



OPEN

Elevated third trimester corticosteroid levels are associated with fewer offspring infections

Nicole Prince¹, Rachel S. Kelly¹, Su H. Chu¹, Priyadarshini Kachroo¹, Yulu Chen¹, Kevin M. Mendez¹, Sofina Begum¹, Hans Bisgaard², Klaus Bønnelykke², Min Kim², Ofer Levy³, Augusto A. Litonjua⁴, Craig E. Wheelock^{5,6,7}, Scott T. Weiss¹, Bo L. Chawes^{2,8} & Jessica A. Lasky-Su^{1,8}✉

Respiratory infections are a leading cause of morbidity and mortality in early life, and recurrent infections increase the risk of developing chronic diseases. The maternal environment during pregnancy can impact offspring health, but the factors leading to increased infection proneness have not been well characterized during this period. Steroids have been implicated in respiratory health outcomes and may similarly influence infection susceptibility. Our objective was to describe relationships between maternal steroid levels and offspring infection proneness. Using adjusted Poisson regression models, we evaluated associations between sixteen androgenic and corticosteroid metabolites during pregnancy and offspring respiratory infection incidence across two pre-birth cohorts (N = 774 in VDAART and N = 729 in COPSAC). Steroid metabolites were measured in plasma samples from pregnant mothers across all trimesters of pregnancy by ultrahigh-performance-liquid-chromatography/mass-spectrometry. We conducted further inquiry into associations of steroids with related respiratory outcomes: asthma and lung function spirometry. Higher plasma corticosteroid levels in the third trimester of pregnancy were associated with lower incidence of offspring respiratory infections ($P = 4.45 \times 10^{-7}$ to 0.002) and improved lung function metrics ($P = 0.020$ – 0.036). Elevated maternal androgens were generally associated with increased offspring respiratory infections and worse lung function, with some associations demonstrating nominal significance at $P < 0.05$, but these trends were inconsistent across individual androgens. Increased maternal plasma corticosteroid levels in the late second and third trimesters were associated with lower infections and better lung function in offspring, which may represent a potential avenue for intervention through corticosteroid supplementation in late pregnancy to reduce offspring respiratory infection susceptibility in early life.

Clinical Trial Registry information: VDAART and COPSAC were originally conducted as clinical trials; VDAART: ClinicalTrials.gov identifier NCT00920621; COPSAC: ClinicalTrials.gov identifier NCT00798226.

Abbreviations

BMI	Body mass index
COPSAC	Copenhagen prospective studies on asthma in childhood
EDTA	Ethylenediaminetetraacetic acid
FDR	False discovery rate

¹Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, 181 Longwood Avenue, Boston, MA 02115, USA. ²Copenhagen Prospective Studies on Asthma in Childhood (COPSAC), Herlev and Gentofte Hospital, University of Copenhagen, 2820 Gentofte, Denmark. ³Precision Vaccines Program, Boston Children's Hospital and Harvard Medical School, Boston, MA, USA. ⁴Division of Pediatric Pulmonary Medicine, Department of Pediatrics, Golisano Children's Hospital and University of Rochester Medical Center, Rochester, NY, USA. ⁵Unit of Integrative Metabolomics, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden. ⁶Department of Respiratory Medicine and Allergy, Karolinska University Hospital, Stockholm, Sweden. ⁷Gunma University Initiative for Advanced Research (GIAR), Gunma University, Maebashi, Japan. ⁸These authors jointly supervised this work: Bo L. Chawes and Jessica A. Lasky-Su. ✉email: rejas@channing.harvard.edu

FEV ₁	Forced expiratory volume in 1 s
FVC	Forced vital capacity
GA	Gestational age
GW	Gestational weeks
IRR	Incidence rate ratio
IU	International units
OR	Odds ratio
RPM	Revolutions per minute
SD	Standard deviation
UPLC-MS/MS	Ultrahigh-performance liquid chromatography-tandem mass spectrometry
VDAART	Vitamin D antenatal asthma reduction trial
WHO	World Health Organization

Respiratory infections during childhood are a major cause of morbidity and mortality in early life¹. In 2019 alone, there were an estimated 33 million episodes of acute lower respiratory infections in children between ages 0–5 years globally, resulting in > 100,000 preventable deaths². Further, recurrent infections in early childhood can affect long-term health and increase the risk of developing chronic diseases later in life³. Recurrent respiratory infection incidence has been observed in subsets of highly susceptible children⁴, but this phenomenon is not well understood. Some epidemiological risk factors have been observed, such as daycare attendance, Caesarean section delivery, and maternal smoking in pregnancy⁵, but the underlying biology associated with increased infection proneness remains elusive. Exploring biological factors related to infection proneness could inform novel clinical strategies to prevent infection proneness.

