Sex-Dimorphic Effects of Prenatal Treatment With Dexamethasone

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Context: Dexamethasone (DEX) is used to prevent virilization in female fetuses at risk of congenital adrenal hyperplasia (CAH). Given that treatment has to be started before the genotype is known, 7 out of 8 fetuses will be exposed to DEX without benefit.

Objective: To evaluate long-term cognitive effects of prenatal DEX therapy in healthy (non-CAH) DEX-treated children.

Design and Setting: Observational study with patient and control groups from a single research institute.

Participants: Healthy (non-CAH) DEX-treated subjects (n = 34) and untreated population controls (n = 66) from Sweden, aged 7–17 years.

Intervention: DEX-treatment used in unborn children at risk of CAH, during first trimester of fetal life.

Main Outcome Measures: Standardized neuropsychological tests and questionnaires were used.

Results: DEX treatment has widespread negative effects in girls. In Wechsler Intelligence Scales for Children-III scale subtests, we observed significant interactions between DEX and GENDER (coding, $P = .044$; block design, $P = .013$; vocabulary, $P = .025$) and a trend for the subtest digit span ($P = .074$). All interactions were driven by DEX effects in girls, but not boys, with DEX-treated females showing lower scores than female untreated controls (coding, $P = .068$, $d = 0.66$; block design, $P = .021$, $d = 0.81$; vocabulary, $P = .014$, $d = 0.84$; digit span, $P = .001$, $d = 1.0$). Likewise, DEX-treated girls tend to have poorer visual spatial working memory performance than controls (span board test forward: $P = .065$, $d = .80$). We observed no effects on long-term memory, handedness, speed of processing, nor self-perceived or parentally reported scholastic performance.

Conclusions: Early prenatal DEX exposure affects cognitive functions in healthy girls, ie, children who do not benefit from the treatment. It can therefore not be considered safe to use this therapy in the context of CAH. **(*J Clin Endocrinol Metab* 101: 3838–3846, 2016)**

Congenital adrenal hyperplasia (CAH) is one of the most common causes of ambiguous genitalia in females. Affected individuals have mutations in one of the genes that code for enzymes needed for cortisol synthesis in the adrenal cortex. In over 90% of the cases, the deficient enzyme is 21-hydroxylase encoded by the 21-hydroxylase gene (*CYP21A2*, 6p21.3). The reduced production of cortisol and aldosterone in the classic form of

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Abbreviations: CAH, congenital adrenal hyperplasia; CBCL, Child Behavior Checklist; DEX, dexamethasone; GC, glucocorticoid; GR, GC receptor; HPA, hypothalamic-pituitary-adrenal; MR, mineralocorticoid receptor; NEPSY, A Developmental Neuropsychological Assessment; WISC-III, Wechsler Intelligence Scales for Children-III.

21-hydroxylase deficiency results in excess levels of adrenal androgens which virilize the female fetus already in utero. Genital plastic surgery may therefore be needed in the more severe cases.

In order to prevent virilization, physicians all over the world, have offered, for more than 30 years now, the synthetic glucocorticoid (GC) dexamethasone (DEX) to pregnant mothers at risk of having a child with classic CAH. DEX passes through the placenta and decreases fetal ACTH production thereby suppressing the fetal production of androgens. The treatment has been shown to be effective if administered from early pregnancy (gestational week 7), resulting in a mean Prader score of 2 compared with a score of 4–5 in untreated cases (1–3). Due to the fact that treatment has to be started before the genotype of the fetus is known, 7 out of 8 treated fetuses will be subjected to high doses of DEX during early embryogenesis without any benefit. Even with early fetal sex typing using cell-free fetal DNA from maternal blood, unnecessary treatment in healthy girls (ie, not affected by CAH) during the first trimester of pregnancy cannot be avoided (4). This dilemma emphasizes the importance of risk-benefit assessments of antenatal therapy in the context of CAH.

In our effort to systematically evaluate the short- and long-term effects of prenatal DEX therapy in the context of CAH, we have investigated the somatic effects of DEX therapy and cognitive and behavioral functions in DEX-treated children at risk of CAH in Sweden. In previous reports, we found a significant negative effect on short-term memory/verbal working memory in healthy children treated during the first trimester of fetal life, but long-term memory and learning as well as full-scale IQ, were comparable with untreated controls (5). DEX-exposed children were not born preterm and were not significantly small for gestational age. No major long-lasting somatic effects were seen in the treated women, although 1 out of 4 short-term and all full-term-treated mothers experienced acute glucocorticoid-related side effects during treatment (6).

In a Polish cohort of DEX-treated girls (16 CAH; 9 CAH-DEX; 8 healthy-DEX; mean age, 12 y), healthy-DEX girls (treated short term) showed poorer results on visual perception and visual memory tasks than treated CAH girls (full term) and performed at the same level as CAH girls not exposed to DEX in utero (7). A limitation of this study is that it lacked a control group of healthy individuals. In an American cohort of 67 DEX-treated children (of which 51 were healthy short-term treated), no significant effects of DEX were observed when assessing working memory in the short-term treated group, whereas long-term-treated CAH girls (n = 8) had poorer mental processing/short-term memory than non-DEX CAH girls (n = 15) (8).

