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Association of Prenatal Acetaminophen Exposure Measured in Meconium With Risk of Attention-Deficit/Hyperactivity Disorder Mediated by Frontoparietal Network Brain Connectivity

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⊞ **Supplemental content**

IMPORTANCE Despite evidence of an association between prenatal acetaminophen exposure and attention-deficit/hyperactivity disorder (ADHD) in offspring, the drug is not contraindicated during pregnancy, possibly because prior studies have relied on maternal self-report, failed to quantify acetaminophen dose, and lacked mechanistic insight.

OBJECTIVE To examine the association between prenatal acetaminophen exposure measured in meconium (hereinafter referred to as meconium acetaminophen) and ADHD in children aged 6 to 7 years, along with the potential for mediation by functional brain connectivity.

DESIGN, SETTING, AND PARTICIPANTS This prospective birth cohort study from the Centre Hospitalier Université de Sherbrooke in Sherbrooke, Québec, Canada, included 394 eligible children, of whom 345 had meconium samples collected at delivery and information on ADHD diagnosis. Mothers were enrolled from September 25, 2007, to September 10, 2009, at their first prenatal care visit or delivery and were followed up when children were aged 6 to 7 years. When children were aged 9 to 11 years, resting-state brain connectivity was assessed with magnetic resonance imaging. Data for the present study were collected from September 25, 2007, to January 18, 2020, and analyzed from January 7, 2019, to January 22, 2020.

EXPOSURES Acetaminophen levels measured in meconium.

MAIN OUTCOMES AND MEASURES Physician diagnosis of ADHD was determined at follow-up when children were aged 6 to 7 years or from medical records. Resting-state brain connectivity was assessed with magnetic resonance imaging; attention problems and hyperactivity were assessed with the Behavioral Assessment System for Children Parent Report Scale. Associations between meconium acetaminophen levels and outcomes were estimated with linear and logistic regressions weighted on the inverse probability of treatment to account for potential confounders. Causal mediation analysis was used to test for mediation of the association between prenatal acetaminophen exposure and hyperactivity by resting-state brain connectivity.

RESULTS Among the 345 children included in the analysis (177 boys [51.3%]; mean [SD] age, 6.58 [0.54] years), acetaminophen was detected in 199 meconium samples (57.7%), and ADHD was diagnosed in 33 children (9.6%). Compared with no acetaminophen, detection of acetaminophen in meconium was associated with increased odds of ADHD (odds ratio [OR], 2.43; 95% CI, 1.41-4.21). A dose-response association was detected; each doubling of exposure increased the odds of ADHD by 10% (OR, 1.10; 95% CI, 1.02-1.19). Children with acetaminophen detected in meconium showed increased negative connectivity between frontoparietal and default mode network nodes to clusters in the sensorimotor cortices, which mediated an indirect effect on increased child hyperactivity (14%; 95% CI, 1%-26%).

CONCLUSIONS AND RELEVANCE Together with the multitude of other cohort studies showing adverse neurodevelopment associated with prenatal acetaminophen exposure, this work suggests caution should be used in administering acetaminophen during pregnancy. Research into alternative pain management strategies for pregnant women could be beneficial.

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A cetaminophen is one of the most commonly used drugs during pregnancy, with use reported by more than half of pregnant women in some populations.[1,2] It is the only recommended over-the-counter pain reliever during gestation, because other analgesics, such as ibuprofen and aspirin, may cause miscarriage or birth defects.[3-7] Despite acetaminophen's widespread use and reputation as a safe drug during pregnancy, concerns over the long-term effects of prenatal exposure on respiratory and neurodevelopmental outcomes have risen during the past several decades.[8-10]

One major concern is that acetaminophen may impair fetal brain development, both directly by inducing oxidative stress and apoptosis in the brain[11-13] and indirectly via disruption of important developmental hormones, such as testosterone.[14,15] Indeed, recent meta-analyses of observational studies support an association between prenatal acetaminophen exposure and 3 neurodevelopmental outcomes: attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder, and hyperkinetic disorder/hyperactivity symptoms.[10,16] All 8 studies in these meta-analyses, however, used maternal self-report of acetaminophen exposure. Inaccurate maternal self-report may introduce information bias. Genetic and environmental factors may also affect acetaminophen metabolism, thereby altering the amount of the drug that reaches the fetus. Differential metabolism is not accounted for when acetaminophen is measured by maternal self-report.