The maternal environment during pregnancy can impact offspring respiratory^{6,7} and immune health⁸, and may similarly influence susceptibility to respiratory infections. Steroid signaling has been specifically implicated in respiratory and developmental outcomes that are closely related to early life respiratory infections^{9,10}. However, studies addressing the connections between endogenous steroid levels in pregnancy and offspring respiratory and immune outcomes have produced conflicting results. While prenatal androgens promote fetal lung branching morphogenesis¹¹, elevated levels may impair offspring immune response to pathogens post-birth¹². Corticosteroids during pregnancy similarly represent a double-edged sword, as they are supplemented in late pregnancy to facilitate lung maturation¹³ but increase the risk of offspring respiratory diseases, such as asthma^{9,14}. Given the intimate links between steroid levels in pregnancy and offspring respiratory and immune outcomes^{15,16}, steroid metabolites may be relevant in childhood respiratory infection proneness and could represent targets with therapeutic potential.

In this exploratory study, we investigated associations between maternal plasma levels of androgen and corticosteroid metabolites and the number of respiratory infections that occurred in offspring. We incorporated two pre-birth cohorts of mother–child pairs from the Vitamin D Antenatal Asthma Reduction Trial (VDAART) and the Copenhagen Prospective Studies on Asthma in Childhood (COPSAC) to span early (10–18 gestational weeks [GW]), middle (22–26 GW), and late (32–38 GW) pregnancy periods. We conducted further inquiry into associations of steroid metabolites with asthma and lung function outcomes to assess potential broader implications of maternal steroids on offspring respiratory health. To our knowledge, ours is the first study to systematically assess the relationships between endogenous androgen and corticosteroid profiles during pregnancy and childhood infection proneness.

Methods

Cohort descriptions. VDAART was a randomized, double-blind, parallel-design study conducted at three study sites across the United States that preferentially recruited asthmatic mothers (ClinicalTrials.gov identifier: NCT00920621)¹⁷. VDAART recruited pregnant women at 10–18 GW and randomized them to Vitamin D supplementation at 4000 IU/day or placebo; all women received 400 IU/day vitamin D supplementation as part of usual pregnancy care. Blood samples were collected in EDTA tubes during enrollment between 10 and 18 GW and again between 32 and 38 GW; plasma was separated through centrifugation at 2000 RPM at 4 °C, and samples were stored at –80 °C until metabolomic profiling. For simplicity, throughout the manuscript, the earlier VDAART pregnancy time point (10–18 GW) will be referred to as “T1”, and the later VDAART pregnancy time point (32–38 GW) will be referred to as “T3”.

COPSAC was a single-center, pre-birth cohort study, as described previously¹⁸. As part of the COPSAC study, mothers were randomized to vitamin D₃ supplementation of 2400 IU/day vs. placebo; all women received 400 IU/day of vitamin D₃ as part of usual pregnancy care. Blood samples were collected in EDTA tubes from mothers during the 22–26 GW enrollment visit; plasma was separated and stored at –80 °C until processing¹⁹. Since this pregnancy time point occurs between the two VDAART time points, the COPSAC pregnancy period of 22–26 GW will be referred to as “T2” for simplicity.

Infection proneness, asthma, and lung function outcomes in offspring. Respiratory infections in VDAART offspring were recorded between the birth of the child and age 6 years using standardized quarterly questionnaires completed by caregivers. In order to match the COPSAC reporting period, infections occurring only between birth and age 3 years were evaluated in this study. Respiratory infections in COPSAC offspring were recorded during scheduled clinical visits every 6 months to 1 year through parent report, beginning at birth until age 3 years. For both cohorts, the infection proneness outcome is a continuous variable representing the count of total reported respiratory infections reported, including upper respiratory infections, lower respiratory infections, colds, and ear infections. Asthma status in VDAART and COPSAC was recorded as positive if

the child was diagnosed by a physician with asthma and/or recurrent wheeze any time between birth and age 6 years. Lung function spirometry measurements were collected in both cohorts at age 6 years, including forced expiratory volume in the first second (FEV₁), forced vital capacity (FVC) and the ratio of FEV₁/FVC; further details of spirometry measurement are available in Supplemental Methods S1.

Steroid metabolite measurement by UPLC-MS/MS. Metabolomic profiles for VDAART and COPSAC plasma samples were generated by Metabolon, Inc. (NC, USA) using ultrahigh-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). A total of 16 steroid metabolites were included in analysis, including 14 androgenic and 2 corticosteroid metabolites. Of these, 15 were Tier 1 assignments by Metabolon, with full structure identification matched to a standard. One androgenic steroid metabolite met putative Tier 2 identification by matching to a literature database, and is denoted with a superscript hash sign in Tables. Details of UPLC-MS/MS methods are available in Supplemental Methods S1.