Children at risk of premature birth and respiratory distress syndrome who are treated prenatally with betamethasone or DEX have reduced early neonatal mortality and morbidity (9). In a recent metaanalysis, at follow-up, GC-treated preterm infants showed less cerebral palsy, severe disability and less of a psychomotor development index below 70 compared with untreated infants (9). However, in a follow-up study examining brain structures with magnetic resonance imaging at the age of 6–10 years, betamethasone-exposed children (born at term) had an 8% thinner rostral anterior cingulate cortex, an area important for emotional regulation, compared with children born at term and not exposed to GC (10). In addition, a thinner left rostral anterior cingulate cortex was associated with a risk for affective problems regardless of GC exposure (10). Recently, it was also shown that DEX given postnatally to extremely preterm babies resulted in smaller brain volumes at 18 years of age compared with children born preterm without receiving DEX therapy (11).

It is not yet fully understood how fetal brain development is affected by GCs. GCs influence neural function via a number of neurotransmitter systems important for attention, perception, and memory. Normal neurogenesis is dependent on GC action, because GCs promote terminal maturation, remodeling of axons and dendrites, and affect cell survival (12). The exact molecular mechanisms whereby GCs modulate memory are yet to be defined, but epigenetic mechanisms, such as DNA methylation, are involved in memory formation. The interplay between the hippocampus and neocortex and the transient activity dependent DNA methylation and demethylation within the hippocampus precede the stable, long-lived alterations in double-stranded DNA methylation and neocortical plasticity that lead to cortical consolidation (13).

There are numerous animal studies that show both structural and functional effects on the brain after prenatal GC exposure (14, 15). The GC receptor (GR) is widely distributed within the brain, whereas the mineralocorticoid receptor (MR) is mainly located in the limbic structures. Neurons within the amygdala, hippocampus, and the prefrontal cortex coexpress both MR and GR at high levels (16–18), and these areas are important for executive functioning, emotional regulation, and memory. The hippocampus, important for learning, memory consolidation, and long-term memory, is vulnerable to high doses of GC. Reduction of the hippocampal volume has been identified in rodents and primates exposed to prenatal DEX, which has also been related to degeneration of neural progenitor cells in the hippocampus. Moreover, DEX affects spatial learning

and short-term memory (19–22). Other studies have shown effects on motor, affective, and cognitive behaviors in primates, as well as lower expression of genes important for synaptic plasticity (23, 24), a key mechanism for memory consolidation and storage into long-term memory (13). However, in the 2-year-old common marmoset exposed to prenatal DEX, the volume of the dentate granule cell layer in the hippocampus as well as neuronal proliferation and differentiation was not altered (25). Given to pregnant mice, DEX leads to decreased blood vessel density and blood brain barrier integrity within the paraventricular nucleus of the hypothalamus in the off-spring (26). This may be one mechanism for altered function of neuroendocrine neurons and subsequent long-term behavioral and physiological consequences observed after excessive GC during prenatal development.

The underlying neurobiological origin for selective effects on working memory in humans, but not on long-term memory, is not known, but working memory seems to be a vulnerable function that is disturbed not only in the setting of prenatal GC treatment but also in postnatal hypercortisolism. Patients with Cushing's syndrome have impairments of attention, nonverbal memory, and verbal working memory at diagnosis, and several years after surgery, they still exhibit deficits in short-term memory (27). In a recent report assessing short-term memory in children with CAH (7–11 y), the authors conclude that the impairment of short-term memory/working memory observed in CAH children is most probably due to effects of GC excess or early salt-wasting crises (28).

The present study comprises all Swedish children, age 7–17 years, subjected to prenatal therapy with DEX (during 1984–2010) during the first trimester of fetal life and at risk of CAH. The purpose of the investigation was to assess the long-term impact of fetal GC exposure on cognition during childhood and adolescence. We hypothesized that early prenatal GC treatment would affect cognitive functions. We present data on cognitive functions and scholastic performance of children not having CAH, meaning the children that do not benefit at all from antenatal DEX treatment.

Materials and Methods

Subjects

Since 1984, in 77 pregnancies in Sweden DEX, therapy was used to avoid virilization in female fetuses with CAH. Four mothers were treated twice. Four of the pregnancies resulted in miscarriages or termination, which gives a total of 73 cases who have received prenatal DEX treatment. The mothers were treated with DEX at a dose of 20 μg/kg · d from gestational week 6.1 (SD, 0.93) and treatment was terminated in gestational week 13.0 (SD, 2.1).

In this report, we present the neurocognitive outcome for an extended cohort consisting of all children aged 7–17 years at risk of, but not having, CAH and who were treated with DEX during the first trimester of fetal life during the period of 1984–2010 (ie, short-term DEX without CAH). The first 40 women and fetuses (out of which 27 were short-term treated fetuses without CAH) were treated before 1997, and the follow-up of these children was presented in our first reports on neurocognitive outcomes (5, 29, 30). The rest of the cases in the current extended study were treated after 1997 (n = 33, out of which 30 did not have CAH and were treated short term).

We were not able to reach 6 DEX-treated mothers (7 treated pregnancies) in this extended study. In addition, 1 family declined participation and 1 child had died in an accident before 7 years of age. The participation rate was 65% among the DEX-treated subjects and 55% for controls. The reason for refusal among controls is not known, but the length of the neuropsychological assessment (2 h) and the fact that the evaluation also included other analyses, such as blood sampling, could be factors of importance. The socioeconomic background of the controls who did not respond to the invitation letter was not studied. For a detailed description of the study group in our first evaluation of the DEX cohort, see Hirvikoski et al (5).

In total, 100 subjects (34 DEX-treated subjects [DEX], 16 females and 18 males; 66 population controls [C], 36 females and 30 males) were assessed between the ages of 7 and 17 years (average test age, 10.5 y; SD, 2.6 y). The groups did not differ in terms of age, birth weight and length, gestational age, or parental education (Table 1).

