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In utero exposure to glucocorticoids and risk of anxiety and depression in childhood or adolescence

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ABSTRACT

Glucocorticoid use is prevalent in pregnant women, but whether *in utero* exposure impacts mental health in the offspring has not been fully explored. The aim of this study was to investigate if *in utero* exposure to synthetic glucocorticoids increases the risk of anxiety and depression in childhood or adolescence. The study was conducted as a nationwide cohort study, including negative control exposure analyses and a sibling design to optimize control of confounding. The study population comprised 1,275,909 children born in 1996–2015 in Denmark (median follow-up of 13 years). Exposure was divided into systemic and local glucocorticoid exposure, levels of cumulative dose, generic type and according to trimester of exposure. The comparison cohort was children without exposure born to maternal never-users. Negative control exposures included children without glucocorticoid exposure born to: maternal users of non-steroidal anti-inflammatory drugs or immunotherapy during pregnancy, maternal former users of systemic glucocorticoids, maternal users of systemic glucocorticoids in the postnatal period, and fathers who were prescribed glucocorticoids. The sibling design compared siblings with and without exposure. 9307 (0.7%) children were exposed to systemic glucocorticoids and 116,389 (9.1%) children were exposed to local glucocorticoids. High-dose systemic glucocorticoids (≥500 mg prednisolone equivalents) increased the risk of anxiety compared to the comparison cohort [aIRR 1.79 (95% CI: 1.36–2.37), cumulative risk 16% vs. 7.8% by age 20]. A similar result was found for depression [aIRR 1.45 (95% CI: 0.80–2.63), cumulative risk 3.6% vs. 2.6% by age 20]. The association with anxiety was consistent in the sibling design [aIRR 1.83 (95% CI: 1.03–3.66), exposed siblings (≥ 500 mg) vs. unexposed]. Sex did not modify the associations. Negative control exposure analyses indicated robustness towards confounding from genetics and family environment. No association was found with low doses of systemic exposure or local use. In conclusion, potential adverse mental health effects of *in utero* exposure to high-dose glucocorticoids merit clinical attention.

1. Introduction

Women of childbearing age might receive treatment with glucocorticoids for imminent preterm birth, asthma, autoimmune diseases, allergies, or skin conditions (UpToDate, 2019). Often, this treatment must be continued during pregnancy to control the disease. Whether *in utero* exposure to synthetic glucocorticoids impacts mental health in the offspring has not yet been fully explored.

During pregnancy, the fetus is protected from excessive maternal cortisol exposure by placental 11 β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which converts cortisol to inactive cortisone (Salvante et al., 2017). The expression and activity of 11β-HSD2 are affected by multiple factors, including gestational age, sex of the fetus, and maternal glucocorticoid levels (Moisiadis and Matthews, 2014b; O'Donnell et al., 2012; Salvante et al., 2017; van Beek et al., 2004). 11β-HSD2 also converts synthetic glucocorticoids, however, affinity is highly dependent on generic glucocorticoid type. Beta- and dexamethasone have low affinity and large proportions pass the placenta. Prednisolone has high affinity for 11b-HSD2, but enzymatic saturation after high-dose or long-term treatment can lead to greater placental bypass (Seckl, 2004). Through fetal programming, the *in utero* environment plays a role in determining health later in life, mediated through epigenetic changes

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and alterations in hormonal axes set points (Braun et al., 2013; Moisiadis and Matthews, 2014a, 2014b; Seckl, 2004; Tegethoff et al., 2009). In both human and animal studies, excess endogenous glucocorticoid exposure *in utero* (stress) and exogenous glucocorticoid exposure (pharmacological treatment) have been found to reduce birth weight and alter the sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis (Alexander et al., 2012; Braun et al., 2013; Crombie et al., 2021; Edelmann et al., 2016; Ilg et al., 2019; Irwin et al., 2021; Khan et al., 2011; McLaughlin et al., 2021; Moisiadis and Matthews, 2014a, 2014b; Murphy et al., 2012; Osborne et al., 2018; Provençal et al., 2020; Rodriguez et al., 2019; Seckl, 2004; Tegethoff et al., 2009). Altered HPA axis reactivity has been linked to anxiety and depressive disorders (Braun et al., 2013; Craske et al., 2017). Likewise, small for gestational age (SGA) is a predictor for mental disorders later in life (Abel et al., 2010). Previous studies showed an association between maternal glucocorticoid treatment and mental disorders in offspring (Khalife et al., 2013; Räikkönen et al., 2020; Wolford et al., 2020). These studies examined betamethasone and dexamethasone, which are mainly used antenatal for imminent preterm birth and constitute a minor part of glucocorticoid treatment regimens during pregnancy. More knowledge is needed on glucocorticoid exposure during other stages of pregnancy, for other indications, and in relation to different doses, other types of administrations, and generic types of glucocorticoids. In this population-based cohort study we aimed to improve the knowledge on the association between *in utero* exposure to glucocorticoids and the risk of anxiety or depression in childhood or adolescence. Anxiety and depression are among the most common mental disorders in childhood and adolescence (Dalsgaard et al., 2020), despite the fact that peak incidence occurs later in life.

2. Materials and methods

2.1. Setting

This study is based on Danish healthcare data collected during 1996–2018. The Danish healthcare system provides tax-supported health services to all residents, guaranteeing access to health care free of charge (Laugesen et al., 2021). A unique civil registration number is assigned to all Danish residents at birth or upon emigration (Laugesen et al., 2021). The civil registration number is used in registries to record use of health services, allowing continuous follow-up of the population and permitting accurate and unambiguous linkage of information among relevant registries at the individual level (Laugesen et al., 2021).

2.2. Study population

We used the Danish Medical Birth Registry (Laugesen et al., 2021) to identify a cohort of all children born in Denmark from 1 January 1996 until 31 December 2015 (n = 1,281,223). Stillbirths and children with an invalid civil registration number were excluded [n = 5314 (0.4%)], hence, the final study population consisted of 1,275,909 children (Fig. 1). The Registry contains information on all deliveries in Denmark. Each record includes the civil registration numbers of the newborn and the parents, as well as information on the pregnancy, the delivery, and infant and maternal characteristics.