Metabolite associations with childhood outcomes. Regression analyses estimated associations between steroid metabolites and childhood outcomes at each of the three maternal time points using Poisson (infection proneness), logistic (asthma), or linear (lung function) regression models. Potential confounding factors were selected based on previous research (Fig. 1)⁶. VDAART models were adjusted for maternal age, race, ethnicity, pre-pregnancy BMI, vitamin D level (nanograms of 25 hydroxyvitamin D [25(OH)D] per milliliter of blood), maternal education status, birth order of child, and smoking during pregnancy; an additional variable for gestational days at collection was included for T1 only, as this time point spanned a longer eight-week period. COPSAC was homogenous with respect to race and ethnicity, so models used the same covariates as VDAART models except race and ethnicity. A restricted analysis of non-asthmatics mothers was also conducted for the infection proneness outcome to control for potential confounding by asthma status or asthma medication use (details in Supplemental Methods S1). Analyses were conducted using the stats package in R version 4.0.3²⁰, and incidence rate ratios (IRRs), odds ratios (ORs), linear regression coefficients, and P-values are reported, as appropriate. Correction for false discovery rate (FDR) was performed using the Benjamini–Hochberg Procedure.

Mediation of the relationships between maternal steroids and infection proneness was assessed for the following potential mediators: gestational age at delivery, pregnancy-induced hypertension, gestational diabetes, pre-term labor, delivery mode, maternal asthma, and child daycare attendance during the first 3 years of life. Mediation analyses were performed using the stats²⁰ and mediation²¹ packages in R 4.0.3.

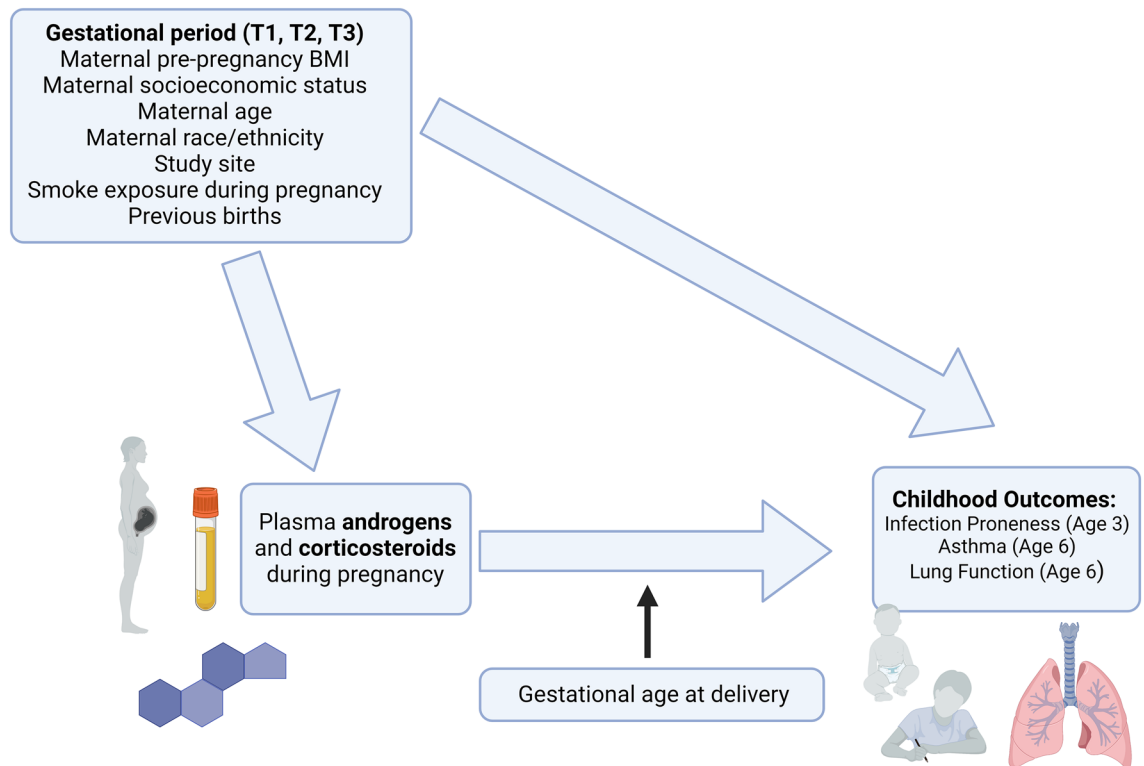


Figure 1. Overview of study design. Relationships between maternal steroids during pregnancy and childhood respiratory outcomes were estimated using two pre-birth cohorts of mother–child pairs. Regression models were adjusted for potential confounding variables, and mediation by gestational age at delivery was evaluated for steroid–outcome associations. Figure created in BioRender.

Ethics approval and consent to participate. All portions of this study were conducted in accordance with the Declaration of Helsinki. Local institutional review boards approved the protocol, and written informed consent was obtained from all individuals. The institutional review boards at each participating VDAART Clinical Center and the Data Coordinating Center at Brigham and Women's Hospital approved protocols of the trial. This project was additionally approved by Brigham and Women's Hospital IRB protocol 2018P000478. The COPSAC study was conducted in accordance with the Declaration of Helsinki and was approved by the Copenhagen Ethics Committee (KF 01-289/96) and the Danish Data Protection Agency (2008-41-1754).