Owing to limitations of prior studies, the US Food and Drug Administration[17] and the Society for Maternal-Fetal Medicine[18] have not changed their recommendations to reflect the potential harm of prenatal acetaminophen to neurodevelopment. The Society for Maternal-Fetal Medicine[18] cited maternal self-report of acetaminophen use, lack of quantification of acetaminophen dose, and measurement of outcomes using questionnaires as 3 limitations of previous studies. A recent study in the Boston Birth Cohort[19] addressed these limitations by finding a positive association between acetaminophen metabolites measured in cord plasma and physician diagnosis of ADHD.

Despite growing evidence of an association between prenatal acetaminophen exposure and increased risk for ADHD, several limitations in prior studies remain. First, the Boston Birth Cohort study is the only one that used a direct measurement of acetaminophen levels.[19] No single observational study is sufficient for causal inference, and more observational studies using direct measurements of fetal acetaminophen exposure are needed. Second, owing to the half-life of acetaminophen of less than 3 hours,[20] a cord plasma measurement may only reflect acetaminophen use shortly before and immediately after birth.[19] A direct measurement of fetal acetaminophen exposure that reflects longer-term exposure throughout pregnancy is warranted. Third, no prior studies have examined the potential mechanisms mediating the association of prenatal acetaminophen exposure with neurodevelopment, a key component for assessing the potential for causation.[21] Neuroimaging research has repeatedly documented altered connectivity in important brain networks (eg, default mode, salience, frontoparietal) in individuals with

Key Points

Question Is prenatal acetaminophen exposure measured in meconium associated with attention-deficit/hyperactivity disorder (ADHD), and is the association mediated by changes in functional brain connectivity?

Findings In this birth cohort study of 345 children, acetaminophen exposure detected in meconium was associated with increased odds of ADHD and altered brain connectivity between the frontoparietal and default mode networks to sensorimotor cortices. Altered frontoparietal-sensorimotor cortex connectivity mediated an association of prenatal acetaminophen exposure with hyperactivity.

Meaning The findings that ADHD and related brain phenotypes are associated with prenatal acetaminophen exposure measured directly in meconium suggest that the safety of the drug's use during pregnancy should be reevaluated.

ADHD,[22-24] yet to date no studies have examined functional connectivity in association with prenatal acetaminophen exposure.

In an ongoing prospective birth cohort, we addressed the first 2 limitations by evaluating the association between ADHD and acetaminophen levels measured directly in meconium, the first feces of newborn infants. Chemicals in meconium are known to have passed through the fetus and into the fetal intestinal tract.[25-27] In addition, meconium measurements reflect cumulative exposures during the last two-thirds of pregnancy, because drugs and drug metabolites are deposited in meconium during that period.[28] We addressed the third limitation by conducting the first study using neuroimaging to assess the potential mediating role of functional connectivity in the association between prenatal acetaminophen exposure and child hyperactivity.