2.3. Exposure and comparison cohorts

We defined *in utero* glucocorticoid exposure based on maternal

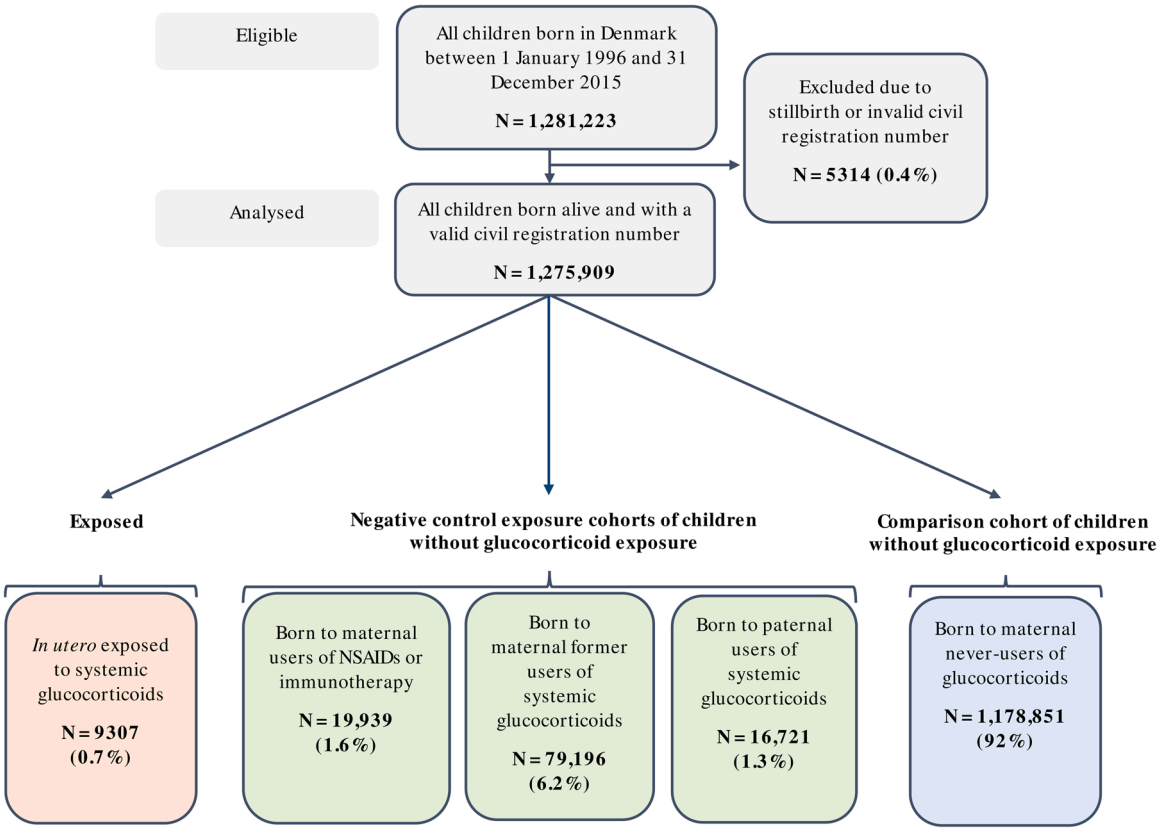

Fig. 1. Flow chart. The negative control exposure cohorts are not mutually exclusive. Median and (interquartile range) of follow-up were: 12 years (7–16 years) for children *in utero* exposed to systemic glucocorticoids, 13 years (8–18 years) for children in the comparison cohort, 12 years (8–18 years) for children born to maternal former users of systemic glucocorticoids (6–24 months since most recent prescription), 11 years (7–15 years) for children born to maternal former users of systemic glucocorticoids (> 24 months since most recent prescription), 13 years (9–17 years) for children born to paternal users of systemic glucocorticoids, and 14 years (8–18 years) for children born to maternal users of non-steroidal anti-inflammatory drugs (NSAIDs) or immunotherapy.

redeemed prescriptions or hospital records for glucocorticoid treatment during pregnancy, identified in the Danish National Prescription Registry or the National Patient Registry (Laugesen et al., 2021). Start of pregnancy was defined as the first day of the last menstrual period, calculated using the gestational age of the child at birth. We considered both systemic glucocorticoids (oral or injectable) and locally acting glucocorticoids (inhaled, intestine or skin), but assessed them separately (Supplemental Table 1). Injectable glucocorticoids refer to intra-articular, intramuscular, and intravenous routes of administration. Glucocorticoids acting on the intestine refer to drugs with oral or rectal routes of administration, but with release of the active substance locally in the intestine (e.g., for treatment of inflammatory bowel disease or haemorrhoids). The Prescription Registry contains detailed nationwide data on drug prescriptions redeemed at Danish outpatient pharmacies (Laugesen et al., 2021). The Danish National Patient Registry holds information on antenatal glucocorticoid treatment for imminent preterm birth. Other types of in-hospital glucocorticoid treatment is typically not recorded.

We defined the following exposure categories based on time of exposure:

- Children exposed any time during the pregnancy: if the mother redeemed one or more prescriptions or had a hospital record of glucocorticoid treatment from start of pregnancy until delivery.
- Children exposed during the 1st trimester only: if the mother redeemed one or more prescriptions during the first 84 days (first 12 weeks) of pregnancy, with no further prescriptions or records during the rest of the pregnancy.
- Children exposed during the 2nd trimester only: if the mother redeemed one or more prescriptions or had a hospital record of glucocorticoid treatment between day 85 and day 196 (weeks 13–28), and no further prescriptions or records during the rest of the pregnancy.
- Children exposed during the 3rd trimester only: if the mother redeemed one or more prescriptions or had a hospital record of glucocorticoid treatment between day 197 and the delivery date (last 12 weeks of pregnancy), with no further prescriptions or records during the rest of the pregnancy.
- Children exposed during multiple trimesters: if the mother redeemed one or more prescriptions or had a hospital record of glucocorticoid treatment in more than one trimester according to the definitions above.

We further evaluated the cumulative systemic glucocorticoid dose expressed in prednisolone- equivalents (peq) calculated as the total number of tablets/injections during pregnancy multiplied by the strength of the tablets/injections and the peq conversion factor (Supplemental Table 2). By this calculation, cumulative systemic exposure dose took discrete values (Supplemental Table 3). Based on the exposure distribution, we categorized cumulative dose as follows: < 250 mg, 250–499 mg, and ≥ 500 mg peq. A cumulative exposure dose of ≥ 500 mg peq was defined as high-dose glucocorticoid exposure. Last, we conducted analyses according to generic systemic glucocorticoid type.

The comparison cohort consisted of children without glucocorticoid exposure and born to maternal never-users, i.e., mothers who did not redeem a prescription or have a hospital record of glucocorticoid treatment any time before or during pregnancy.

2.4. Negative control exposure cohorts

We established four different negative control exposure cohorts of children without glucocorticoid exposure. These cohorts included children born to: maternal users of non-steroidal anti-inflammatory drugs (NSAIDs) or immunotherapy during pregnancy, maternal former (pre-pregnancy) systemic glucocorticoid users, maternal postnatal systemic glucocorticoid users, or fathers who were prescribed systemic glucocorticoids during the pregnancy of their partner. We expected no biological associations when comparing these negative control exposure cohorts with the comparison cohort. The purpose of such comparisons therefore was to disentangle potential confounding issues. Maternal glucocorticoid users, maternal users of NSAIDs or immunotherapy, former maternal users, maternal postnatal users and paternal users may be more similar with regard to measured and unmeasured characteristics than never users. Likewise, they may share common traits, such as genetics, family environment and treatment indication. Findings of associations in the negative control exposure cohorts vs. the comparison cohort would indicate confounding from such characteristics/factors in our main analysis.

We defined the negative control exposure cohorts as follows:

- Children without in utero glucocorticoid exposure whose mother used NSAIDs or immunotherapy during pregnancy: if the mother redeemed at least one prescription for these agents or was treated with them in a hospital settings any time during pregnancy (Supplemental Table 1) and did not redeem prescriptions or have any hospital records for glucocorticoids from the start of pregnancy until delivery.

Children without *in utero* glucocorticoid exposure born to maternal former users of glucocorticoids were divided into:

- Children whose mother used glucocorticoids $>$ 6 – 24 months before pregnancy: the mother redeemed her most recent prescription 6–24 months before the start of pregnancy.
- Children whose mother used glucocorticoids $>$ 24 months before pregnancy: the mother redeemed her most recent prescription $>$ 24 months before the start of pregnancy.

Children whose mother used glucocorticoids 0–6 months before pregnancy, defined as the mother redeeming her most recent prescription 0–6 months before the start of pregnancy and none during the pregnancy, were not included in the analyses due to uncertainty about exposure status. *I.e.*, uncertainty about whether the oocyte was exposed to glucocorticoids in the periconceptional critical window of development or whether the mother used glucocorticoids in the beginning of the pregnancy or not.

- Children without in utero glucocorticoid exposure whose mother used systemic glucocorticoids in the postnatal period: if the mother redeemed one or more prescriptions for systemic glucocorticoids from birth to one year after birth, but did not redeem a prescription or have a hospital records for glucocorticoids during pregnancy.
- Children without in utero glucocorticoid exposure with fathers who were prescribed systemic glucocorticoids during the pregnancy of their partner: if the father redeemed one or more prescriptions for systemic glucocorticoids during the pregnancy of his partner, but never before the start of pregnancy (i.e., only new users were considered). We examined only paternal new use, to ensure that these children served solely as a negative control exposure cohort. This was in light of evidence that fetal programming may be transmitted via paternal germline lineages also (Moisiadis et al., 2017). We excluded paternal users if the mother also used systemic glucocorticoids during pregnancy.

2.5. Outcomes

The outcomes of depression (ICD-10 codes: F32, F33) and anxiety (ICD-10 codes: F40-F48, F93) were based on inpatient and outpatient records in the Danish National Patient Registry, using both primary and secondary diagnoses (Laugesen et al., 2021). The registry contains data on patients discharged from all Danish hospitals since 1977 and on visits

to outpatient clinic and emergency departments since 1995. Information includes the civil registration number of the patient, dates of admission and discharge, and primary and secondary diagnoses classified according to the *International Classification of Diseases. Eighth Revision* (ICD-8) until the end of 1993, and the *Tenth Revision* (ICD-10) thereafter (Laugesen et al., 2021).