Table 1. Demographic Data

	DEX (f)	C (f)	DEX (m)	C (m)	*P* (DEX)	*P* (GENDER)	*P* (DEX × GENDER)
N	16	36	18	30			
Age (y) (SD)	9.69 (2.50)	10.71 (2.79)	10.45 (3.04)	10.61 (2.17)	$F_{1,96} = 1.12$, $P = .294$	$F_{1,96} = 0.35$, $P = .556$	$F_{1,96} = 0.59$, $P = .446$
% Parental higher education[a]	45%	47%	65%	23%	$F_{1,54} = 2.30$, $P = .135$	$F_{1,54} = 0.03$, $P = .858$	$F_{1,54} = 2.69$, $P = .107$
Birth length (cm) (SD)	50.3 (2.6)	49.4 (3.3)	50.4 (2.0)	49.9 (2.8)	$F_{1,83} = 1.27$, $P = .262$	$F_{1,83} = 0.27$, $P = .606$	$F_{1,83} = 0.07$, $P = .787$
Birth weight (g) (SD)	3479 (457)	3327 (687)	3618 (466)	3486 (730)	$F_{1,84} = 1.00$, $P = .320$	$F_{1,84} = 1.11$, $P = .295$	$F_{1,84} = 0.00$, $P = .944$
Gestational week (SD)	40.5 (1.4)	38.5 (3.3)	39.7 (1.3)	39.4 (2.8)	$F_{1,77} = 3.26$, $P = .075$	$F_{1,77} = 0.00$, $P = .983$	$F_{1,77} = 1.71$, $P = .194$

Group averages (±SDs) for age, parental education, and birth data by treatment and gender groups. DEX, DEX-treated subjects; C, untreated controls; m, male; f, female.

[a] Education at college level.

Procedures

Since 1999, physicians in Sweden have only offered DEX treatment as part of a clinical study, PREDEX. The purpose has been to evaluate the efficacy of the treatment and the short- and long-term safety for both mothers and children. The children have been followed until adulthood with behavioral, cognitive, and physiological testing. Owing to results from our previous studies, no treatment has been initiated since 2010 in Sweden.

All families were initially contacted with an invitational letter. The participants received 50 euros for their participation in the neuropsychological assessment plus reimbursement of travel expenses. The population controls were identified through the Swedish population registry. They were randomly selected from the population in Stockholm and were matched for gender and age. All children were evaluated by trained psychologists using standardized and normed neuropsychological tests. The entire test time was approximately 2 hours. All parents gave their written informed consent and the study was approved by the Regional Ethics Committee of Stockholm.

Outcome measures

To assess cognitive functions, standardized neuropsychological tests were used. For estimations of psychometric intelligence, we used 2 subtests from the Wechsler Intelligence Scales for Children-III (WISC-III): vocabulary, for estimation of verbal psychometric intelligence; and block design, for estimation of nonverbal psychometric intelligence (31). Handedness was measured using the Manual Preference Test (32). Executive functions were tested by a series of different tests: nonverbal processing speed by WISC-III coding; verbal working memory by WISC-III digit span (31). Visual spatial working memory was assessed by the span board test from the Wechsler Nonverbal Scale of Ability (33). Verbal processing speed, both reading speed and speeded naming of colors, and also impulse inhibition were tested with the Stroop Color and Word Test (34). To test memory performance (encoding/learning, long-term memory, and memory interference effect) 2 subtests from A Developmental Neuropsychological Assessment (NEPSY), list learning and memory for faces (35) were used. To evaluate scholastic performance the parents were asked to fill out the questionnaire Child Behavior Checklist (CBCL) (36) and the children themselves estimated their scholastic performance by completing the Scholastic Competence Subscale from the Self-Perception Profile for Children. The questionnaire consists of school-related items tapping the child's perception of his or her competence within the realm of scholastic performance (37).

Statistical analyses

General two-way ANCOVAs with factors DEX (treated, nontreated) and GENDER (female, male) were used to analyze data from all tests in SPSS 23 (IBM). Age at the time of testing was included as a covariate of no interest to correct for age effects on test scores. A 2-tailed α-level of $P < .05$ was used for comparisons between DEX treated and nontreated subjects. Interactions between DEX and GENDER were followed up for GENDER-specific tests of DEX effects when interaction reached a 2-tailed $P < .10$. Effect sizes were calculated as Cohen's d where positive effect sizes represent higher test scores in controls and negative effect sizes higher test scores in patients. Effects were categorized as large with $d \geq 0.80$ and small with $d < 0.20$ (38).

Results

We present test results for WISC-III, NEPSY, span board, and Stroop Color and Word Test, followed by parental and the children's self-ratings of scholastic performance.