Methods

Study Population

This observational analysis was conducted in the Gestation and the Environment cohort in Sherbrooke, Québec, Canada. Women 18 years or older with no known thyroid disease enrolled at the Research Center of the Centre Hospitalier Université de Sherbrooke from September 25, 2007, to September 10, 2009, at their first prenatal care visit or delivery, and were followed up when children were aged 6 to 7 years. Families are currently completing a fourth follow-up assessment (starting February 3, 2018; children aged approximately 9-11 years). As a part of this fourth assessment, children are asked to undergo a magnetic resonance imaging (MRI) assessment. Parents were asked to not give their children ADHD medication on the day of the scan. On the day of the scan, parents were questioned to confirm adherence to this instruction. The eligible study sample consisted of 394 individuals for whom meconium measurements were collected at delivery. The final sample size was 345 children, because ADHD status was unknown for 49 individuals owing to loss to follow-up. At the time of this report, 76 children have undergone functional MRIs

(February 3, 2018, to January 18, 2020), 48 of whom had meconium samples collected in infancy; only children with both meconium samples and MRIs were included in the MRI analyses. Parents signed informed consent forms at each follow-up, and children provided written consent at the follow-up at ages 9 to 11 years. All study protocols were approved by the institutional review boards of the University of Sherbrooke, Harvard T. H. Chan School of Public Health, Boston, Massachusetts, and Columbia University, New York, New York. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

Exposure Assessment

Meconium was collected from the diapers of newborn infants after delivery and stored at −80 °C until analysis. Acetaminophen was extracted from less than 120 mg of meconium and analyzed with ultraperformance liquid chromatography mass spectrometry following the methods described elsewhere.[29] Acetaminophen was detected in 199 of the 345 samples (57.7%), with a recovery of 104% and repeatability of ±15%. The limit of detection (LOD) was 2 ng/g; the limit of quantification, 5 ng/g. In addition, clinical files from the hospital database and medical records were used to determine administration of acetaminophen during labor (yes or no).

Outcome Assessment

At a scheduled cohort follow-up when children were aged 6 to 7 years, parents were asked on a questionnaire if their child had physician-diagnosed ADHD. In total, 176 parents provided information at the 6- to 7-year follow-up. For those who did not complete the 6- to 7-year follow-up visit (n = 169), physician diagnosis of ADHD was obtained from reviewing medical records from Centre Hospitalier Universitaire de Sherbrooke pediatric clinics, which are available in the hospital database. In addition, among the 48 children in the MRI analysis subsample, 46 completed the Behavioral Assessment System for Children Parent Report Scale (BASC3-PRS) at ages 9 to 11 years. In the BASC3-PRS, parents answer a range of questions concerning the behavior of their children that are combined into various rating scales, including scales for attention problems and hyperactivity.[30]

MRI Assessment

At ages 9 to 11 years, T1-weighted structural MRI and functional images were acquired on a 3-T whole-body scanner with a 32-channel head coil (Ingenia; Philips Healthcare). The Conn toolbox[31] was used for preprocessing, and seed-based analyses were conducted. Forty-eight participants had both resting-state MRI data and meconium samples collected. The eMethods and eTable 1 in the Supplement provide details on MRI acquisition, preprocessing, and head motion, as well as demographic characteristics of the children undergoing scanning.

Covariates

Covariate data were obtained from questionnaires given during pregnancy and after delivery. Covariates were child sex, familial income (dichotomized at the sample median), and maternal characteristics, including age at delivery, educational status (college/university vs no college/university), prepregnancy body mass index, smoking during pregnancy (yes or no), and alcohol use during pregnancy (yes or no). A sensitivity analysis including ADHD in the mother (self-reported, obtained from questionnaire) as an additional covariate was conducted, but this variable was excluded from the final models because data were only available for 155 individuals. Controlling for maternal ADHD in this subset altered the estimate for acetaminophen level in meconium by just 2%, and the shift was away from the null. Missing covariate data were imputed with the median of continuous variables and the mode of categorical variables.