2.6. Confounding

To ascertain possible confounding we used directed acyclic graphs (DAGs) (Supplemental Fig. 1) and identified variables that were both risk factors for anxiety or depression and determinants of glucocorticoid exposure. Variables included calendar year of birth (1996–2000, 2001–2005, 2006–2010, 2011–2015), maternal and paternal age at birth (restricted cubic spline with three knots), maternal pre-pregnancy body mass index (BMI) (restricted cubic spline with three knots), maternal smoking, single or multiple pregnancy, potential treatment indications (obstructive pulmonary disease including asthma or chronic obstructive pulmonary disease, inflammatory bowel disease, rheumatic disease, renal disease, or skin disease), comorbidities such as maternal type I, type II, or gestational diabetes, maternal and paternal mood or anxiety disorders, schizophrenia spectrum disorders, substance use disorders, other mental health disorders, maternal infections during pregnancy and maternal use of antiepileptic drugs or anxiolytics/hypnotics during pregnancy. Comorbidities were detected by either hospital records (inpatients and outpatient records) or relevant drug use (Supplemental Table 4). We included measures of socioeconomic position from the Danish social and demographic registries, including marital status, maternal country of birth (Denmark, other country), employment status, income and maternal and paternal highest educational level at the birth. Marital status was classified into three categories (married or in a civil partnership; single, widow, divorced, or not registered/annulled civil partnership; unknown). Employment status was categorized as employed, unemployed, early retirement, state pension, under education, or missing. Level of income was categorized according to percentiles as either low (0 – <25th percentile), intermediate (≥25th - <75th percentile), high (≥75th – 100th percentile), or missing. For both employment status and level of income we used data from the year previous to the birth. Highest educational level was classified as low (primary and lower secondary education), medium (upper secondary education or professional degree), and high (university education at the bachelor's degree level or higher).

2.7. Birth outcomes

We assessed the following delivery and birth characteristics, as captured in the Danish Medical Birth Registry: sex of the child, birth order (1, ≥2), gestational age (<28, 28–31, 32–36, ≥37 weeks), birth weight, small for gestational age (SGA), Apgar score after 5 min (<7, 7–10), and caesarean section status. Preterm birth (gestational age < 37 weeks) and SGA were considered potential mediators.

2.8. Statistical analyses

We first evaluated time trends in the use of glucocorticoids during pregnancy from 1996 to 2017. We calculated the prevalence of use each year among pregnant women and examined oral, injectable, and inhaled glucocorticoids as well as glucocorticoids applied to the intestinal mucosa or the skin. All pregnant women (whether they had a still birth or a live birth) were included in this analysis.

We then described our study population (all liveborn children from 1996 to 2015) according to birth, infant, maternal, and paternal characteristics, as well as glucocorticoid exposure *in utero*. We followed all children from date of birth until date of diagnosis of anxiety or depression, emigration, death, or the end of follow-up on 31 December 2018, whichever came first. We computed and plotted cumulative risk (%) of anxiety and depressive disorders by 5, 10, 15, and 20 years of age taking into account competing risk by death (using the Aalen Johansson estimator). In order to comply with Danish legislation, the full cumulative risk curves were not shown due to sensitive or micro data in the plots. We used multilevel Poisson regression to compare children exposed *in utero* with the comparison cohort. Likewise, the negative control exposure cohorts were compared with the comparison cohort. As women may have more than one child, we used a multilevel model to account for clustering. We computed both crude and adjusted incidence rate ratios (IRRs) with 95% confidence intervals (CIs), adjusting for potential confounding as described in Section 2.5. As fetal programming may be sex-dependent (Buss et al., 2012; Kim et al., 2017; McLaughlin et al., 2021; Sandman et al., 2013), we stratified all our analyses by sex to investigate whether associations were modified by the sex of the child.

2.8.1. Sibling design

To further control for time-stable and family-related confounding, such as genetics and family environment, we conducted a sibling analysis. Such an analysis is stratified by sibling pairs within families, and each family has its own baseline rate function reflecting the shared environment and genetics of the family. Only siblings discordant for exposure contribute to the effect estimates. We used a conditional Poisson regression with a separate ID for each family identified by the mother's civil registration number. We then computed crude and adjusted IRR, adjusting for parity and the covariates described in Section 2.5. Based on findings in the main analyses, we compared siblings exposed to high-dose systemic glucocorticoids to their sibling without exposure. To account for secular trends in health seeking behaviour we adjusted for parity and calendar year of birth. Likewise, we repeated the sibling analysis comparing siblings where only the oldest sibling was exposed and younger siblings unexposed and vice versa.

2.8.2. Mediation

Based on findings in the main analyses, we decomposed the overall association (total effect) between high-dose systemic glucocorticoid and anxiety into direct effects and indirect effects acting through the mediators of interest (preterm birth and SGA). The overall association (total effect), in the presence of a mediator with which the exposure might interact, can be decomposed into four components: the controlled direct effect (due to neither mediation nor interaction), reference interaction (due to interaction only), mediated interaction (due to mediated interaction) and pure indirect effect (due to mediation only). To investigate potential mediation by preterm birth and SGA, we decomposed the overall association (total effect) into these four components using the med4way command (Discacciati et al., 2018; VanderWeele, 2014). For the mediator model, we specified a logistic regression and for the outcome model, we specified a Cox-regression. The controlled direct effects were estimated by fixing mediator levels to preterm birth and SGA, respectively. We estimated risk ratios and overall attributable proportions in adjusted models. The adjusted models included covariates as described in Section 2.5.

2.9. Sensitivity analyses

To ensure the validity of our results, we conducted several sensitivity analyses. First, to account for maternal BMI, we adjusted for maternal BMI in a subcohort of children born in 2004 or later. (Maternal BMI was not recorded before 2004 in the Medical Birth Registry). Second, to understand the influence of each covariate on the effect estimates, we evaluated the association between each covariate and the exposure, and each covariate and the outcomes. We also adjusted for each covariate separately. Third, we increased the specificity of our exposure definition by defining *in utero* exposure as maternal redemption of two or more prescriptions for glucocorticoids during pregnancy. Fourth, we increased the specificity of the outcome definition by including only

primary diagnosis codes for anxiety and depression. Fifth, we used a family ID identified by both maternal and paternal civil registration numbers in the sibling analysis (i.e., identified full siblings).

Statistical analyses were conducted using Stata version 16.

3. Results

3.1. Time trends

Glucocorticoid use among pregnant women was frequent, with prevalence ranging from 0.4% to 1.2% for oral use, 1.7–3.2% for inhaled use, 0.3–0.7% for injectable use, 6.6–11% for glucocorticoids applied to the skin, and 4.5–6.8% for glucocorticoids applied to the intestine (Fig. 2). Use of oral and intestinal glucocorticoids increased during the 1996–2017 period, while use of inhaled glucocorticoids, injectable glucocorticoids, and glucocorticoids applied to the skin declined (Fig. 2).

3.2. Exposure

We identified 1,275,909 children in our study population (Fig. 1), with a median follow-up time of 13 years for both outcomes (IQR: 8–18 years). Among these, 34,724 children developed anxiety only, 4830 depression only, and 6956 developed both anxiety and depression during follow-up. In our study population, 9307 children (0.7%) were exposed to systemic glucocorticoids *in utero* and 116,389 (9.1%) to local glucocorticoids *in utero*. The median cumulative systemic glucocorticoid dose was 200 mg peq (IQR: 200–500 mg) and the most frequently used generic types of systemic glucocorticoids were betamethasone (n = 4622) and prednisolone (n = 3257) (Table 1). The negative control exposure cohorts consisted of children without *in utero* glucocorticoid exposure, including 79,196 (6.2%) children born to maternal former users of systemic glucocorticoids, 11,565 (0.9%) children born to maternal postnatal users of systemic glucocorticoids, 19,939 (1.6%) children born to maternal users of NSAIDs or immunotherapies during pregnancy, and 16,721 (1.3%) children born to paternal users of systemic glucocorticoids (Table 1).

3.3. Birth outcomes and parental characteristics

Children born following *in utero* exposure to systemic glucocorticoids were more often born prematurely (43%) and SGA (13%) than children without exposure in the comparison cohort (7% premature and 9.6% SGA) and in the negative control exposure cohorts (6–10% premature and 1.0–1.3% SGA) (Table 1).

