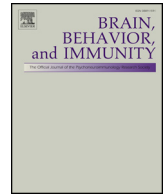




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Sex-specific association between prenatal life stress exposure and infant pro-inflammatory cytokine levels during acute respiratory infection

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ABSTRACT

Background: Prenatal life stress exposure is linked to dysregulated immune function and chronic inflammatory disease in offspring, but we know little about its effects on infant immune response during viral infection.

Method: To address this issue, we examined associations between prenatal life stress exposure and infant upper-airway inflammatory markers during acute respiratory infection (ARI) using data from a prospective, population-based birth-cohort study ($N = 180$). Infant inflammation was measured as a continuous latent factor within a structural equation modeling framework using nasal wash concentrations of interleukin-1 β , interleukin-6, and tumor necrosis factor- α . We hypothesized that infants exposed to prenatal life stress would have greater levels of nasal inflammation during ARI and increased risk for ARI-related morbidity in early childhood.

Results: Our findings contradicted these hypotheses and provided evidence of sexually dimorphic effects of prenatal stress exposure on infant immune functioning during ARI. Among boys, but not girls, prenatal stress was negatively associated with nasal inflammation and indirectly associated with both lower ARI severity and reduced likelihood of subsequent ARI-related hospitalization in the 2nd and 3rd years of life.

Conclusion: These data suggest that prenatal stress exposure may be beneficial for infant boys in the context of respiratory viral infections; however, it will be critical to determine if these benefits are offset by increased risk for chronic inflammatory diseases in later childhood. As the participants in this cohort are being followed longitudinally through age 8, we will be able to evaluate long-term health outcomes in future studies.

1. Background

Burgeoning evidence suggests that exposure to prenatal adversity alters the development of the fetal immune system (Andersson et al., 2016b; Cao-Lei et al., 2016; Howerton and Bale, 2012; Mattes et al., 2009; Peters et al., 2012; Veru et al., 2015; Wright et al., 2010) and confers increased risk for chronic inflammatory diseases (e.g., Andersson et al., 2016a). As these effects on immune function can persist into adulthood (Entringer et al., 2008; Plant et al., 2016), prenatal adversity may lead to lifespan health problems in offspring.

Despite their ubiquity and lasting health implications (Nair et al., 2010), little research has evaluated the effect of prenatal adversity on immune functioning during infant respiratory infections. Viral respiratory infections leading to bronchiolitis substantially increase risk

for pediatric asthma (Feldman et al., 2014), which is among the most burdensome of all pediatric diseases (Asher and Pearce, 2014). Investigators from the Urban Environment and Childhood Asthma (URECA) cohort evaluated the effect of prenatal stress on offspring immune response to respiratory viruses using experimental methods: stimulating cord blood mononuclear cells (Wright et al., 2010) and peripheral blood mononuclear cells (Ramratnam et al., 2017) *ex vivo* with respiratory syncytial virus (RSV) and human rhinovirus (HRV). They found no evidence that prenatal adversity altered immune response to these stimuli. However, we know of no studies that have evaluated the effects of prenatal stress on infant immune response during naturally occurring respiratory infections.

To address this issue, we examined associations between prenatal life stress exposure and markers of infant nasal inflammation during

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acute respiratory infection (ARI) in a prospective, population-based birth cohort study specifically designed to characterize infant respiratory infections. We hypothesized prenatal life stress would be associated with greater concentrations of pro-inflammatory cytokines and increased risk for markers of ARI illness severity. As child biological sex often moderates the effect of prenatal adversity on child outcomes (Sutherland and Brunwasser, 2018), we estimated separate effects for boys and girls.

2. Method

2.1. Participants

Data were drawn from the Infants Susceptibility to Pulmonary Infections and Asthma following RSV Exposure (INSPIRE) study. Study procedures, described in detail elsewhere (Larkin et al., 2015), were approved by Vanderbilt University's Institutional Review Board. All participating families provided informed consent. The study enrolled uniparous, term, and otherwise healthy infants in Middle Tennessee ($N = 1951$). Children with ARI in the first year of life completed evaluations with research team nurses and provided nasal wash samples. Families completed annual follow-up assessments reporting on prior-year health outcomes. The present study included 180 infants who: (a) had an ARI detected during the first year of life, (b) provided a nasal wash specimen that tested positive for either RSV or HRV by reverse transcription polymerase chain reaction, and (c) had a caregiver who reported whether major life stressors occurred during the target pregnancy. Participant characteristics are provided in Supplemental Table 1.