Results

A total of 774 mother–child pairs were included at VDAART T1 and 779 mother–child pairs were included at VDAART T3, which differed slightly in makeup due availability of blood samples for metabolomic profiling; 742 mothers had plasma samples at both time points. VDAART T1 and T3 were similar in all respects other than gestational stage and vitamin D level at time of blood collection ($P < 0.001$; Table 1). COPSAC mothers had lower BMI ($P < 0.001$) and were older ($P < 0.001$) than VDAART mothers; COPSAC offspring were delivered later than VDAART offspring ($P < 0.001$), and vitamin D levels in COPSAC mothers were significantly different from either VDAART time point ($P < 0.001$). COPSAC mothers demonstrated higher socioeconomic indices of income ($P < 0.001$) and education level ($P < 0.001$). There were also fewer asthmatic mothers in the COPSAC cohort (26.6%) than in VDAART (40.6% and 39.9% at T1 and T3, respectively). Children in VDAART experienced an average of 5.2 respiratory infections per year during follow-up, and COPSAC children experienced an average of 5.0 respiratory infections per year during follow-up. A total of 349 (45.1%) of children in VDAART experienced asthma/wheeze by age 6 years while 149 (21.8%) of COPSAC children were diagnosed with asthma by a doctor by age 6 years.

Maternal steroid associations with infection proneness. Associations between maternal corticosteroids and offspring infection proneness differed across pregnancy time periods. At VDAART T1, neither corticosteroid was associated with infection proneness at $P < 0.05$ (Table 2). At COPSAC T2, elevated cortisone was associated with reduced offspring infections ($P = 0.007$), and at VDAART T3, elevated levels of both cortisone and cortisol were associated with reduced offspring infections (P -values: 4.45×10^{-7} and 0.002, respectively); these associations retained significance at $FDR < 0.05$. In the restricted analysis of non-asthmatic mothers, only cortisone at VDAART T3 was associated with lower numbers of infections at $P < 0.05$ (Supplemental Table S1). However, increased cortisol showed a borderline association with reduced infections in the restricted analysis at T2 ($P = 0.071$). The relationships between third trimester corticosteroids and offspring respiratory infections were partially mediated by offspring gestational age at delivery (GA). At VDAART T3, 23.3% of the total effect of cortisol on infection proneness was mediated by GA ($P = 0.002$), and 9.6% of the total effect of cortisone on infection proneness was mediated by GA ($P = 0.002$). Pregnancy-induced hypertension, gestational diabetes, pre-term labor, delivery mode, maternal asthma, and child daycare attendance during the first 3 years of life, but none of these variables significantly mediated these relationships at $P < 0.05$ (data not shown).

Elevated maternal androgens at T1 and T2 were generally associated with higher numbers of offspring infections, but results were inconsistent across individual androgen metabolites (Table 2, Supplemental Table S1). Across time points, 3 of 14 androgenic steroid metabolites at T1 (P -value range: 0.005–0.040), 5 of 13 at T2 (P -value range: 1.06×10^{-6} to 0.004), and 2 of 14 at T3 (P -value range: 0.002–0.016) were associated with increased infection proneness at $P < 0.05$. No androgen metabolites were consistently associated across all 3 time periods at a nominal $P < 0.05$ threshold. Trends in analyses restricted to non-asthmatic mothers were largely consistent in the direction of effect (Supplemental Table S1). Relationships between androgenic steroids and infections were not mediated by gestational age at delivery.

Maternal steroids associations with other outcomes. Associations of steroids with childhood asthma were largely non-significant (Supplemental Table S2) except at VDAART T1. Increased levels of corticosteroids at T1 were associated with higher risk of offspring asthma (cortisone: OR [95% CI] 1.77 [1.10, 2.84], $P = 0.019$; cortisol: OR [95% CI] 1.77 [1.08, 2.9], $P = 0.024$), but these results were not consistent with infection proneness findings. No other associations were observed with asthma at a nominal level of $P < 0.05$.

Cortisone and cortisol at T3 were associated with improved $\%FEV_1$ ($P = 0.036$ and 0.020, respectively), but these associations did not survive correction for multiple testing. The associations between androgens and lung function were inconsistent across individual metabolites and gestational periods, but results are available in Supplemental Tables S3, S4, and S5.

Discussion

Our study represents a novel investigation of associations between endogenous maternal steroid levels during pregnancy and respiratory infection proneness in offspring, which have not previously been explored. Maternal steroid levels can significantly impact fetal respiratory and immune health¹⁰, but the relationships of steroids with early life infections are not as well understood. Existing literature has established connections between maternal steroid levels during pregnancy with asthma²² and immune⁸ outcomes separately, but respiratory infection proneness represents an overlap in these respiratory and immune domains that remains elusive. Moreover, prior studies have generated conflicting findings regarding the beneficial and harmful effects of these steroids^{9,11–14}, leaving their impact on childhood respiratory infections unclear. Our results suggest that higher corticosteroid levels in late pregnancy were beneficial in reduced offspring respiratory infections. This study represents an initial step towards understanding the contributing factors that influence childhood infection proneness, which may hold clinical relevance in efforts towards reducing the burden of early life respiratory infections.