Maternal glucocorticoid users during pregnancy and maternal former users were more alike concerning recorded characteristics, than were maternal glucocorticoid users during pregnancy and maternal never-users (Tables 2a,2b). Thus, maternal users and former users were slightly older at birth (median age 30–32 vs. 29 years), had a higher educational level, were more frequently overweight or obese (37–39% vs. 30%), and had a higher prevalence of psychiatric illness, infections during pregnancy (24–35% vs. 22%), and diabetes (6–10% vs. 4%), compared to maternal never-users (Tables 2a,2b).

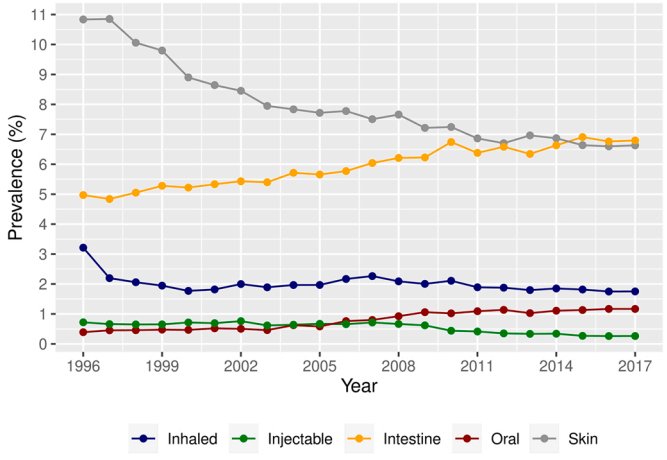

Fig. 2. Prevalence of glucocorticoid use among pregnant women, Denmark, 1996–2017. Injectable glucocorticoids refer to intra-articular, intramuscular and intravenous routes of administration. Glucocorticoids acting on the intestine refer to drugs with oral or rectal routes of administration, but with release of the active substance locally in the intestine (e.g. for treatment of inflammatory bowel disease or haemorrhoids).

Table 1
Glucocorticoid exposure among 1,275,909 children born in Denmark in 1996–2015.

Exposure	N (%)
All children	1,275,909 (100)
Children exposed to glucocorticoids *in utero*	
Exposed to systemic glucocorticoids *in utero*	9307 (0.7)
Time of exposure	
1st trimester only	2770 (0.2)
2nd trimester only	1710 (0.1)
3rd trimester only	4073 (0.3)
Multiple-trimester exposure	754 (0.06)
Generic systemic glucocorticoid type	
Prednisolone only	3257 (0.3)
Prednisone only	583 (0.05)
Methylprednisolone only	492 (0.04)
Betamethasone only	4622 (0.4)
Dexamethasone only	26 (0.0)
Hydrocortisone only	110 (0.01)
Triamcinolone only	86 (0.01)
Multiple types	131 (0.01)
Cumulative systemic glucocorticoid dose in mg prednisolone-equivalents[a], median and IQR	200 mg (200–500 mg)
Exposed to local glucocorticoids only	116,389 (9.1)
Negative control exposure cohorts	
Maternal use of NSAIDs or immunotherapy during pregnancy	19,939 (1.6)
Former maternal systemic glucocorticoid use, < 6 months since most recent prescription[b]	8555 (0.7)
Former maternal systemic glucocorticoid use, 6–24 months since most recent prescription	18,892 (1.5)
Former maternal systemic glucocorticoid use, > 24 months since most recent prescription	60,304 (4.7)
Maternal postnatal systemic glucocorticoid use, 0–1 year after birth	11,565 (0.9)
Paternal systemic glucocorticoid use	16,721 (1.3)
Comparison cohort of children without *in utero* glucocorticoid exposure	1,178,851 (92)

[a] The cumulative systemic glucocorticoid dose in prednisolone-equivalents was calculated by multiplying the number of pills/injections, dose per pill/injection, and prednisolone conversion factor for the cumulative prescriptions during pregnancy.

[b] Not included in the analyses due to uncertainty in exposure status. Abbreviation: IQR, interquartile range

3.4. In utero glucocorticoid exposure and risk of anxiety and depression

The association between *in utero* glucocorticoid exposure, and anxiety or depression depended on route of administration, cumulative glucocorticoid dose, and trimester of exposure.

3.4.1. Route of glucocorticoid administration, cumulative dose and generic type

Low cumulative dose of systemic glucocorticoid exposure or local exposure was not associated with anxiety or depression (Fig. 3 and Fig. 4). However, *in utero* exposure to cumulative high-dose systemic glucocorticoids increased risk of both anxiety disorders and depression (Fig. 3 and Fig. 4). For anxiety, risk by 20 years of age was 16% (95% CI: 12%−20%) for high-dose systemic glucocorticoid exposure and 7.8%

Table 2a
Birth and infant characteristics of 1,275,909 children born in Denmark from 1996 to 2015, according to exposure.

	N (%)					
	Children exposed to systemic glucocorticoids	Negative control exposure cohorts (children without glucocorticoid exposure)				Comparison cohort (children without glucocorticoid exposure)
		Maternal former use (>24 months before pregnancy)	Maternal former use (6–24 months before pregnancy)	Maternal use of NSAIDs or immunotherapy during pregnancy	Paternal use of systemic glucocorticoids	Maternal never-use of glucocorticoids
All births	9307 (100)	60,304 (100)	18,892 (100)	19,939 (100)	16,721 (100)	1,178,851 (100)
Sex						
Male	4788 (51)	30,699 (51)	9692 (51)	10,386 (52)	8456 (51)	604,742 (51)
Birth year						
1996–2000	970 (10)	2605 (4.3)	4288 (23)	6089 (31)	4866 (29)	325,593 (28)
2001–2005	2040 (22)	14,142 (23)	4435 (23)	4917 (25)	4070 (24)	302,343 (26)
2006–2010	3054 (33)	20,770 (34)	5201 (28)	4853 (24)	4541 (27)	292,341 (25)
2011–2015	3243 (35)	22,787 (38)	4968 (26)	4080 (20)	3244 (19)	258,574 (22)
Birth order						
1	5190 (56)	23,791 (39)	8648 (46)	8314 (42)	6602 (39)	482,276 (41)
≥ 2	3750 (40)	35,315 (59)	9583 (51)	9940 (50)	8770 (52)	597,042 (51)
Missing	367 (4.0)	1198 (2.0)	661 (3.5)	1685 (8.5)	1349 (8.1)	99,533 (8.4)
Gestational age, weeks						
< 28	697 (7.6)	221 (0.4)	89 (0.5)	69 (0.4)	30 (0.2)	3710 (0.3)
28–31	890 (9.7)	484 (0.8)	134 (0.7)	186 (1.0)	83 (0.5)	8351 (0.7)
32–36	2349 (25)	4299 (7.2)	1470 (7.9)	1504 (7.7)	945 (5.7)	73,171 (6.4)
≥ 37	5284 (57)	54,629 (92)	16,926 (90)	17,791 (91)	15,400 (94)	1,066,759 (93)
Birth weight in g, median and IQR	2920 (2000–3490)	3500 (3100–3850)	3480 (3100–3840)	3470 (3100–3830)	3530 (3180–3900)	3500 (3150–3860)
Missing	68 (0.7)	704 (1.2)	193 (1.0)	225 (1.3)	74 (1.3)	22,285 (1.9)
SGA	1247 (13)	5525 (9.2)	1873 (9.9)	2192 (11)	1607 (9.6)	113,143 (9.6)
Apgar score after 5 min						
< 7	291 (3.1)	522 (0.9)	183 (1.0)	189 (1.0)	132 (0.8)	8779 (0.8)
7–10	8747 (94)	59,100 (98)	18,241 (97)	18,285 (92)	15,402 (92)	1,081,646 (92)
Missing	269 (2.9)	882 (1.1)	468 (2.5)	1465 (7.4)	1187 (7.1)	88,426 (7.5)
Caesarean section	3438 (37)	14,503 (24)	4448 (24)	4399 (22)	2969 (18)	201,425 (17)
Multiple pregnancy	1975(21)	2971 (4.9)	862 (4.6)	520 (2.6)	518 (3.1)	45,926 (3.9)

Abbreviations: IQR, interquartile range; NSAIDs, non-steroidal anti-inflammatory drugs. SGA, small for gestational age (defined as birth weight < the 10th percentile for infants of the same gestational age, sex, and birth year). Children whose mother used glucocorticoids 0–6 months before pregnancy, but not during pregnancy, were not included due to uncertainty about exposure status.