2.2. Measures

2.2.1. Life stress exposure

Several major life stressors affecting the mother during the target pregnancy were assessed at 1-year follow-up: separation/divorce, death of a loved one, high stress job, financial troubles, unemployment, partner unemployment, or any other (participant-provided) stressors. As few families ($n = 14$; 7.8%) reported multiple stressors, we coded prenatal life stress as a binary exposure (0 = unexposed; 1 = exposed).

2.2.2. Nasal inflammation

ARI inflammation was measured using nasal wash concentrations of three pro-inflammatory cytokines: interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF)- α . These cytokines have well-established roles in upregulating inflammatory activity in response to viral invaders (Moldoveanu et al., 2008) and mediating the effects of stress on physical health (Slavich and Irwin, 2014). During the winter viral season of the child's first year of life, we conducted surveillance, including bi-weekly phone assessments, to identify children with likely ARI. In-person visits were scheduled when parents reported any major ARI symptom (i.e., wheezing, difficulty breathing, or a positive RSV test), or multiple minor symptoms (i.e., congestion/runny nose, cough, ear infection, or hoarse cry). Nasal wash samples were collected using 5 mL of sterile saline. Samples were stored at 4 °C for transport and then snap frozen at -80 °C. Cytokine concentrations (quantified using fluorescence intensity) were evaluated in samples testing positive for either RSV or HRV in two replicates with Luminex xMap multi-analyte bead assays (Turi et al., 2018; Won et al., 2012).

2.2.3. ARI-related illness severity

We created two outcome variables as proxies for ARI-related illness severity. The first was an ordinal outcome coding whether the child had an acute medical visit for the target ARI: 0 = no visit ($n = 55$); 1 = outpatient ($n = 99$); 2 = emergency department/hospitalization ($n = 26$). Additionally, we derived a binary outcome variable coding whether children had any subsequent ARI-related hospitalizations in

the second or third years of life ($n = 67$) based on parent report at the Year 2 and Year 3 follow-up visits.

2.2.4. Covariates

We adjusted for the following factors: (1) maternal marital status (married vs. unmarried); (2) prenatal maternal antidepressant use (none vs. any); (3) target child birth method (vaginal vs. cesarean); (4) insurance type (Medicaid vs. private); (5) maternal asthma; (6) infant age at target ARI; and (7) number of days between parent-reported onset of ARI symptoms and nasal wash sample collection.

2.3. Data analysis

We tested hypotheses using multiple-group structural equation modeling (SEM) with lavaan version 0.6–2.1261 (Rosseel, 2012) in R version 3.4.1. Nasal inflammation was estimated as a continuous latent factor, with nasal wash concentrations of IL-1 β , IL-6, and TNF- α treated as measurable markers of this unobserved construct. We standardized the latent factor ($M = 0$; $SD = 1$) so that the coefficient for the regression of latent inflammation factor on prenatal stress (\hat{d}) represented the standardized mean difference between exposed vs. unexposed infants.

First, we tested our latent variable formulation, including whether the factor structure was invariant (consistent) across child sexes. We specified four competing invariance models to determine whether allowing factor loadings, indicator intercepts, and indicator disturbances to vary for boys and girls improved model fit (Vandenberg and Lance, 2000). We compared these models using chi-squared likelihood ratio tests (χ^2_{LRT}), with significant tests indicating inconsistency in the factor structure across sexes.

We next specified three models. The primary model (Model 1; Supplemental Fig. 1) evaluated the adjusted effect of prenatal stress on the latent nasal inflammation variable: $Stress^a \rightarrow Inflammation$. Model 2 evaluated whether prenatal stress exposure was indirectly associated with having a medical visit for the target ARI via the latent infant inflammation factor: $Stress^a \rightarrow Inflammation^b \rightarrow Medical\ Visit$. Finally, Model 3 evaluated whether prenatal stress was indirectly associated with subsequent ARI hospitalization in the 2nd and 3rd years of life via the latent inflammation factor: $Stress^a \rightarrow Inflammation^b \rightarrow Hospitalization$.

Parameters were estimated using maximum likelihood when all outcomes were continuous (Model 1) and diagonally-weighted least squares in models with discrete outcomes (models 2 and 3). Model adequacy was assessed using chi-square model fit tests (χ^2_{FIT}), with significant tests indicating model misspecification. Confidence intervals for indirect effects were calculated using the Distribution of the Product method (Tofighi and MacKinnon, 2011). Because we estimated associations of interest separately for girls and boys, we set $\alpha = 0.025$. We provide detailed modeling information (Supplemental Appendix) and full model programming code (Supplemental Code) as online supplements. Descriptive statistics necessary to replicate our statistical models (e.g., observed covariance matrixes, fit indices, weight matrixes, and residual covariance matrixes) are in a data file linked to this manuscript.