Table 2. Group Averages and Effect Sizes for All Test Scores

	Females (n = 52)			Males (n = 48)		
	DEX (n = 16)	C (n = 36)	Cohen's *d*	DEX (n = 18)	C (n = 30)	Cohen's *d*
WISC-III, scaled scores						
Coding	10.2 (2.74)	12.0 (2.69)	0.66	9.9 (2.69)	9.3 (2.69)	−0.22
Block design	9.4 (2.96)	11.7 (2.92)	0.81	11.9 (2.84)	11.1 (2.92)	−0.28
Vocabulary	8.8 (2.26)	10.7 (2.23)	0.84	10.9 (2.16)	10.6 (2.23)	−0.14
Digit span	7.8 (3.02)	10.8 (2.98)	1.00	9.6 (2.89)	10.3 (2.98)	0.24
NEPSY						
Faces learning	11.51 (3.37)	11.21 (3.32)	−0.09	10.76 (3.32)	10.99 (3.32)	0.07
Faces LTM	14.0 (1.85)	13.9 (1.82)	−0.05	13.6 (1.83)	14.5 (1.82)	0.49
List learning (raw score) (max 5 × 15 words)	51.5 (9.09)	52.3 (8.94)	0.09	46.2 (8.96)	48.1 (8.95)	0.21
List interference	10.3 (2.68)	11.8 (2.67)	0.56	10.5 (2.65)	10.5 (2.63)	0
List LTM	11.75 (2.46)	11.61 (2.42)	−0.06	10.47 (2.43)	10.68 (2.43)	0.09
Span board test						
Forward (T)	39.4 (16.95)	52.8 (16.67)	0.80	45.3 (14.74)	45.1 (16.68)	−0.01
Backward (T)	54.2 (16.42)	51.5 (16.15)	−0.17	57.6 (16.18)	49.3 (16.16)	−0.51
Stroop test						
Word reading (T)	46.5 (8.01)	50.0 (8.01)	0.44	45.3 (8.02)	46.8 (8.04)	0.19
Color naming (T)	42.4 (6.73)	45.6 (6.73)	0.48	42.1 (6.75)	40.4 (6.63)	−0.25
Interference (T)	53.0 (5.52)	52.8 (5.52)	−0.04	53.8 (5.53)	51.4 (5.52)	−0.43
SPPC school performance	3.3 (0.43)	3.3 (0.42)	0	3.1 (0.24)	3.2 (0.43)	0.27
CBCL school performance	4.8 (0.99)	4.8 (0.97)	0	4.8 (0.97)	5.0 (0.97)	0.21

Estimated marginal means (and SDs) corrected for age at the time of testing. Effect sizes (Cohen's *d*) are shown for all findings. Positive effect sizes represent higher scores in controls (C), whereas negative effect sizes represent higher scores in the DEX-treated group (DEX). The effect sizes for WISC-III subscales are moderate to large (coding, $d = .66$; block design, $d = .81$; vocabulary, $d = 0.84$; digit span, $d = 1.0$) with DEX-treated girls performing worse than untreated controls. For span board test forward, the result is similar with a large negative effect of DEX ($d = .80$) in treated girls. CBCL, Child Behavior Checklist; LTM, long-term memory; NEPSY, A Developmental NEuroPSYchological Assessment; SPPC, Self-Perception Profile for Children; T, T-scores; WISC-III, Wechsler Intelligence Scales for Children-III.

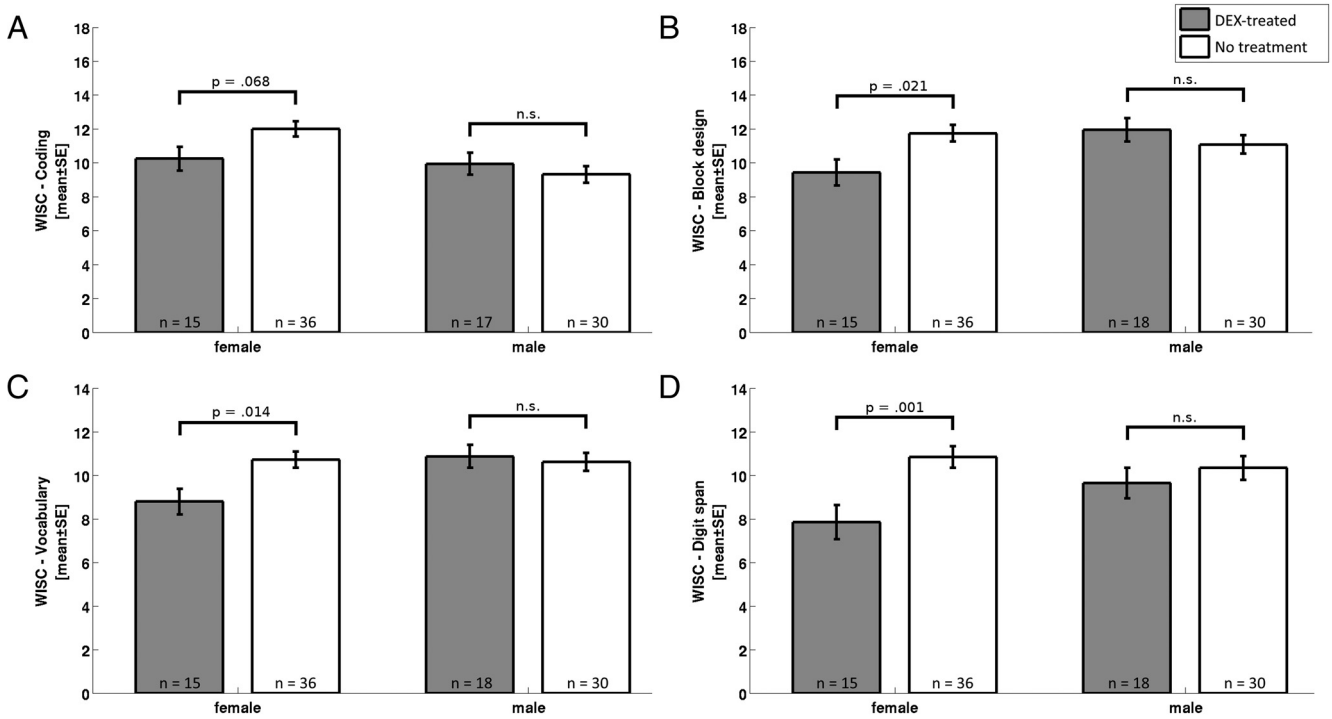

Figure 1. Group averages for WISC-III. Coding (A), block design (B), vocabulary (C), and digit span (D). On all 4 tests, DEX-treated girls scored lower than female controls. No differences were observed between male DEX-treated and male control subjects. Estimated marginal means (test age, 11 y) and SEs are displayed. WISC-III, Wechsler Intelligence Scales for Children-III.