(95% CI: 7.7%−7.8%) for the comparison cohort (Fig. 5). The adjusted IRR was 1.79 (95% CI: 1.36–2.37) when comparing children exposed to high-dose systemic glucocorticoid with the comparison cohort (Fig. 3). For depression, risk by 20 years of age was 3.6% (95% CI: 1.7%−6.5%) for high-dose systemic exposure and 2.6% (95% CI: 2.5%−2.6%) for the comparison cohort (Fig. 5). The adjusted IRR was 1.45 (95% CI: 0.80–2.63) comparing children exposed to high-dose systemic glucocorticoid with the comparison cohort (Fig. 4). Estimates were similar for prednisolone and betamethasone (Fig. 3 and Fig. 4). Further, sex did not modify the IRRs substantially (Fig. 3 and Fig. 4). As expected, the absolute risks of anxiety and depression were generally higher in females than in males. In females, the risk of anxiety at 20 years of age was 20% (95% CI: 14%−27%) in the cohort with high-dose glucocorticoid exposure vs. 10% (95% CI: 9.9%−10%) in the comparison cohort. In males, the risk of anxiety at 20 years of age was 10% (95% CI: 6.1%−14%) in the cohort with high-dose exposure vs. 5.7% (95% CI: 5.6%−5.8%) in the comparison cohort. For depression, the risks were 4.8% (95% CI: 2.0%−9.2) vs. 3.7% (95% CI: 3.6%−3.8%) in females and 2.1% (95% CI: 0.4%−7.0%) vs. 1.5% (95% CI: 1.4%−1.6%) in males (Supplemental Fig. 2). Adjusting additionally for maternal BMI did not change the associations (Supplemental Table 5 and 6) and increasing the specificity of the exposure and outcome definitions did not change estimates substantially.

3.4.2. *Trimester of exposure*

With respect to trimester of exposure, 2nd and 3rd trimester exposure, as well as multiple trimester exposure were associated with increased risk of anxiety (Fig. 3). For depression, we only found an association for 2nd trimester of exposure (Fig. 4). Timing of exposure and cumulative glucocorticoid dose were however associated. Thus, the effect of timing is difficult to separate from the effect of cumulative dose. High-dose exposure was present in 8.6% of children exposed in 1st trimester only, in 11% of children exposed in 2nd trimester only, in 4.0% of children exposed in 3rd trimester only, and in 94% of children exposed in multiple trimesters.

3.4.3. *Negative control exposure analyses and sibling design*

All negative control exposure analyses yielded null associations (Fig. 3 and Fig. 4). In the sibling design, we identified 1491 siblings who were discordant for exposure (651 who were exposed to cumulative high-dose systemic glucocorticoids and 840 without exposure). Of the 651 exposed children, 253 (39%) were the oldest sibling. For anxiety disorders, we found an adjusted IRR of 1.83 (95% CI: 1.03–3.63), comparing siblings with high-dose exposure vs. siblings without exposure. Estimates did not change, when comparing oldest exposed siblings with younger unexposed siblings and vice versa, or when using both maternal and paternal civil registration number to identify siblings (full siblings) (Supplemental Table 7). We were unable to conduct the sibling

Table 2b
Parental characteristics of 1,275,909 children born in Denmark in 1996–2015, according to exposure.

	N (%)					
	Children exposed to systemic glucocorticoids	Negative control exposure cohorts (children without glucocorticoid exposure)				Comparison cohort (children without glucocorticoid exposure)
		Maternal former use (>24 months before pregnancy)	Maternal former use (6–24 months before pregnancy)	Maternal use of NSAIDs or immunotherapy during pregnancy	Paternal use of systemic glucocorticoid	Maternal never-use of glucocorticoid
All births	9307 (100)	60,304 (100)	18,892 (100)	19,939 (100)	16,721 (100)	1,178,851 (100)
Maternal characteristics						
Age at birth (years), median and IQR	32 (28–35)	31 (28–34)	30 (27–34)	30 (26–34)	30 (27–34)	29 (27–33)
Marital status						
Married/civil partnership	4369 (47)	29,333 (49)	8866 (47)	8884 (45)	8217 (49)	570,073 (48)
Single	4826 (52)	30,607 (51)	9950 (53)	10,983 (55)	8367 (50)	587,761 (50)
Unknown	112 (1.2)	364 (0.6)	76 (0.4)	72 (0.4)	137 (0.8)	21,017 (1.8)
Country of origin						
Denmark	7690 (83)	55,607 (92)	16,827 (89)	17,037 (85)	14,560 (87)	991,581 (84)
Employment status						
Employed	6368 (68)	44,449 (74)	13,245 (70)	12,155 (61)	11,836 (71)	805,729 (68)
Unemployed	1509 (16)	9189 (15)	2935 (16)	3346 (17)	2408 (14)	173,574 (15)
Early retirement	1006 (11)	4614 (7.7)	1969 (10)	3706 (19)	1860 (11)	142,672 (12)
State pension	NA	NA	NA	24 (0.1)	NA	413 (0.04)
Under education	328 (3.5)	1772 (2.9)	633 (3.4)	618 (3.1)	524 (3.1)	44,425 (3.8)
Missing	86 (0.9)	276 (0.5)	106 (0.6)	90 (0.5)	85 (0.5)	12,038 (1.0)
Highest educational level						
Low	3405 (37)	23,300 (39)	7348 (39)	8473 (42)	6445 (39)	451,867 (38)
Medium	3675 (39)	27,526 (45)	9139 (48)	9132 (46)	8180 (49)	520,067 (44)
High	1805 (19)	8708 (14)	2033 (11)	1643 (8.2)	1561 (9.3)	147,330 (13)
Missing	422 (4.5)	770 (1.3)	372 (2.0)	691 (3.5)	535 (3.2)	59,587 (5.1)
Income						
Low	2115 (23)	10,287 (17)	3938 (21)	5252 (26)	3672 (22)	297,332 (25)
Intermediate	4163 (45)	33,167 (55)	10,441 (55)	10,892 (55)	8952 (54)	578,461 (49)
High	2977 (32)	16,806 (28)	4508 (24)	3760 (19)	4005 (24)	289,017 (25)
Missing	52 (0.6)	44 (0.07)	NA	35 (0.2)	92 (0.6)	14,041 (1.2)
Pre-pregnancy body mass index (kg/m^2), median IQR	23 (21–26)	24 (21–27)	24 (21–28)	24 (21–28)	23 (21–27)	23 (21–26)
< 18.5	195 (3.7)	1687 (3.3)	439 (3.7)	384 (3.6)	375 (4.1)	29,126 (4.3)
18.5–24	2894 (55)	28,342 (55)	6412 (54)	5428 (51)	5273 (57)	396,821 (59)
25–29	1096 (21)	11,201 (22)	2692 (23)	2331 (22)	1897 (21)	130,487 (19)
≥ 30	815 (16)	7492 (15)	1878 (16)	1919 (18)	1897 (13)	75,491 (11)
Missing (2004 and onwards)	212 (4.1)	2153 (4.2)	506 (4.2)	513 (4.9)	440 (4.8)	38,895 (5.8)
Not recorded before 2004	1945	9429	6965	9364	7547	508,031
Smoking during pregnancy						
Yes	1289 (14)	8308 (14)	2991 (16)	4774 (24)	2706 (16)	177,647 (15)
Missing	708 (7.6)	1868 (3.1)	1997 (11)	2306 (12)	1604 (10)	125,431 (11)
Obstructive pulmonary disease	289 (3.1)	2067 (3.4)	603 (3.2)	247 (1.2)	154 (0.9)	8667 (0.7)
Inflammatory bowel disease	60 (0.6)	228 (0.4)	94 (0.5)	106 (0.5)	29 (0.5)	977 (0.08)
Rheumatic disease	482 (5.2)	1161 (1.9)	388 (2.1)	406 (2.0)	97 (0.6)	1161 (1.9)
Renal disease	156 (1.7)	518 (0.9)	131 (0.7)	114 (0.6)	76 (0.5)	4060 (0.4)
Skin disease	196 (2.1)	1244 (2.1)	355 (1.9)	404 (2.0)	195 (1.2)	14,619 (1.2)
Diabetes (type I, II, or gestational)	895 (9.6)	4039 (6.7)	1047 (5.5)	993 (5.0)	628 (3.8)	43,657 (3.7)
Infections during pregnancy	3215 (35)	13,068 (24)	5358 (28)	8706 (44)	4916 (29)	257,054 (22)
Mood or anxiety disorder	1648 (18)	12,387 (21)	3288 (17)	3611 (11)	2025 (12)	124,088 (11)
Schizophrenia spectrum disorder	340 (3.7)	2250 (3.7)	629 (3.3)	863 (4.3)	378 (2.3)	22,125 (1.9)
Substance use disorder	114 (1.2)	563 (0.9)	178 (0.9)	279 (1.4)	96 (0.6)	6694 (0.6)
Other mental health disorders	412 (4.4)	2617 (4.3)	654 (3.5)	871 (4.4)	456 (2.7)	29,514 (2.5)
	47 (0.5)	236 (0.4)	89 (0.5)	178 (0.9)	70 (0.4)	3571 (0.3)