3. Results

3.1. Infant inflammation measured as a latent factor

We applied Box-Cox power transformations to all three cytokines (Supplemental Table 2). There was no evidence that separate factor loadings or intercepts were required for girls and boys when estimating the latent inflammation factor model. However, allowing separate indicator disturbances significantly improved model fit: $\chi^2_{LRT}(3) = 11.56$, $p = .01$. Therefore, our final latent factor model held factor loadings and intercepts equal for girls and boys, but estimated separate

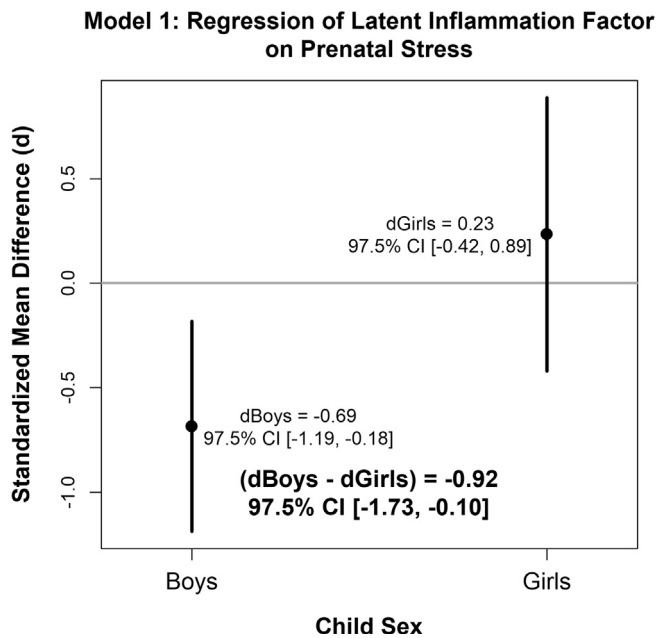


Fig. 1. Sex Differences in the Effect of Prenatal Stress Exposure on Nasal Inflammation during ARI. Prenatal stress exposure was negatively associated with nasal inflammation for infant boys, adjusting for all covariates listed in Section 2.2.4, whereas the association was not significant for girls.

disturbances, yielding no evidence of misspecification: $\chi^2_{Fit}(4) = 3.09$, $p = .54$. The latent inflammation factor accounted for the majority of variance in the cytokine measures: McDonald's $\omega_{Girls} = 0.84$, 95% CI [0.78, 0.90]; $\omega_{Boys} = 0.83$, 95% CI [0.78, 0.89].

3.2. Model 1: Primary model

Model 1 regressed the latent inflammation factor on prenatal stress and all covariates: $\chi^2_{Fit}(43) = 44.44$, $p = .41$. There was a significant negative association between prenatal stress exposure and inflammatory activity for boys ($\hat{d}_{Boys} = -0.69$, 97.5% CI [-1.19, -0.18]), but no significant association for girls ($\hat{d}_{Girls} = 0.23$, 97.5% CI [-0.42, 0.89]). The effect of prenatal stress exposure differed significantly for boys versus girls: $\hat{d}_{Boys} - \hat{d}_{Girls} = -0.92$, 97.5% CI [-1.73, -0.10] (Fig. 1). Supplemental Table 2 provides raw descriptive statistics for each cytokine by prenatal stress exposure and child biological sex: stress-exposed boys tended to have lower levels of all three pro-inflammatory cytokines.

3.3. Model 2: Indirect effect on ARI-related medical visits

In Model 2 ($\chi^2_{Fit}[56] = 35.00$, $p = .99$), the association between the latent inflammation factor and the medical-provider visit outcome variable (proxy for ARI severity) was positive and significant: $\hat{b} = 0.22$, 97.5% CI: [0.06, 0.39]. Prenatal stress exposure was associated with decreased probability of having a medical visit for the target ARI indirectly via its negative association with the latent inflammation factor for boys ($\hat{a} \times \hat{b} = -0.15$, 97.5% CI [-0.32, -0.02]) but not girls ($\hat{a} \times \hat{b} = 0.01$, 97.5% CI [-0.05, 0.11]) (Fig. 2a).