Cohort characteristics	VDAART		COPSAC
	T1: 10–18 GW (n = 774)	T3: 32–38 GW (n = 779)	T2: 22–26 GW (n = 729)
Maternal variables			
Pre-pregnancy BMI kg/m ² , mean (SD)	28.4 (7.7)	28.3 (7.7)	24.0 (5.9)
Age at enrollment yrs, mean (SD)	27.3 (5.5)	27.4 (5.5)	31.9 (4.5)
Gestational days at blood collection, mean (SD)	99.4 (19.2)	237.9 (11.6)	170.2 (6.2)
Race, n (%)			NA
Black	336 (43.4)	340 (43.6)	–
White	315 (40.7)	314 (40.3)	–
Other	123 (15.9)	125 (16.0)	–
Ethnicity, n (%)			NA
Hispanic or Latino	213 (27.5)	214 (27.5)	–
Not Hispanic or Latino	561 (72.5)	565 (72.5)	–
Study site, n (%)			NA
Boston	234 (30.2)	226 (29.0)	–
San Diego	259 (33.5)	269 (34.5)	–
St. Louis	281 (36.4)	284 (36.5)	–
Income category, n (%) ¹			
Low	331 (56.2)	329 (55.9)	65 (9.5)
Medium	171 (29.0)	172 (29.2)	364 (53.0)
High	87 (14.8)	88 (14.9)	258 (37.6)
Education category, n (%) ²			
Low	474 (61.2)	482 (61.9)	54 (7.8)
Medium	191 (24.7)	183 (23.5)	444 (64.4)
High	109 (14.1)	114 (14.6)	191 (27.7)
Vitamin D level at time of collection (ng/mL), mean (SD)	22.9 (10.3)	33.0 (14.6)	30.1 (10.2)
Mothers in 4000 IU/day treatment group, n (%)	387 (50.0)	391 (50.2)	293 (51.0)
Mothers in 2.4 g/day fish oil group, n (%)	–	–	340 (49.7)
Maternal Asthma Diagnosis, n (%)	314 (40.6)	311 (39.9)	192 (26.6)
Smoking during pregnancy, n (%) ³	117 (15.1)	120 (15.4)	58 (8.3)
C-section delivery, n (%)	228 (29.5)	233 (29.3)	161 (21.9)
Child variables			
Gestational age at delivery weeks, mean (SD)	39.0 (2.0)	39.2 (1.6)	39.8 (1.9)
Number of older siblings, mean (SD)	0.9 (1.1)	0.9 (1.1)	0.9 (1.0)
Daycare attendance, n (%)	360 (46.5)	353 (45.3)	676 (93.0)
Number of respiratory infections, 0–3 years mean (SD)	15.6 (8.8)	15.6 (8.9)	14.9 (9.3)
Respiratory infections/year, mean (SD)	5.2 (2.9)	5.2 (3.0)	5.0 (3.1)
Child asthma diagnosis, n (%)	349 (45.1%)	344 (44.6%)	149 (21.8%)

Table 1. VDAART and COPSAC cohort characteristics by gestational period. *Yrs* years, *SD* standard deviation, *BMI* body mass index. ¹Income in VDAART was categorized based on reported household yearly income in USD: Low (< \$50,000/year), Medium (\$50,000–\$100,000/year), or High (> \$100,000/year); Income in COPSAC was categorized based on reported household yearly income in Euros: Low (< €50,000/year), Medium (€50,000–€110,000/year), or High (> €110,000/year). ²Maternal educational status was categorized based on maximum education level reported and categorized in VDAART as: Low (primary school, secondary school, or some college/junior college), Medium (technical/trade school or bachelor's degree), or High (graduate degree); in COPSAC, education levels were categorized as: Low (primary school, secondary school, or college graduate), Medium (trade school or bachelor's degree), or High (Master's or other graduate degree). ³In VDAART, smoking or exposure to smoking in the home was recorded during enrollment questionnaires; in COPSAC, maternal smoking during pregnancy was recorded by COPSAC physicians.

Increased levels of corticosteroids in the third trimester of pregnancy were robustly associated with reduced offspring respiratory infections in this study. Corticosteroids have been well described for their clinical utility in respiratory health²³, and our results support a role for corticosteroids in alleviating the burden of early life respiratory infections. Although some associations between corticosteroids and offspring infections did not meet nominal significance in the restricted analysis of non-asthmatic mothers, this may be a consequence of reduced statistical power, as the directions of effect were consistent with overall analyses. Steroid supplementation in pregnancy promotes fetal lung maturation, particularly in pregnancies at high risk of preterm labor¹³, and our formal mediation findings indicate gestational age at delivery was a significant mediator of the relationship between third trimester corticosteroids and infections, which further emphasizes the role of corticosteroids close to term.