(*continued on next page*)

Table 2b (*continued*)

	N (%)					
Use of antiepileptic drugs during pregnancy						
Use of anxiolytics/ hypnotics during pregnancy	149 (1.6)	381 (0.6)	211 (1.1)	654 (3.3)	145 (0.9)	6966 (0.6)
Paternal characteristics						
Age at birth (years), median and IQR	33 (30–38)	33 (30–37)	33 (29–36)	32 (28–36)	32 (29–36)	32 (29–36)
Highest educational level at birth						
Low	2476 (27)	16,224 (27)	5509 (29)	6667 (33)	4987 (30)	341,970 (29)
Medium	4302 (46)	32,980 (55)	10,440 (55)	10,387 (52)	9641 (58)	608,417 (52)
High	1795 (19)	8653 (14)	2154 (11)	1652 (8.3)	1596 (9.5)	154,421 (13)
Missing	734 (7.9)	2447 (4.1)	789 (4.2)	1233 (6.2)	497 (3.0)	74,043 (6.3)
Mood or anxiety disorder	863 (9.3)	6076 (11)	1641 (9.3)	1773 (8.9)	1725 (10)	84,396 (7.2)
Schizophrenia spectrum disorder	247 (2.7)	1590 (2.6)	450 (2.4)	635 (3.2)	544 (3.3)	25,191 (2.1)
Substance use disorder	197 (2.1)	1477 (2.5)	449 (2.4)	691 (3.5)	473 (2.8)	22,633 (1.9)
Other mental health disorders	176 (1.9)	1079 (1.8)	317 (1.7)	400 (2.0)	299 (1.8)	16,368 (1.4)

Abbreviations: IQR, interquartile range; NSAIDs, non-steroidal anti-inflammatory drugs. Highest educational level at birth: low (primary and lower secondary education), medium (upper secondary education or professional degree) and high (university education at bachelor's degree level or higher. Children whose mother used glucocorticoids 0–6 months before pregnancy, but not during pregnancy, were not included due to uncertainty in exposure statu

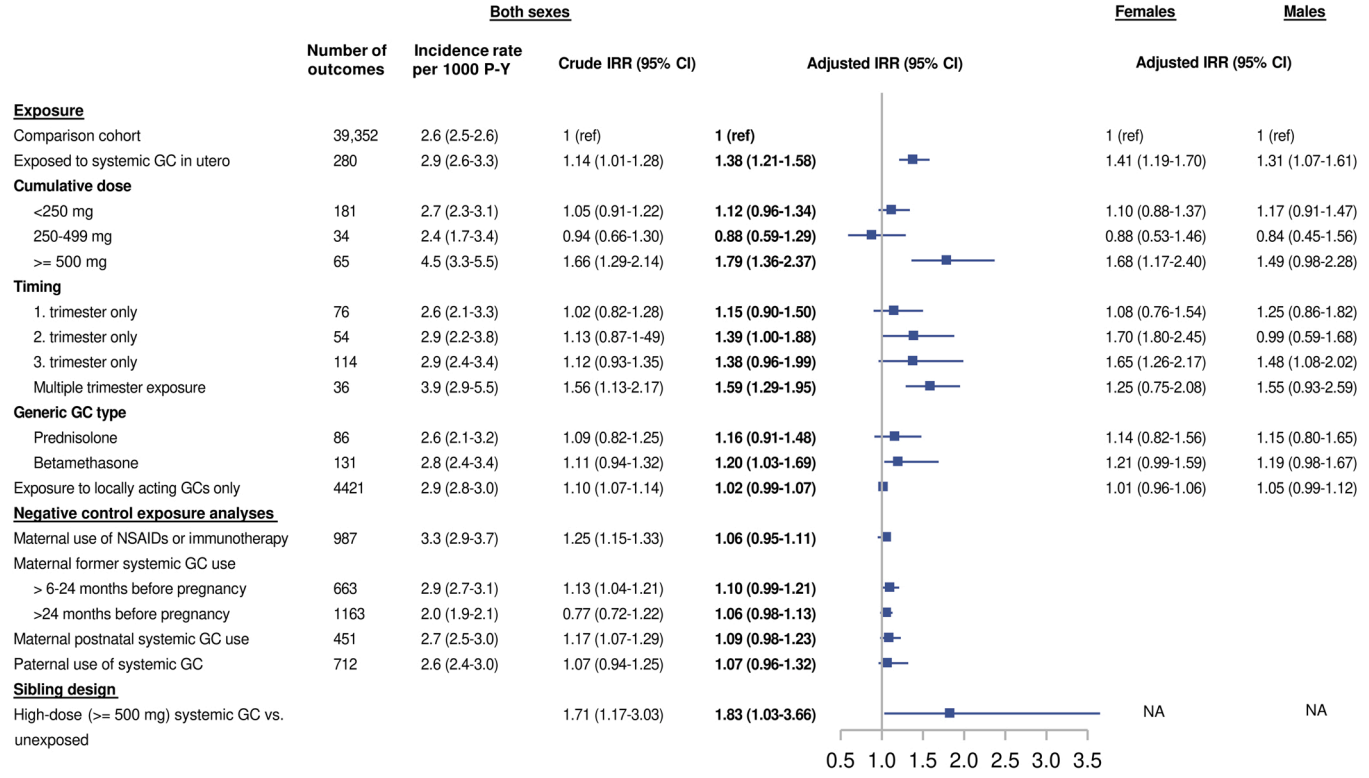

Fig. 3. Associations between *in utero* exposure to glucocorticoids and anxiety disorders. Adjusted for calendar year of birth, maternal and paternal age (cubic spline with three knots), highest maternal and paternal educational level, maternal income, employment status, marital status, country of origin, maternal treatment indications, multiple pregnancy, maternal infections during pregnancy, maternal diabetes, maternal smoking, and maternal and paternal mood or anxiety disorders, schizophrenia spectrum disorder, substance use disorder, other mental health disorders, maternal use of anxiolytics/hypnotics, and maternal use of antiepileptic drugs during pregnancy. The sibling design is further adjusted for parity. Abbreviations: CI, confidence interval. GC, glucocorticoids. IRR, incidence rate ratio. P-Y, person years.

design for depression due to few outcomes.

3.4.4. Mediation

Supplemental Table 8 shows the associations between *in utero* exposure to high-dose systemic glucocorticoids and anxiety decomposed by the potential mediators preterm birth and SGA. Mediation by preterm birth accounted for 3.6% of the overall association and mediation by SGA accounted for 1.4%. We were unable to investigate mediation for