3.4. Model 3: Indirect association with subsequent ARI hospitalizations

In Model 3 ($\chi^2_{Fit}[56] = 32.96$, $p = .99$), the association between the latent inflammation factor and subsequent ARI-related hospitalization in the 2nd-3rd years of life was positive and significant: $\hat{b} = 0.20$, 97.5% CI: [0.02, 0.39]. Boys exposed to prenatal stress had a lower likelihood of ARI-related hospitalization in the 2nd and 3rd years of life relative to unexposed boys with the association being transmitted

indirectly via the latent inflammation factor: $\hat{a} \times \hat{b} = -0.15$, 97.5% CI [-0.35, -0.004]. For girls, the indirect association was not significant: $\hat{a} \times \hat{b} = 0.01$, 97.5% CI [-0.07, 0.13] (Fig. 2b).

4. Discussion

The present data yielded three main findings, which were contrary to hypotheses but consistent with research showing that prenatal adversity exposure may have implications for immune development and that these effects are sex-dependent. First, infant boys exposed to prenatal stress had lower levels of nasal inflammatory activity during ARI relative to their unexposed counterparts. Second, among boys, prenatal stress exposure was indirectly associated with decreased probability of (a) medical visits for the target ARI and (b) subsequent ARI-related hospitalizations through its effect on infant ARI nasal inflammation. Third, among girls, prenatal stress exposure was not associated with any outcomes. In sum, these data suggest that prenatal stress exposure may have a beneficial effect for infant boys during ARI by modifying the immune response to viral infection.

Evolutionary theory may help reconcile these findings with prior research linking prenatal adversity to exaggerated pro-inflammatory responses in healthy samples (e.g., Wright et al., 2010). Offspring of mothers experiencing prenatal adversity are more likely to be born into dangerous postnatal environments where there is high risk for injury and infection (Slavich and Irwin, 2014; Viltart and Vanbesien-Mailliot, 2007). Having immune defenses that are primed to fight infection may increase chances of survival, which may explain why prior studies found elevated inflammatory markers among stress-exposed offspring during healthy states (Andersson et al., 2016a; Wright et al., 2010). If stress-exposed boys do indeed have primed immune systems, this may help them fight viruses more efficiently. This may explain why stress-exposed boys exhibited less nasal inflammation in this study by the time samples were collected (i.e., $M = 2.86$, $SD = 1.39$ days after onset of ARI symptoms). It is not clear, however, why we would not see this same effect in girls, though the female fetus is generally more adaptive to intrauterine adversity and less susceptible to heightened inflammation (Bale, 2011; Clifton, 2010; Sandman et al., 2013). Importantly, the potential benefits of a hypersensitive pro-inflammatory response in fighting ARI may come with a substantial long-term tradeoff in the form of chronic inflammatory diseases (Flanigan et al., 2018; Viltart and Vanbesien-Mailliot, 2007). Testing this theory would require measuring infant immune functioning both during and outside of illness states, as well as long-term inflammatory diseases outcomes. This will be possible in future studies with INSPIRE, as children will complete annual assessments of immune-mediated diseases and provide a variety of biomarker samples through age eight.

Our findings were inconsistent with those from the URECA cohort, which found no evidence of prenatal stress-related differences in HRV or RSV immune response (Ramratnam et al., 2017; Wright et al., 2010). This may be attributable to significant methodological differences. First, the URECA study experimentally stimulated samples from healthy children *ex vivo* with RSV and HRV, whereas we evaluated unstimulated samples from children with naturally occurring ARI. Second, we measured immune functioning using nasal wash assays, whereas URECA used blood samples. It may be that nasal wash and blood samples capture different aspects of immune functioning (e.g., local vs. systemic inflammation). Finally, the URECA investigators did not report evaluating a stress \times sex interaction, though they did test several other interactions. It is possible that a sex-specific effect was present but not tested.

