have had an impact on the outcomes. We cannot rule out an independent effect of the tocolytic treatment on our results. However, because two pharmacologically different tocolytics were used in a balanced manner, a significant bias is unlikely. Given the complex and long-lasting nature of neurodevelopment, residual confounding is likely. We did not adjust the level of significance for multiple comparisons, which potentially raises the risk of false-positive results. Lastly, we cannot establish a causal relationship between antenatal exposure to BM and the outcomes explored due to the observational nature of our study.

5 | CONCLUSION

In our follow-up study of individuals aged 14 to 15 years, prenatal exposure to GC prophylaxis for respiratory distress syndrome was not associated with changes in IQ scores and ANS activity compared to an unexposed reference group. In contrast, GC exposed adolescents demonstrated a decelerated electrocortical activity which may indicate aberrations in functional brain development with currently unknown clinical significance. Furthermore, antenatal GC exposure was not associated with primary markers of the metabolic syndrome. Due to the small sample size of our cohort and the lower participation rates especially of children with IQ scores at the extreme ends compared to the first examination 6 years before, it is important to interpret our results with caution. Our findings will need to be replicated in a larger sample before firm clinical conclusions can be drawn.

AUTHOR CONTRIBUTIONS

The idea of this study was conceived by Matthias Schwab and Ekkehard Schleussner. Matthias Schwab, Ekkehard Schleussner, Heike Hoyer, Dirk Hoyer, Sven Rupprecht, Carolin Ligges and Isabel Muth designed the study. Isabel Muth, Sven Rupprecht, Steffen Kluckow, Torsten Schultze and Amani Cornelius recruited the participants and/or performed the tests. Dirk Hoyer, Karin Schiecke and Heike Hoyer analyzed the data. Florian Rakers, Heike Hoyer, Ekkehard Schleussner and Matthias Schwab interpreted the data and wrote the manuscript. All authors critically revised the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have nothing to disclose.

ETHICS STATEMENT

The study was approved by the Jena University Hospital's ethics committee (Nr: 4160-07/14) on July 15, 2014, and all participants provided informed consent.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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