	Both sexes				Females	Males
	Number of outcomes	Incidence rate per 1000 P-Y	Crude IRR (95% CI)	Adjusted IRR (95% CI)	Adjusted IRR (95% CI)	
Exposure						
Comparison cohort	11,558	0.8 (0.7-0.8)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Exposed to systemic GC in utero	51	0.6 (0.4-0.7)	0.92 (0.74-1.14)	0.93 (0.66-1.32)	1.07 (0.72-1.32)	0.90 (0.35-1-20)
Cumulative dose						
<250 mg	26	0.4 (0.3-0.6)	0.52 (0.36-0.77)	0.70 (0.42-1.17)	0.91 (0.53-1.57)	0.57 (0.27-1.30)
250-499 mg	12	0.8 (0.5-1.5)	1.15 (0.65-2.02)	1.16 (0.52-2.58)	1.03 (0.39-2.76)	1.26 (0.32-5.06)
>= 500 mg	13	0.9 (0.5-1.5)	1.20 (0.70-2.07)	1.45 (0.80-2.63)	1.58 (0.79-3.18)	1.44 (0.66-3.77)
Timing						
1. trimester only	18	0.6 (0.4-1.0)	0.85 (0.48-1.50)	0.84 (0.45-1.57)	0.99 (0.49-1.96)	0.83 (0.33-2.41)
2. trimester only	10	1.0 (0.5-2.0)	1.38 (0.72-2.65)	1.37 (0.79-2.54)	1.27 (0.57-2.83)	1.30 (0.63-3.95)
3. trimester only	17	0.8 (0.3-1.7)	1.06 (0.48-2.37)	0.95 (0.51-1.77)	1.13 (0.56-2.25)	0.91 (0.23-3.65)
Multiple trimester exposure	6	0.7 (0.3-1.5)	0.89 (0.40-1.99)	0.95 (0.48-2.10)	0.87 (0.28-2.71)	0.58 (0.08-4.19)
Generic GC type						
Prednisolone	27	0.8 (0.6-1.2)	1.10 (0.76-1.61)	1.10 (0.71-1.72)	1.06 (0.61-1.83)	1.16 (0.55-2.46)
Betamethasone	18	0.8 (0.5-1.3)	0.99 (0.65-1.72)	0.98 (0.62-1.79)	0.99 (0.59-1.83)	1.01 (0.60-1.85)
Exposure to locally acting GCs only	1370	0.9 (0.8-0.9)	1.10 (1.04-1.17)	1.12 (1.04-1.22)	1.15 (1.05-2.30)	1.09 (0.93-1.25)
Negative control exposure analyses						
Maternal use of NSAIDs or immunotherapy	264	1.0 (0.9-1.1)	1.38 (1.22-1.57)	1.10 (0.95-1.30)		
Maternal former systemic use						
> 6-24 months before pregnancy	155	0.7 (0.6-0.8)	0.91 (0.78-1.49)	0.93 (0.72-1.32)		
>24 months before pregnancy	183	0.3 (0.3-0.4)	0.49 (0.40-0.60)	0.95 (0.82-1.11)		
Maternal postnatal systemic GC use	114	0.8 (0.6-0.9)	1.04 (0.87-1.26)	0.98 (0.79-1.22)		
Paternal use of systemic GC	213	0.9 (0.8-1.1)	1.12 (0.88-1.46)	1.04 (0.95-1.69)		

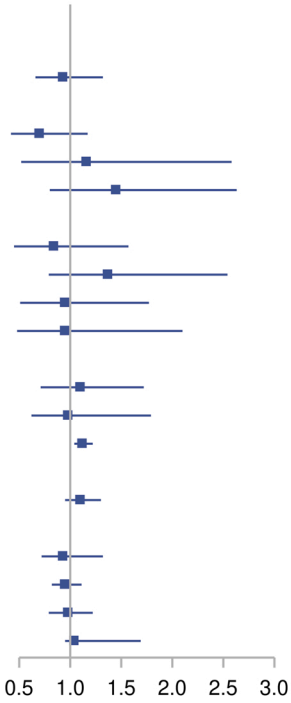

Fig. 4. Association between *in utero* exposure to glucocorticoids and depression. Adjusted for calendar year of birth, maternal and paternal age (cubic spline with three knots), highest maternal and paternal educational level, maternal income, employment status, marital status, country of origin, maternal treatment indications, multiple pregnancy, maternal infections during pregnancy, maternal diabetes, maternal smoking, and maternal and paternal mood or anxiety disorders, schizophrenia spectrum disorder, substance use disorder, other mental health disorders, maternal use of anxiolytics/hypnotics, and maternal use of antiepileptic drugs during pregnancy. Abbreviations: CI, confidence interval. GC, glucocorticoids. IRR, incidence rate ratio. NA, not available (due to Danish legislation), P-Y, person years.

the outcome depression due to few data.

4. Discussion

In utero exposure to cumulative high-dose systemic glucocorticoids increased the risk of subsequent anxiety disorders by 1.8-fold compared to children without *in utero* exposure. Exposure to lower systemic doses and local glucocorticoids did not. These results showed robustness in the negative control exposure and sibling design analyses. Similar results were found for depression; however, the estimates had low precision.

4.1. Limitations

As our study was conducted in the setting of a universal tax-supported healthcare system, selection bias is unlikely to explain our findings (Laugesen et al., 2021). We used prescription redemption and hospital records as a proxy for glucocorticoid use. While the Prescription Registry is virtually complete for medications dispensed in community pharmacies, it does not capture in-hospital medication use (Laugesen et al., 2021). The Patient Registry contains information on antenatal glucocorticoid treatment for imminent preterm birth, but no other glucocorticoid treatment regimens. A few maternal glucocorticoid users might therefore have been misclassified as non-users. Additionally, non-users might have been misclassified as users if they redeemed a glucocorticoid prescription but did not adhere to the treatment. We would expect misclassification of the exposure to be non-differential. It is also likely that not all outcomes were captured, as they were defined only by hospital diagnoses; hence, we did not capture diagnoses in general practice. Maternal glucocorticoid users were older, more educated, and had higher prevalence of morbidity compared to mothers in our comparison cohort. Thus, increased healthcare seeking among maternal glucocorticoid users might have biased our estimates upwards. Our negative control analyses, however, did not support such bias. Despite that the estimates for depression had low statistical precision, our findings are an important contribution to existing knowledge (Hernán, 2021). Yet, studies with longer follow up are warranted. We controlled for measured relevant confounding. Further, our negative control exposure and sibling design analyses confirmed the robustness of our findings towards confounding from genetics, family environment and maternal disease. Still, confounding by treatment indication and underlying disease severity, comorbidity, lifestyle and genetics cannot be ruled out. We were unable to include information on lifestyle, such as alcohol consumption, but a former study showed that drinking alcohol was less frequent among glucocorticoid users compared to non-users in women of reproductive age (Laugesen et al., 2019).

4.2. Mechanisms and interpretation of our findings

Several mechanisms may contribute to our findings. First, the effects of *in utero* glucocorticoid exposure on the neuroendocrine function are evident from both animal and human studies. Exposure to excess glucocorticoids may impact the development of the fetal HPA axis by altering glucocorticoid receptor isoform expression as well as glucocorticoid receptor density in regions such as the paraventricular nucleus of the hypothalamus, hippocampus, and amygdala. Such modifications have a consequence for HPA axis feedback sensitivity, leading to increased cortisol levels in postnatal life. (Alexander et al., 2012; Braun et al., 2013; Crombie et al., 2021; Davis et al., 2011; Edelmann et al., 2016; Ilg et al., 2019; Irwin et al., 2021; McLaughlin et al., 2021; Moisiadis and Matthews, 2014a, 2014b; Osborne et al., 2018; Provençal