Our findings reinforce recommendations to evaluate biological sex as a potential moderator in adversity-based prenatal programming (Bale, 2011; Clifton, 2010; Sandman et al., 2013). Two recent studies reported sex-dependent associations with prenatal stress exposure conferring risk for early childhood wheeze (Rosa et al., 2016) and

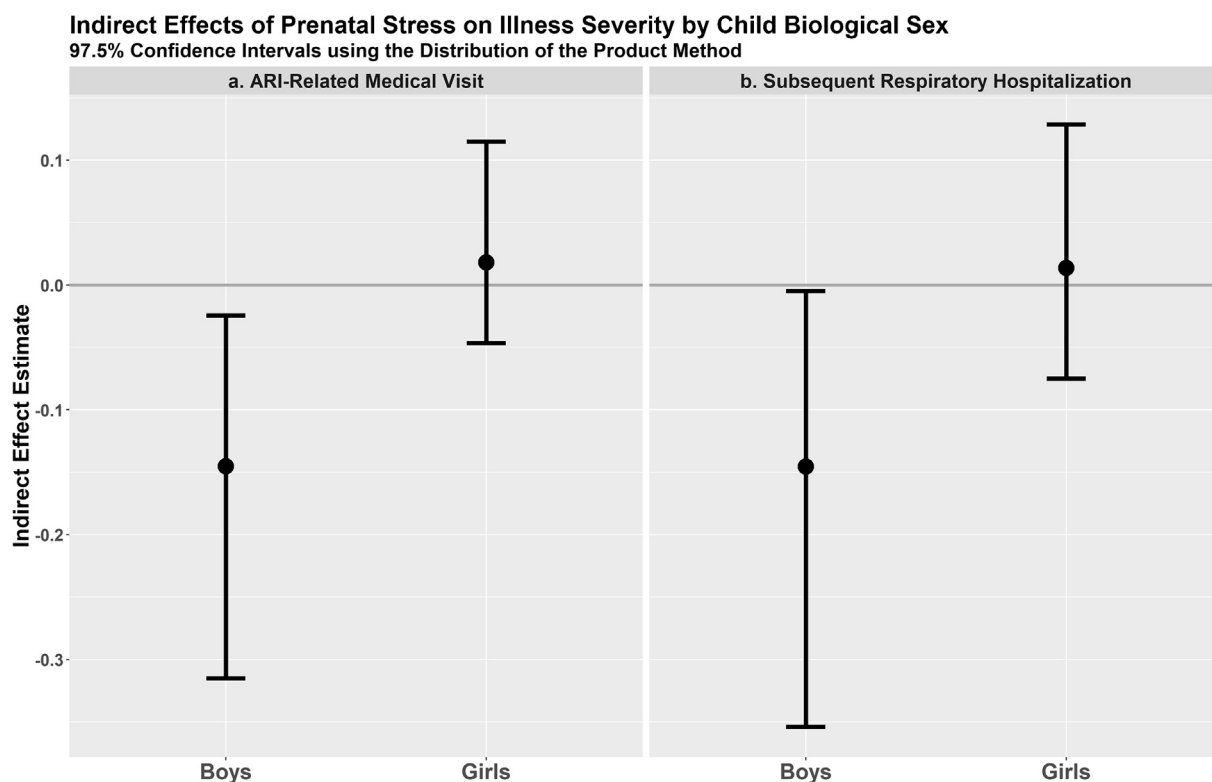


Fig. 2. Indirect Effects of Prenatal Stress Exposure on Respiratory Illness Outcomes. Among boys, but not girls, there were significant negative indirect effects of prenatal stress exposure through the latent inflammation factor on ARI-related medical visits (Panel a) and subsequent respiratory hospitalizations in the second and third years of life (Panel b).

asthma (Lee et al., 2016) specifically among boys. As the INSPIRE participants age, we will be able to evaluate whether prenatal stress exposure predicts asthma and other chronic diseases, and whether the observed sexually-dimorphic effects on ARI-related immune function help elucidate disease pathways.

We view these findings as intriguing but preliminary given several limitations. First, the sample size was relatively small. Second, prenatal stress exposure was measured retrospectively and did not assess the severity, gestational timing, or chronicity of prenatal life stressors. It will be important to account for these dimensions in future studies (Slavich, 2019). Third, we only had inflammatory markers for this subset of participants during ARI and not during healthy states. Finally, although our models were consistent with the data, we cannot know whether they accurately represent underlying causal structures.

Notwithstanding these limitations, this study provides novel evidence that prenatal adversity may alter infant immune functioning during ARI in a sex-dependent manner. The study is the first, to our knowledge, to evaluate associations between prenatal life stress exposure and infant nasal inflammation and symptom severity during naturally occurring ARI. These findings will need to be validated in larger samples with pre- and post-infection measures and with more granular prenatal stress instruments administered during pregnancy. Additionally, it will be critical to evaluate whether the potential benefits of prenatal stress exposure for boys in terms of early life ARI severity are offset by increased risk for inflammatory disease.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jbbi.2018.12.002>.

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