Group scores for all tests are shown in Table 2 and are omitted from the text.

Wechsler Intelligence Scales for Children-III

In 3 out of 4 tests from the WISC-III Scale (coding, nonverbal processing speed; block design, nonverbal intelligence; vocabulary, verbal intelligence), we observed significant interactions between DEX and GENDER (coding, $F_{1,93} = 4.16$, $P = .044$; block design, $F_{1,94} = 6.44$, $P = .013$; vocabulary, $F_{1,94} = 5.16$, $P = .025$) (Figure 1). In the fourth test (digit span, verbal working memory), we observed a near significant interaction between DEX and GENDER (digit span, $F_{1,94} = 3.27$, $P = .074$) (Figure 1). Digit span (verbal working memory) was the only test that showed a negative main effect of DEX ($F_{1,94} = 8.35$, $P = .005$). A follow-up analysis identified that, on all 4 tests, female DEX-treated children scored lower than female controls, although in 1 test, this was only a trend (coding, $F_{1,48} = 3.49$, $P = .068$; block design, $F_{1,48} = 5.70$, $P = .021$; vocabulary, $F_{1,48} = 6.52$, $P = .014$; digit span, $F_{1,48} = 12.55$, $P = .001$). None of the follow-up analyses in the male groups were significant (all $P > .10$).

Handedness

The Manual Preference Test showed no difference between groups ($P > .10$).

A Developmental Neuropsychological Assessment

No effects of DEX were observed on measures of immediate memory/learning, long-term memory, and memory interference effects assessed with the NEPSY subtests, list learning and memory for faces (all $P > .10$).

Span board test

The interaction between DEX and GENDER on visual spatial working memory assessed with the forward span board test was approaching significance ($F_{1,95} = 3.73$, $P = .056$) but not with the backward test ($F_{1,95} = 0.67$, $P = .416$) (Figure 2). In the forward span board test, DEX-treated girls scored lower than female controls ($F_{1,48} = 7.92$, $P = .007$), whereas there was no difference in the male group ($F_{1,46} = 0.002$, $P = .964$). A main effect of DEX approached, but did not reach, statistical significance for forward span board scores ($F_{1,95} = 3.48$, $P = .065$), whereas no effect was seen on backward span board scores ($F_{1,95} = 2.54$, $P = .114$).

Stroop

No effects of DEX or interactions between DEX and GENDER were observed for speed of processing or impulse inhibition assessed with the Stroop measures of speeded reading of words, speeded naming of colors, and Stroop interference (all $P > .10$).

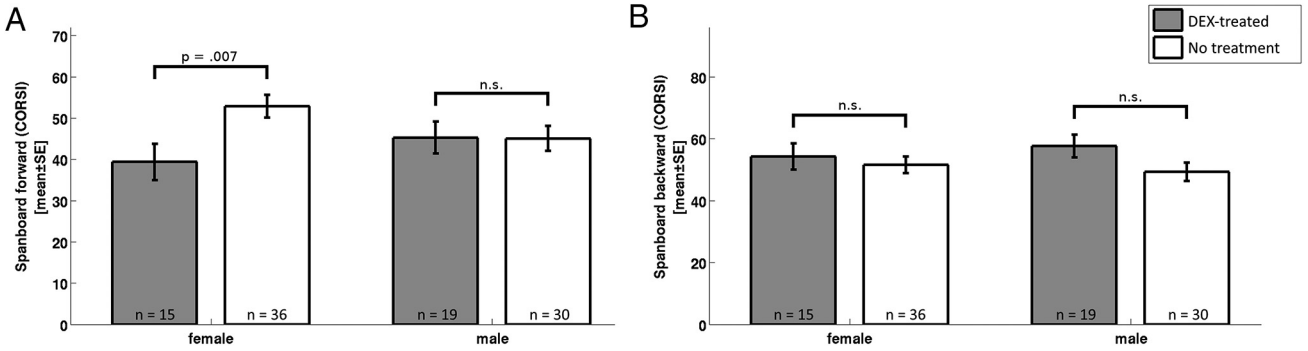

Figure 2. Group averages for span board forward (A) and backward (B). DEX-treated girls scored lower than female controls on span board forward ($P = .007$). No differences were observed between male DEX-treated and male control subjects. Estimated marginal means (test age, 11 y) and SEs are displayed.

Scholastic ability

Most of the children over 7 years of age completed the Scholastic Competence Subscale from the Self-Perception Profile for Children (n = 93). DEX-treated children did not differ from population controls on self-perceived scholastic ability or parental estimations of scholastic performance assessed with the CBCL (all $P > .10$).

Effect sizes

Cohen's d effect sizes were calculated for all comparisons (Table 2). The effect sizes for WISC-III subscales were moderate to large within females (coding, $d = 0.66$; block design, $d = 0.81$; vocabulary, $d = 0.84$; digit span, $d = 1.0$), with DEX-treated girls performing worse than untreated controls. For span board test forward, we could see a similar result with a large negative effect of DEX ($d = 0.80$) in treated girls.