Steroid metabolite	VDAART T1 (N = 774) 10–18 GW			COPSAC T2 (N = 729) 22–26 GW			VDAART T3 (N = 779) 32–38 GW		
	IRR (95% CI)	P-value	FDR	IRR (95% CI)	P-value	FDR	IRR (95% CI)	P-value	FDR
16 α -hydroxy DHEA 3-sulfate	1.01 (0.97,1.05)	0.561	0.641	0.97 (0.92,1.01)	0.157	0.236	1.04 (0.99,1.08)	0.091	0.290
Androstenediol (3beta,17beta) monosulfate (1)	1.01 (0.97,1.06)	0.530	0.641	<i>1.04 (1,1.08)</i>	<i>0.081</i>	<i>0.136</i>	1.01 (0.98,1.05)	0.570	0.684
Androstenediol (3beta,17beta) monosulfate (2)	1.02 (0.99,1.06)	0.219	0.396	1.05 (1.02,1.08)	0.002	0.007	0.98 (0.95,1.01)	0.181	0.364
Androstenediol (3alpha, 17alpha) monosulfate (3)	1.04 (1.01,1.08)	0.021	0.171	<i>1.04 (1,1.08)</i>	<i>0.064</i>	<i>0.136</i>	1.05 (1.01,1.08)	0.016	0.062
Androstenediol (3alpha, 17alpha) monosulfate (2)	1.05 (1.01,1.09)	0.005	0.086	1.01 (0.98,1.04)	0.361	0.451	1.06 (1.02,1.09)	0.002	0.013
Androstenediol (3beta,17beta) disulfate (2)	1.05 (1,1.09)	0.040	0.212	<i>1.05 (1,1.11)</i>	<i>0.076</i>	<i>0.136</i>	1.03 (0.98,1.07)	0.250	0.364
Androstenediol (3beta,17beta) disulfate (1)	1.03 (0.98,1.07)	0.223	0.396	1.07 (1.03,1.12)	0.001	0.005	1.02 (0.98,1.06)	0.373	0.498
5alpha-androstan-3beta,17beta-diol disulfate	1 (0.96,1.04)	0.995	0.995	1.08 (1.04,1.13)	1.21 × 10⁻⁴	0.001	1.02 (0.99,1.06)	0.210	0.364
5alpha-androstan-3beta,17alpha-diol disulfate	1.02 (1,1.05)	0.053	0.213	1 (0.98,1.03)	0.814	0.854	1 (0.97,1.03)	0.971	0.971
5alpha-androstan-3alpha,17alpha-diol monosulfate	1.02 (0.99,1.05)	0.193	0.396	0.9 (0.86,0.94)	1.06 × 10⁻⁶	1.59 × 10⁻⁵	1.01 (0.98,1.04)	0.606	0.684
Epiandrosterone sulfate	1.01 (0.98,1.05)	0.461	0.614	1.06 (1.02,1.1)	0.004	0.011	1.02 (0.99,1.06)	0.249	0.364
Andro steroid monosulfate C19H28O6S (1)*	1.01 (0.98,1.04)	0.601	0.641	1 (0.95,1.04)	0.854	0.854	0.99 (0.95,1.03)	0.641	0.684
Dehydroepiandrosterone sulfate (DHEA-S)	1.03 (0.99,1.07)	0.111	0.301	1.01 (0.97,1.06)	0.557	0.643	1.03 (0.99,1.06)	0.200	0.364
Androsterone sulfate	1.02 (0.98,1.06)	0.294	0.471	NA	NA	NA	1.03 (0.99,1.06)	0.128	0.342
Cortisone	1.05 (0.99,1.11)	0.113	0.301	0.9 (0.85,0.97)	0.002	0.007	0.9 (0.86,0.94)	4.45 × 10⁻⁷	7.11 × 10⁻⁶
Cortisol	1.02 (0.97,1.09)	0.414	0.602	0.97 (0.91,1.03)	0.254	0.346	0.93 (0.89,0.97)	0.002	0.013

Table 2. Associations between maternal steroid metabolites and offspring infections. Incidence rate ratios (IRRs), P-values, and FDR-corrected P-values of associations are shown at T1, T2, and T3 time points. Associations at $P < 0.05$ are bolded. Associations at $0.05 < P < 0.1$ are italicized for COPSAC.

As corticosteroids are utilized in high risk pregnancies to promote fetal lung maturation¹³, we hypothesize that depleted maternal corticosteroid may be associated with offspring infection incidence through gestational age at delivery, which is closely related to lung maturation²⁴. Elevated corticosteroids at T3 were also associated with better offspring lung function and showed no association with asthma. Overall, our results suggest that increased corticosteroids in the third trimester could produce multiple respiratory-related benefits. However, the effects of corticosteroid supplementation in pregnancy are not innocuous²⁵, so it is important to consider risk-versus-benefit in future feasibility analyses. Additionally, increased levels of corticosteroids in the first and early second trimesters were associated with increased risk of offspring asthma. This relationship may be specific to this gestational stage, as this was not observed at T2 or T3, but stresses that other affected outcomes should be considered, as corticosteroids can have many implications for lung-related health^{9,13,14}. Generally, androgens were associated with higher offspring infections and poorer lung function in this study, but comparison across multiple time periods limited interpretation due to lack of consistency. Elevated androgen levels in pregnant mothers have been linked to increased risk of pregnancy complications that can undermine fetal health^{12,26}. Overall, maternal androgens appear to be associated with offspring infections, but much remains to be learned. A targeted analysis capturing a broader range of androgen metabolites may help clarify these relationships.