Statistical Analysis

Data were analyzed from January 7, 2019, to January 22, 2020. To control for potential confounders, we used inverse probability weighting with propensity scores[32-35] in the CBPS R package.[36] Propensity scores (the likelihood of detectable meconium acetaminophen) were estimated using logistic regression models in which exposure (acetaminophen in meconium detected vs not detected) was regressed on child sex and maternal covariates described above. Weights were estimated as $1/p$ for exposed individuals and $1/(1 - p)$ for unexposed individuals, where p indicates the propensity score. Study sample weighting creates a pseudopopulation balanced on measured baseline covariates.[32-34] Standardized mean differences were computed to assess balance of covariates between the exposed and unexposed groups in both weighted and unweighted samples (eTable 2 in the Supplement). In a sensitivity analysis, we excluded all mothers who were administered acetaminophen during delivery to account for potential confounding by indication for use during labor.

To explore a potential dose response association, we repeated models with continuous acetaminophen level in meconium and with acetaminophen categorized into 3 levels: not detected, low (≤69.0 ng/g [the 50th percentile of exposure]), and high (>69.0 ng/g). Continuous acetaminophen was $\log_2 n$ transformed, with 146 values below the LOD imputed with $LOD/\sqrt{2}$ and 13 values below the LOQ imputed with $LOQ\sqrt{2}$. We modeled continuous acetaminophen with both a linear regression and a generalized additive model, including a penalized spline term. A likelihood ratio test was used to compare these linear and nonlinear models.

Based on the outcome of interest (ie, ADHD in children), resting-state analyses focused on connectivity in 3 classical brain networks often implicated in ADHD: the default mode, salience/cingulo-opercular, and frontoparietal/central executive networks.[22] Seed-based functional connectivity analyses were restricted to regions of interest constituting the aforementioned networks (from the Conn-provided atlas) and compared participants with (n = 25) and without (n = 23) prenatal acetaminophen exposure. Analyses controlled for confounders using propensity scores calculated specifically for the 48 participants with MRI data. Scores included the previously detailed variables, as well as child age at scan. Analyses were thresholded at a voxel level of $P < .001$ (uncorrected) and at a cluster level of $P < .05$ (corrected for false discovery rate

Table 1. Characteristics of Study Population Stratified by Prenatal Acetaminophen Exposure
in the GESTE Cohort[a]

Characteristic	Acetaminophen exposure		All (N = 345)	P value
	No (n = 146)	Yes (n = 199)		
Sex				
Female	73 (50.0)	95 (47.7)	168 (48.7)	.68
Male	73 (50.0)	104 (52.3)	177 (51.3)	
Maternal age at delivery, mean (SD) [range], y	29.2 (4.7) [18.0-43.0]	28.8 (5.0) [19.0-41.0]	29.0 (4.9) [18.0-43.0]	.45
Maternal educational level				
No college or university	70 (47.9)	82 (41.2)	152 (44.1)	.21
College or university	76 (52.1)	117 (58.8)	193 (55.9)	
Family income, CAD$/y				
NA or missing	19	16	35	
≤60 000[b]	64 (50.4)	96 (52.5)	160 (51.6)	.72
>60 000	63 (49.6)	87 (47.5)	150 (48.4)	
Maternal BMI				
NA	10	5	15	
Mean (SD) [range]	25.2 (6.4) [17.8-60.5]	26.6 (7.8) [17.6-89.4]	26.0 (7.3) [17.6-89.4]	.10
Smoked during pregnancy				
NA	4	2	6	
No	121 (85.2)	169 (85.8)	290 (85.5)	.88
Yes	21 (14.8)	28 (14.2)	49 (14.5)	
Alcohol use during pregnancy				
NA	4	2	6	
No	106 (74.6)	158 (80.2)	264 (77.9)	.22
Yes	36 (25.4)	39 (19.8)	75 (22.1)	

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); GESTE, Gestation and the Environment; NA, not available.

[a] Study sample data before imputation and inverse probability weighting are shown. P values are calculated from χ^2 goodness of fit tests for binary variables and 2-sample t tests for continuous variables.

[b] CAD$60 000 is equivalent to US$45 769.