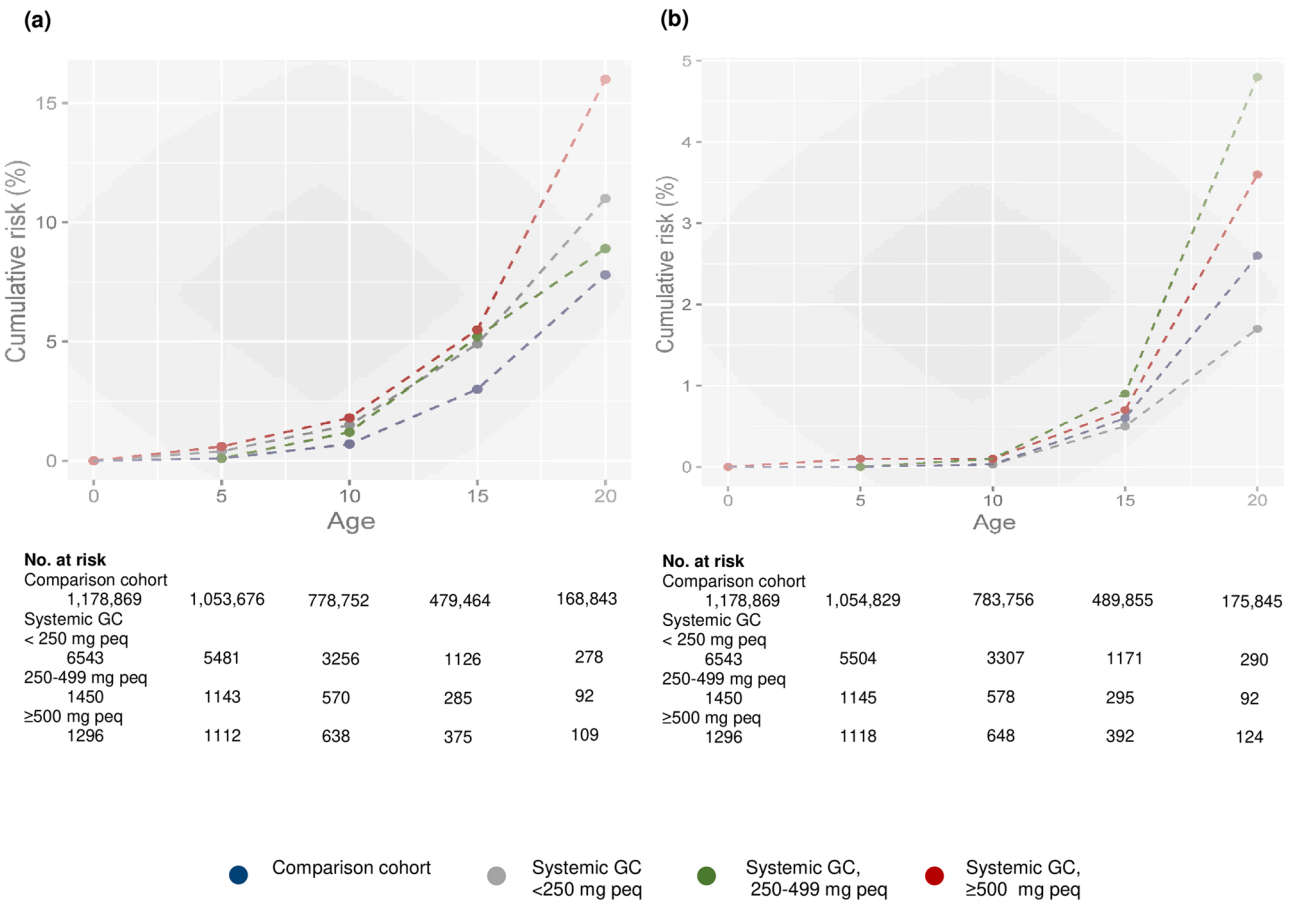

Fig. 5. Cumulative risk (%) of anxiety and depressive disorders by 5, 10, 15, and 20 years of age. (a) Anxiety. (b) Depression. The full cumulative risk curves are not shown due to sensitive or micro data in the plots, in compliance with Danish legislation. Abbreviations: GC, glucocorticoids. PEQ, prednisolone-equivalents.

et al., 2020; Saif et al., 2016; Seckl, 2004; Tegethoff et al., 2009). Further, *in utero* exposure may disrupt the circadian regulation of the HPA axis (Edelmann et al., 2016; Ter Wolbeek et al., 2015). Altered HPA axis sensitivity and disrupted circadian regulation of cortisol have been linked to both anxiety and depressive disorders (Braun et al., 2013; Craske et al., 2017). *In utero* exposure to excess glucocorticoid can further induce structural and functional changes in the brain and affect neural connectivity in a sex- and time-specific manner (Buss et al., 2012; Davis et al., 2013; Kim et al., 2017; Seckl, 2004). For instance, prenatal exposure to excess maternal cortisol has been linked to larger amygdala volume (Buss et al., 2012) and altered neural connectivity (Kim et al., 2017) in girls, but not in boys. Further, synthetic glucocorticoid exposure has shown bilateral cortical thinning with most pronounced effect in the rostral anterior cingulate cortex (Davis et al., 2013). These changes are associated with paediatric anxiety and depression (Boes et al., 2008; Swartz et al., 2014). Second, accumulating evidence suggests that inflammation and metabolic disturbances are involved in the development of psychiatric disorders (Osborne et al., 2018). Thus, biological mechanisms linking glucocorticoids, inflammation, metabolic disturbances, and psychiatric disorders may play a role in our findings. Third, *in utero* exposure to excess glucocorticoids is associated with increased risk of preterm birth and SGA (Khan et al., 2011; Murphy et al., 2012; Rodriguez et al., 2019), which are predictors for mental disorders later in life (Abel et al., 2010). Our study confirmed that preterm birth and SGA occurred more frequently among exposed children compared to children without exposure. However, mediation by preterm birth and SGA accounted for little of the overall association. Finally, other pathways than the effect of *in utero* exposure itself, cannot be ruled out. Accumulation of risk factors needs to be considered. As an example, maternal inflammatory or autoimmune disease can disrupt maternal care and impact family life and mental wellbeing. Our study showed an association for high-dose systemic glucocorticoid exposure only. This finding is consistent with enzymatic saturation of placental 11β-HSD2 after high-dose or long-term treatment, leading to greater fetal exposure. In addition, we found the strongest association for anxiety compared to depression. Age of onset are slightly different for the disorders, with an earlier rise in incidence for anxiety (Dalsgaard et al., 2020). Thus, our study has a greater power to detect an association for anxiety than for depression. Further, biological mechanisms, such as altered HPA axis sensitivity and structural changes in the amygdala, might play a more pivotal role for the pathogenesis of anxiety. The temporal associations observed in our study can be explained by either exposure dose or by physiological mechanisms. Glucocorticoid effects are determined by the time of tissue-specific expression of glucocorticoid receptors in the fetus and development of the HPA axis (Bolt et al., 2002). Glucocorticoid-receptor expression in the fetal brain is evident from the end of 1st trimester and onwards.

4.3. Comparison with previous studies

Our findings are in line with former studies on antenatal treatment of women with imminent preterm birth and likewise on maternal stress during pregnancy (Crombie et al., 2021; Irwin et al., 2021; Khalife et al., 2013; McLaughlin et al., 2021; Osborne et al., 2018; Räikkönen et al., 2020; Tuovinen et al., 2021; Van den Bergh et al., 2020; Wolford et al., 2020). We provide new knowledge about synthetic glucocorticoid exposure across all stages of pregnancy and covering a broad spectrum of treatment indications and glucocorticoid formulations. Previous studies assessed a broader spectrum of mental disorders (Khalife et al., 2013; Räikkönen et al., 2020; Wolford et al., 2020), while we focused on

anxiety and depression, partly in order to assess a broader spectrum of exposures in more details. Further, anxiety and depression are among the most common mental disorders in childhood and adolescence (Dalsgaard et al., 2020), although peak incidence occurs later in life.

5. Conclusion

Given the widespread use of glucocorticoids in pregnant women, adverse mental health effects of high-dose *in utero* exposure merit clinical attention. Exposure to low cumulative systemic doses, such as for treatment of imminent preterm birth, and local glucocorticoids do not seem to affect risk of anxiety or depression in the offspring. The implications of this study largely affect pregnant women with autoimmune or inflammatory diseases, who receive high-dose cumulative treatment. Clinicians may consider glucocorticoid-sparring strategies as an alternative for these patients. Yet, risks and benefits should be weighed, as insufficiently treated maternal disease may also affect both mother and fetus.

Funding source and role

This work was supported by a grant from the Novo Nordisk Foundation (grant no. 058005). The funding source had no role in the study design, in the collection, analysis, and interpretation of data; in the writing of the report, or in the decision to submit the paper for publication.

Ethical approval

This study was approved by the Danish Data Protection Agency (Record number: 2016–051–000001, serial number 605). According to Danish legislation, informed consent or approval from an ethics committee is not required for registry-based studies.

Data sharing

Sharing of individual-level data are not permitted due to Danish legislation.

Author's contributions

All authors contributed to the study's conception. KL wrote the initial manuscript and performed the statistical analyses. KL and HTS take responsibility for the integrity of the data. IP, HTS, and JOLJ contributed to the interpretation of results and revised the manuscript critically. All authors approved the final manuscript and accept responsibility to submit for publication. The corresponding author attests that all listed authors meet authorship criteria and that no other persons meeting the criteria have been omitted.

Declaration of Competing Interest

All authors declare no personal conflict of interest. Department of Clinical Epidemiology, Aarhus University Hospital, receives funding for other studies from companies in the form of research grants to (and administered by) Aarhus University. None of these studies have any relation to the present study. IP has received payment for participation in a workshop at Novo Nordisk, but it was not in any way related to the present study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2022.105766.

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