Our study features several strengths. Both VDAART and COPSAC collected comprehensive information on respiratory infections, asthma, and lung function metrics during follow-up, enabling comparison of multiple, related childhood outcomes²⁷. The children included in these cohorts experienced a number of infections consistent with WHO observations for infants²⁸, suggesting our results may be generalizable to other populations. However, VDAART and COPSAC suffered from low response to questionnaire data regarding maternal use of inhaled and oral corticosteroid medication during pregnancy, so this variable could not be explicitly included in our analyses. Maternal asthma status served as a proxy to mitigate potential confounding, and restricted analysis of non-asthmatic mothers generally reflected the same trends. Additionally, VDAART infection data was collected through non-verified caregiver reporting. While the total number of all infection types was similar per year between VDAART and COPSAC, incidence of infection subtypes (e.g., LRI, URI) differed greatly across racial and ethnic divides in VDAART. This limited our ability to investigate the role of maternal steroids in specific infection types that could be highly relevant to long-term respiratory health. Finally, follow-up studies using targeted, quantified measurement are necessary to validate the results from this study which were assessed using global, untargeted metabolomic data.

Conclusions

Overall, this is the first study to characterize associations between maternal androgen and corticosteroid metabolites with offspring infection proneness, which represents an urgent public health issue. Clinical intervention could combat the burden of respiratory infection proneness in early life and reduce the high number of preventable deaths each year attributed to respiratory infections. Overall, our findings raise the possibility that corticosteroid supplementation in the third trimester of pregnancy may reduce offspring infection incidence

with potential benefits for lung function. Future studies will address the feasibility of prenatal corticosteroid administration to reduce childhood respiratory infection proneness.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Received: 7 March 2023; Accepted: 6 June 2023