[FDR]), and the eMethods in the Supplement provide details on regions of interest and data analyses. After connectivity analyses, we tested associations between connections that differed between the prenatal acetaminophen-exposed vs unexposed children and BASC3-PRS hyperactivity and attention problems scores at ages 9 to 11 years. We performed logistic regressions on BASC3-PRS scores categorized as above or below the median.

Finally, we performed causal mediation analyses examining connectivity between the frontoparietal network and right precentral/frontal gyrus, because connectivity between these regions was (1) significantly different between exposed and unexposed children and (2) significantly associated with hyperactivity. The purpose of our mediation analyses was not to investigate the total effect of acetaminophen on hyperactivity but rather to investigate processes potentially underlying ADHD. Thus, we did not consider a significant total effect on hyperactivity as a requirement to test for indirect effects. Dropping this requirement reduces type II error associated with the Barron and Kenny causal steps approach.[37] We used the mediation R package,[38] implementing a quasi-Bayesian Monte Carlo method with 1000 simulations, to test whether connectivity mediated the association between prenatal acetaminophen exposure and hyperactivity. This method computes the average direct effect and average causal mediation effect, reflecting direct and indirect (ie, mediated by connectivity) effects of prenatal acetaminophen exposure on hyperactivity. The mediation R package uses information from 2 models with

(1) connectivity as outcome and prenatal acetaminophen level as a covariate, and (2) hyperactivity as outcome and both connectivity and prenatal acetaminophen level as covariates. To assess the potential effect of unobserved pretreatment confounders, we introduced a sensitivity parameter ρ as the correlation between the residuals of the mediator and outcome regressions. We allowed ρ to vary from −0.9 to 0.9 by 0.05-increments to determine what level of confounder-induced correlation would bias results to the null. Two-sided P < .05 indicated significance. Statistical analyses were conducted with R, version 3.5.1.[39]

Results

Among the total study sample of 345 children (168 girls [48.7%] and 177 boys [51.3%]; mean [SD] age, 6.58 [0.54] years at 6- to 7-year follow-up), acetaminophen was detected in the meconium of 199 individuals (57.7%), and ADHD was diagnosed in 33 individuals. This 9.6% ADHD prevalence was comparable to the lifetime 11.3% prevalence in Québec.[40] Baseline covariates stratified by acetaminophen detection are presented in Table 1. Standardized mean differences were less than 0.1 for all covariates after inverse probability weighting, indicating balance between the exposed and unexposed groups (eTable 2 in the Supplement).[41]

Acetaminophen detection in meconium (hereinafter referred to as meconium acetaminophen) was associated with

Table 2. Associations of Prenatal Acetaminophen Exposure With Child ADHD

Acetaminophen exposure detected in meconium	Outcome, No. (%)		Crude OR (95% CI)	P value	Weighted OR (95% CI)[a]	P value
	ADHD	No ADHD				
Binary						
No acetaminophen (n = 146)	8 (5.5)	138 (94.5)	NA	NA	NA	NA
Acetaminophen (n = 199)	25 (12.6)	174 (87.4)	2.48 (1.08-5.67)	.03	2.43 (1.41-4.21)	<.001
Categorical						
No acetaminophen (n = 146)	8 (5.5)	138 (94.5)	NA	NA	NA	NA
Low acetaminophen level (n = 106)	9 (8.5)	97 (91.5)	1.60 (0.60-4.30)	.35	1.44 (0.79-2.63)	.23
High acetaminophen level (n = 93)	16 (17.2)	77 (82.8)	3.60 (1.47-8.76)	.005	4.10 (2.41-6.95)	<.001
P value for trend	NA	NA	NA	.004	NA	<.001
Continuous						
Log^2 (acetaminophen)	NA	NA	1.10 (1.02-1.20)	.02	1.10 (1.02-1.19)	.01

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; NA, not applicable; OR, odds ratio.

[a] Inverse probability weighted for maternal age at birth, maternal body mass index, maternal smoking and alcohol use during pregnancy, maternal educational level, family income, and child sex.