Discussion

We have investigated long-term effects of prenatal DEX treatment given during the first trimester in healthy (non-CAH) children at risk of CAH. We compared their cognitive functions and scholastic ability with those of age- and sex-matched nontreated peers not at risk of CAH. DEX-treated girls performed poorer on several cognitive measures. They scored lower on measures of both verbal and nonverbal intelligence, as well as verbal working memory tasks, compared with a group of untreated female controls. There was also a trend towards poorer performance on nonverbal processing speed in DEX-treated girls, and in addition, visual spatial working memory was negatively affected. In all of these functions, DEX-treated boys performed equally well as control boys. This implies that early DEX exposure during fetal life may have sex-dimorphic effects. These findings extend and are in line with our previous finding of poorer verbal working memory in short-term DEX-treated children (5), but, in the present extended study, we also identified a broader effect on subtests with a strong correlation with full-scale intelligence in girls for the first time. The results are also in line with the findings of Maryniak et al, who concluded that first-trimester DEX exposure in healthy girls (non-CAH) had a negative impact on visual perception and visual memory tasks (7).

Effect sizes of the observed differences are large (Cohen's $d > 0.80$) for all significant findings. There is a 1 SD difference in verbal working memory performance between DEX-treated and untreated girls. For nonverbal and verbal intelligence and visual spatial working memory, the difference is almost as large (Cohen's $d > 0.80$) and, for nonverbal processing speed, the effect size is medium (Cohen's $d = 0.66$). As in our first report, we could not see a negative impact on learning or long-term memory, handedness, or speed of processing and impulse inhibition. In comparison, the difference in working memory between CAH children and their unaffected relatives has been found to be of medium effect size (Cohen's $d = 0.53–0.70$) (28), meaning that the negative effect we see in DEX exposed children not having CAH is not negligible.

The observed effects of DEX on working memory performance are alarming. Working memory/short-term memory involves the ability to retain information for a mental operation during a short period of time and working memory capacity is predictive of academic achievement and intelligence (39). Although working memory performance does not show a gender difference in typically developing children (40), in our study, the reduction in working memory performance was seen only in girls who were exposed to prenatal DEX despite not having CAH. Parental and child assessments of scholastic performance did not differ between treated and nontreated children in the present report, which emphasizes the importance of evaluating children with a multisource assessment and including direct neuropsychological testing. Neuropsychological tests are more sensitive to subtle changes in

cognitive abilities than measures of scholastic performance, which may be too subtle during early education and may have been compensated by increased effort. However, subtle differences in underlying skills will most likely have stronger effects later in life, during second and third level education, when interpersonal differences in terms of intelligence become more apparent. In addition, more general effects on verbal and nonverbal intelligence and effects on the visual spatial working memory were detected. Also for these effects, there seem to be gender typical differences in prenatal programming and development, because the cognitive deficits were exhibited only in girls.

In adults, sex differences with respect to working memory subdomains have been shown. Specifically, males perform better in mathematical (41), spatial, and object working memory tasks (42) and females displaying greater verbal (43) and writing skills than males (44). In a meta-analysis using the BrainMap database and including neuro-functional studies on working memory, it was demonstrated that women activate more extensively limbic structures and prefrontal regions, including bilateral amygdalae, right hippocampus, and cingulate regions, whereas men activate more extensively parietal areas, the right insula, and bilateral thalamus (44). If men and women differ in terms of how neural networks are recruited for specific neurocognitive functions, this may explain differences in vulnerability to endogenous or exogenous factors. It may be that the female fetus, and eventually, women are more vulnerable to the effects of GC on working memory because they rely more on the brain areas that are dense in GR and MR (44).

In children exposed to pregnancy-related anxiety, a reduction of gray matter volume could be seen primarily in girls at 6–9 years of age in areas important for executive functions such as behavioral/emotion regulation (prefrontal cortex, premotor cortex, medial temporal lobe, lateral temporal cortex, postcentral gyrus, cerebellum) (45). Girls also performed poorer on tests assessing executive function (46). In summary, the female fetus may be more susceptible than the male to the consequences of prenatal maternal depression, pre- and postnatal maternal cortisol, and prenatal exogenous GC exposure.

Interventions aimed at enhancing cognitive abilities in GC-treated individuals are important, because tailored cognitive programs and support in school at an early stage may circumvent or ameliorate these deficits. However, not only long-lasting cognitive effects may arise from prenatal GC exposure but effects on other physiological systems too are thought to be already programmed in utero. It is hypothesized that many of the effects of the fetal programming events that persist throughout life are mediated via alterations of the hypothalamic-pituitary-adrenal (HPA) axis. Prenatal GC exposure impacts on brain structures involved in HPA axis regulation (hypothalamus, amygdala, and hippocampus) during critical periods of development. Alterations in GR and MR levels in the hippocampus and apoptosis may thus result in persistent changes in endocrine stress reactivity, because the hippocampus exerts a negative feedback on the HPA axis. Reduced negative feedback will lead to an exaggerated stress response that may persist throughout life and predispose the individual to the metabolic syndrome or psychiatric disorders. In 6- to 11-year-old term children exposed to either betamethasone or DEX during late fetal life, an elevated cortisol response to stress has been shown, especially in girls (47). Again, a sex dimorphism for prenatal GC effects is evident. Whether prenatal treatment with DEX, as used in fetuses at risk for CAH, will lead to alterations in metabolic functions or neuro-structural brain networks is not known at the present time, but this is being investigated within the scope of the Swedish PREDEX study.