Published online: 28 June 2023

References

- Zar, H. J. & Ferkol, T. W. The global burden of respiratory disease-impact on child health. *Pediatr. Pulmonol.* **49**(5), 430–434 (2014).
- Li, Y. *et al.* Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in children younger than 5 years in 2019: A systematic analysis. *Lancet* **399**(10340), 2047–2064 (2022).
- Celedon, J. C. *et al.* Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood. *J. Allergy Clin. Immunol.* **120**(1), 144–149 (2007).
- Pichichero, M. E., Chapman, T. J. & Bajorski, P. Pneumonia, sinusitis, influenza and other respiratory illnesses in acute otitis media-prone children. *Pediatr. Infect. Dis. J.* **40**(11), 975–980 (2021).
- Vissing, N. H., Chawes, B. L., Rasmussen, M. A. & Bisgaard, H. Epidemiology and risk factors of infection in early childhood. *Pediatrics* **141**(6), 1 (2018).
- Huang, M. *et al.* Maternal metabolome in pregnancy and childhood asthma or recurrent wheeze in the vitamin D antenatal asthma reduction trial. *Metabolites* **11**(2), 1 (2021).
- Gatford, K. L., Wooldridge, A. L., Kind, K. L., Bischof, R. & Clifton, V. L. Pre-birth origins of allergy and asthma. *J. Reprod. Immunol.* **123**, 88–93 (2017).
- Coe, C. L. & Lubach, G. R. Prenatal origins of individual variation in behavior and immunity. *Neurosci. Biobehav. Rev.* **29**(1), 39–49 (2005).
- Seckl, J. R. Prenatal glucocorticoids and long-term programming. *Eur. J. Endocrinol.* **151**(Suppl 3), U49–62 (2004).
- Solano, M. E., Holmes, M. C., Mittelstadt, P. R., Chapman, K. E. & Tolosa, E. Antenatal endogenous and exogenous glucocorticoids and their impact on immune ontogeny and long-term immunity. *Semin. Immunopathol.* **38**(6), 739–763 (2016).
- Kimura, Y. *et al.* Expression of androgen receptor and 5alpha-reductase types 1 and 2 in early gestation fetal lung: A possible correlation with branching morphogenesis. *Clin. Sci. (Lond)* **105**(6), 709–713 (2003).
- Muller, W. *et al.* Prenatal androgen exposure modulates cellular and humoral immune function of black-headed gull chicks. *Proc. Biol. Sci.* **272**(1575), 1971–1977 (2005).
- Roberts, D., & Dalziel, S. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database Syst. Rev.* **3**, CD004454 (2006).
- Pole, J. D., Mustard, C. A., To, T., Beyene, J. & Allen, A. C. Antenatal steroid therapy for fetal lung maturation: Is there an association with childhood asthma?. *J. Asthma* **46**(1), 47–52 (2009).
- Ballard, P. L. Hormonal influences during fetal lung development. *Ciba Found. Symp.* **78**, 251–274 (1980).
- Venihaki, M., Carrigan, A., Dikkes, P. & Majzoub, J. A. Circadian rise in maternal glucocorticoid prevents pulmonary dysplasia in fetal mice with adrenal insufficiency. *Proc. Natl. Acad. Sci. U S A* **97**(13), 7336–7341 (2000).
- Litonjua, A. A. *et al.* The Vitamin D Antenatal Asthma Reduction Trial (VDAART): rationale, design, and methods of a randomized, controlled trial of vitamin D supplementation in pregnancy for the primary prevention of asthma and allergies in children. *Contemp. Clin. Trials* **38**(1), 37–50 (2014).
- Bisgaard, H. The Copenhagen prospective study on asthma in childhood (COPSAC): Design, rationale, and baseline data from a longitudinal birth cohort study. *Ann. Allergy Asthma Immunol.* **93**(4), 381–389 (2004).
- Rago, D. *et al.* Fish-oil supplementation in pregnancy, child metabolomics and asthma risk. *EBioMedicine* **46**, 399–410 (2019).
- Team RDC: R: A language and environment for statistical computing. In Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <http://www.R-project.org>; 2010.
- Tingley, D., Yamamoto, T., Hirose, K., Keele, L. & Imai, K. Mediation: R package for causal mediation analysis. *J. Stat. Softw.* **59**(5), 1–38 (2014).
- Blighe, K. *et al.* Vitamin D prenatal programming of childhood metabolomics profiles at age 3 y. *Am. J. Clin. Nutr.* **106**(4), 1092–1099 (2017).
- Sethi, G. R. & Singhal, K. K. Pulmonary diseases and corticosteroids. *Indian J. Pediatr.* **75**(10), 1045–1056 (2008).
- Hussain, M. *et al.* Wnt/beta-catenin signaling links embryonic lung development and asthmatic airway remodeling. *Biochim. Biophys. Acta Mol. Basis Dis.* **1863**(12), 3226–3242 (2017).
- Bandoli, G., Palmsten, K., Forbess Smith, C. J. & Chambers, C. D. A review of systemic corticosteroid use in pregnancy and the risk of select pregnancy and birth outcomes. *Rheum Dis. Clin. N. Am.* **43**(3), 489–502 (2017).
- Jirecek, S. *et al.* Elevated serum concentrations of androgens in women with pregnancy-induced hypertension. *Wien Klin Wochenschr.* **115**(5–6), 162–166 (2003).
- Solano, M. E. & Arck, P. C. Steroids pregnancy and fetal development. *Front. Immunol.* **10**, 3017 (2019).
- Rudan, I., Tomaskovic, L., Boschi-Pinto, C. & Campbell, H. Group WHOCHER: Global estimate of the incidence of clinical pneumonia among children under five years of age. *Bull. World Health Organ.* **82**(12), 895–903 (2004).

Acknowledgements

The authors thank the participants and study staff of the VDAART and COPSAC studies.

Author contributions

N.P. conducted all analyses and prepared the original draft of the manuscript. B.L.C. and J.A.L.S. conceptualized the study, acquired funding for the study, and were major contributors in writing the manuscript. H.B., S.T.W., and A.A.L. acquired funding for VDAART and COPSAC trials and contributed to manuscript revising and editing. R.S.K., S.H.C., P.K., Y.C., K.M.M., S.B., K.B., M.K., O.L., and C.E.W. contributed essential feedback to revise and edit the manuscript. All authors read and approved the final manuscript.

Funding

The Vitamin D Antenatal Asthma Reduction Trial (VDAART) was funded by U01HL091528 from the National Heart, Lung, and Blood Institute (NIH/NHLBI) awarded to STW and AAL. Effort by JALS and metabolomics

measurement for both cohorts was supported by R01HL123915 and R01HL141826 from NIH/NHLBI. OL and JALS are also supported in part by NIH/NIAID grant 1U19AI168643-01 under the Immune Development in Early Life (IDEAL). Effort for PK was supported by K99HL159234 from NIH/NHLBI. NP is supported by NIHT32HL007427 from NIH/NHLBI. CEW was supported by the Swedish Heart Lung Foundation (HLF 20210519, 20200693). BLC was supported by European Research Council (ERC) European Union's Horizon 2020 research and innovation programme: Grant Agreement No. 946228. The external funders played no role in the design or conduct of the study, collection, management, analysis, or interpretation of the data, preparation, review, approval of, or decision to submit the manuscript.

Competing interests

Dr. Scott Weiss receives royalty payments from UpToDate and is on the Scientific Board of Histolix. The other authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-023-36535-0>.

Correspondence and requests for materials should be addressed to J.A.L.-S.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2023