Table 3. Functional Connectivity Differences Between Groups With and Without Acetaminophen Detected in Meconium

| Network	Seed	Region	MNI coordinates			Hemisphere	Cluster size, mm³	Size FDR-corrected P value	Peak t value	Peak z value
			x	y	z					
Default mode	Medial prefrontal cortex (1,55, −3)	Postcentral gyrus, superior parietal lobule, lateral occipital cortex (superior division), supramarginal gyrus, angular gyrus	36	−52	58	Right	1358	<0.01	5.73	4.94
		Postcentral gyrus, superior parietal lobule, lateral occipital cortex (superior division), supramarginal gyrus	−38	−52	64	Left	1104	<0.01	5.89	5.04
		Inferior and middle temporal gyrus (temporo-occipital and posterior divisions), cerebellum crus 1 and 6, lateral occipital cortex (inferior division), temporal occipital fusiform cortex	60	−52	−12	Right	905	<0.01	5.75	4.95
		Precentral gyrus, middle and superior frontal gyrus	26	−14	46	Right	638	<0.01	5.73	4.94
		Precentral gyrus, superior and middle frontal gyrus	−22	−14	48	Left	536	<0.01	5.48	4.77
		Cerebellum 8 and 9	−28	−46	−56	Left	241	0.03	5.30	4.64
Frontoparietal	Left lateral prefrontal cortex (−43,33,28)	Superior frontal, middle frontal, and precentral gyrus	30	2	60	Right	452	0.02	4.62	4.16

Abbreviations: FDR, false discovery rate; MNI, Montreal Neurological Institute.

nearly 2.5-fold increased odds of ADHD at ages 6 to 7 years (odds ratio [OR], 2.43; 95% CI, 1.41-4.21) (**Table 2**) in the weighted sample balanced on covariates. When acetaminophen exposure was categorized into 3 levels, low acetaminophen exposure level did not significantly modify the risk of ADHD compared with no acetaminophen exposure (OR, 1.44; 95% CI, 0.79-2.63). However, high levels of acetaminophen detected in meconium increased the odds of ADHD more than 4-fold (OR, 4.10; 95% CI, 2.41-6.95) (Table 2). When meconium acetaminophen was linearly modeled, each doubling of exposure increased the odds of ADHD by 10% (OR, 1.10; 95% CI, 1.02-1.19) (Table 2). Introducing a nonlinear penalized spline for continuous acetaminophen did not improve the model fit (log likelihood, −109.29 vs −110.64; likelihood ratio test, P = .10) (eFigure in the Supplement). Results did not differ in a sensi-

tivity analysis excluding 44 mothers who were administered acetaminophen at delivery (OR, 2.38; 95% CI, 1.35-4.21).