One limitation of the study includes the relatively low number of individuals included in the follow-up, given the rarity of the disease. The present study is an extension of our previous study and the study group is doubled. Effect sizes of the observed group differences are large and the differences are in accordance with findings in previous studies underscoring the importance of these findings. A larger number of study subjects would probably show more refined differences but it is not likely that the observed effects would disappear. Extended studies in additional, and larger, cohorts around the world are warranted. Another limitation is that the results of the current study are based on children, with an average age around eleven years. Therefore, conclusions about the long-term effects of DEX treatment on scholastic performance are limited to early levels of education (primary education). Differences in cognitive skills, such as observed here, are more likely to manifest themselves at later stages and may still affect final level of education and achieved level in career paths later on.

In conclusion, our results suggest that early prenatal DEX exposure, as employed in prenatal treatment of fetuses at risk for CAH, is likely to affect cognitive functions in healthy girls, ie, children who do not benefit from the treatment. It can therefore not be considered safe to use this therapy in the context of CAH although extended studies in additional cohorts around the world are warranted. If DEX is still offered, it is essential that information about the risks and benefits of the treatment is given to the pregnant couple. The treatment should be questioned and should only be offered within the framework of a clinical study with longitudinal long-term follow-ups of

mothers and children. Given the importance of working memory for academic performance, screening for deficits should be performed and interventional strategies might be needed in DEX-treated cases.

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References

1. **Forest MG, Betuel H, David M.** Prenatal treatment of congenital adrenal hyperplasia due to 21-hydroxylase deficiency: update 88 of the French Multicentric Study. *Endocrinol Res*. 1989;15:277–301.
2. **Lajic S, Bui TH, Holst M, Ritzén M, Wedell A.** [Prenatal diagnostik och behandling av adrenogenitalt syndrom förhindrar virilisering av flickfoster]. *Lakartidningen*. 1997;94:4781–4786.
3. **New MI.** Antenatal diagnosis and treatment of congenital adrenal hyperplasia. *Curr Urol Rep*. 2001;2:11–18.
4. **Tardy-Guidollet V, Menassa R, Costa JM, et al.** New management strategy of pregnancies at risk of congenital adrenal hyperplasia using fetal sex determination in maternal serum: French cohort of 258 cases (2002–2011). *J Clin Endocrinol Metab*. 2014;99:1180–1188.
5. **Hirvikoski T, Nordenström A, Lindholm T, et al.** Cognitive functions in children at risk for congenital adrenal hyperplasia treated prenatally with dexamethasone. *J Clin Endocrinol Metab*. 2007;92:542–548.
6. **Lajic S, Wedell A, Bui TH, Ritzén EM, Holst M.** Long-term somatic follow-up of prenatally treated children with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 1998;83:3872–3880.
7. **Maryniak A, Ginalska-Malinowska M, Bielawska A, Ondruch A.** Cognitive and social function in girls with congenital adrenal hyperplasia – influence of prenatally administered dexamethasone. *Child Neuropsychol*. 2014;20:60–70.
8. **Meyer-Bahlburg HF, Dolezal C, Haggerty R, Silverman M, New MI.** Cognitive outcome of offspring from dexamethasone-treated pregnancies at risk for congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Eur J Endocrinol*. 2012;167:103–110.
9. **Sotiriadis A, Tsiami A, Papatheodorou S, Baschat AA, Sarafidis K, Makrydimas G.** Neurodevelopmental outcome after a single course of antenatal steroids in children born preterm: a systematic review and meta-analysis. *Obstet Gynecol*. 2015;125:1385–1396.
10. **Davis EP, Sandman CA, Buss C, Wing DA, Head K.** Fetal glucocorticoid exposure is associated with preadolescent brain development. *Biol Psychiatry*. 2013;74:647–655.
11. **Cheong JL, Burnett AC, Lee KJ, et al.** Association between postnatal dexamethasone for treatment of bronchopulmonary dysplasia and brain volumes at adolescence in infants born very preterm. *J Pediatr*. 2014;164:737–743.e731.
12. **Yehuda R, Fairman KR, Meyer JS.** Enhanced brain cell proliferation following early adrenalectomy in rats. *J Neurochem*. 1989;53:241–248.
13. **Heyward FD, Sweatt JD.** DNA methylation in memory formation: emerging insights. *Neuroscientist*. 2015;21:475–489.
14. **Harris A, Seckl J.** Glucocorticoids, prenatal stress and the programming of disease. *Horm Behav*. 2011;59:279–289.
15. **Lajic S, Nordenström A, Hirvikoski T.** Long-term outcome of prenatal dexamethasone treatment of 21-hydroxylase deficiency. *Endocr Dev*. 2011;20:96–105.
16. **Colciago A, Casati L, Negri-Cesi P, Celotti F.** Learning and memory: steroids and epigenetics. *J Steroid Biochem Mol Biol*. 2015;150:64–85.
17. **Matsusue Y, Horii-Hayashi N, Kirita T, Nishi M.** Distribution of corticosteroid receptors in mature oligodendrocytes and oligodendrocyte progenitors of the adult mouse brain. *J Histochem Cytochem*. 2014;62:211–226.
18. **de Kloet ER, Joëls M, Holsboer F.** Stress and the brain: from adaptation to disease. *Nat Rev Neurosci*. 2005;6:463–475.
19. **Uno H, Lohmiller L, Thieme C, et al.** Brain damage induced by prenatal exposure to dexamethasone in fetal rhesus macaques. I. Hippocampus. *Brain Res Dev Brain Res*. 1990;53:157–167.
20. **Uno H, Eisele S, Sakai A, et al.** Neurotoxicity of glucocorticoids in the primate brain. *Horm Behav*. 1994;28:336–348.
21. **Noorlander CW, Visser GH, Ramakers GM, Nikkels PG, de Graan PN.** Prenatal corticosteroid exposure affects hippocampal plasticity and reduces lifespan. *Dev Neurobiol*. 2008;68:237–246.
22. **Noorlander CW, Tijsseling D, Hessel EV, et al.** Antenatal glucocorticoid treatment affects hippocampal development in mice. *PLoS One*. 2014;9:e85671.
23. **Hauser J, Knapman A, Zürcher NR, et al.** Effects of prenatal dexamethasone treatment on physical growth, pituitary-adrenal hormones, and performance of motor, motivational, and cognitive tasks in juvenile and adolescent common marmoset monkeys. *Endocrinology*. 2008;149:6343–6355.
24. **Hauser J, Dettling-Artho A, Pilloud S, et al.** Effects of prenatal dexamethasone treatment on postnatal physical, endocrine, and social development in the common marmoset monkey. *Endocrinology*. 2007;148:1813–1822.
25. **Tauber SC, Bunkowski S, Schlumbohm C, et al.** No long-term effect two years after intrauterine exposure to dexamethasone on dentate gyrus volume, neuronal proliferation and differentiation in common marmoset monkeys. *Brain Pathol*. 2008;18:497–503.
26. **Frahm KA, Tobet SA.** Development of the blood-brain barrier within the paraventricular nucleus of the hypothalamus: influence of fetal glucocorticoid excess. *Brain Struct Funct*. 2015;220:2225–2234.
27. **Forget H, Lacroix A, Bourdeau I, Cohen H.** Long-term cognitive effects of glucocorticoid excess in Cushing's syndrome. *Psychoneuroendocrinology*. 2016;65:26–33.
28. **Browne WV, Hindmarsh PC, Pasterski V, et al.** Working memory performance is reduced in children with congenital adrenal hyperplasia. *Horm Behav*. 2015;67:83–88.
29. **Hirvikoski T, Nordenström A, Lindholm T, Lindblad F, Ritzén EM, Lajic S.** Long-term follow-up of prenatally treated children at risk for congenital adrenal hyperplasia: does dexamethasone cause behavioural problems? *Eur J Endocrinol*. 2008;159:309–316.
30. **Hirvikoski T, Lindholm T, Lajic S, Nordenström A.** Gender role behaviour in prenatally dexamethasone-treated children at risk for congenital adrenal hyperplasia - a pilot study. *Acta Paediatr*. 2011;100:e112–e119.
31. **Donders J.** A short form of WISC-III for clinical use. *Psychol Assess*. 1997;9.
32. **Korkman M.** NEPSY: an adaptation of Luria's investigation for young children. *Clin Neuropsychologist*. 1988;2:375–392.
33. **Wechsler D.** *WMS-III: Wechsler Memory Scale*. 3rd ed. San Antonio, TX: Psychological Corporation; 1991.

34. **Golden CJ.** *A Manual for the Stroop Color and Word Test*. Chicago, IL: Stoelting Company; 1978.
35. **Korkman M, Kirk U, Kemp S.** *NEPSY: A Developmental Neuropsychological Assessment*. San Antonio, TX: The Psychological Corporation; 1998.
36. **Achenbach TM.** *Manual for the Child Behavior Checklist/4–18 and 1991 Profile*. Burlington, VT: University of Vermont, Department of Psychiatry; 1991.
37. **Harter S.** *The Self-Perception Profile for Children: Revision of the Perceived Competence Scale for Children*. Denver, CO: University of Denver; 1985.
38. **Cohen J.** *Statistical Power Analysis for the Behavioural Sciences*. 2nd ed. Hillsdale, NJ: Lawrence Erlbaum; 1988.
39. **Conway AR, Kane MJ, Engle RW.** Working memory capacity and its relation to general intelligence. *Trends Cogn Sci*. 2003;7:547–552.
40. **Alloway TP, Gathercole SE, Pickering SJ.** Verbal and visuospatial short-term and working memory in children: are they separable? *Child Dev*. 2006;77:1698–1716.
41. **Lynn R, Irwing P.** Sex differences in general knowledge, semantic memory and reasoning ability. *Br J Psychol*. 2002;93:545–556.
42. **Lejbak L, Crossley M, Vrbancic M.** A male advantage for spatial and object but not verbal working memory using the n-back task. *Brain Cogn*. 2011;76:191–196.
43. **Lewin C, Wolgers G, Herlitz A.** Sex differences favoring women in verbal but not in visuospatial episodic memory. *Neuropsychology*. 2001;15:165–173.
44. **Hill AC, Laird AR, Robinson JL.** Gender differences in working memory networks: a BrainMap meta-analysis. *Biol Psychol*. 2014;102:18–29.
45. **Sandman CA, Glynn LM, Davis EP.** Is there a viability-vulnerability tradeoff? Sex differences in fetal programming. *J Psychosom Res*. 2013;75:327–335.
46. **Buss C, Davis EP, Hobel CJ, Sandman CA.** Maternal pregnancy-specific anxiety is associated with child executive function at 6–9 years age. *Stress*. 2011;14:665–676.
47. **Alexander N, Rosenlöcher F, Stalder T, et al.** Impact of antenatal synthetic glucocorticoid exposure on endocrine stress reactivity in term-born children. *J Clin Endocrinol Metab*. 2012;97:3538–3544.