Functional connectivity analyses revealed that, compared with the unexposed group, children with detectable levels of acetaminophen in meconium demonstrated increased negative connectivity between the medial prefrontal cortex gyrus (default mode network seed) and 6 clusters covering regions of bilateral precentral and postcentral gyri, superior parietal lobules, and supramarginal gyri (t > 5.30; FDR-corrected P < .03). Exposed children also demonstrated increased negative connectivity between the left lateral prefrontal cortex (frontoparietal network seed) and a cluster spanning portions of the right precentral and frontal gyrus (t = 4.62; FDR-corrected P = .02) (**Table 3** and **Figure 1**). There were no differences detected using salience network seeds.
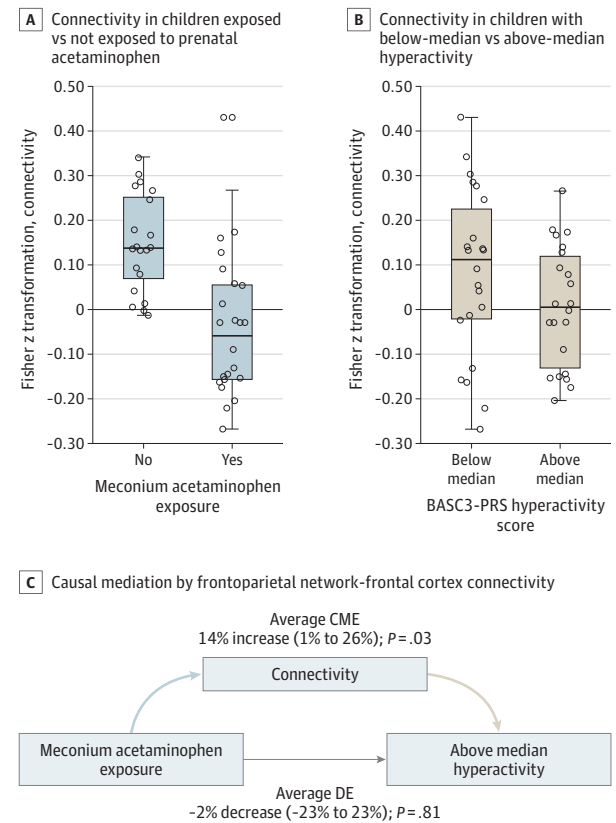
Figure 1. Differences in Resting-State Functional Connectivity Associated With Prenatal Acetaminophen Exposure

A Medial prefrontal cortex seed

B Left lateral prefrontal cortex seed
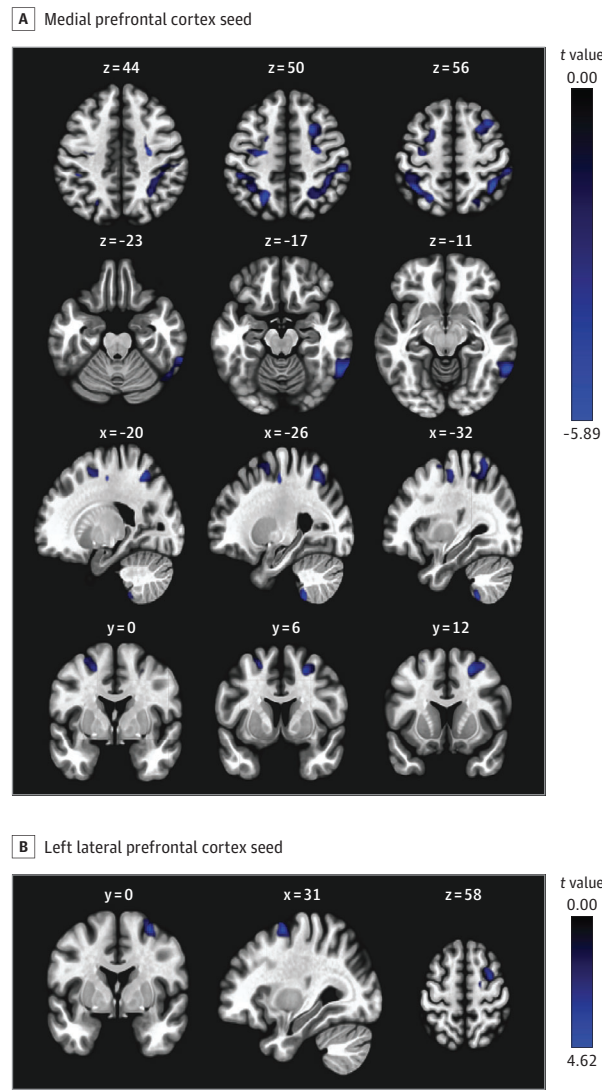
Seed-based functional connectivity contrasts detected differences in resting-state connectivity between children with (n = 25) and without (n = 23) prenatal acetaminophen exposure. Compared with the unexposed group, children with prenatal acetaminophen exposure demonstrated increased negative connectivity between the medial prefrontal cortex gyrus (default mode network seed) and 6 clusters covering regions of bilateral precentral and postcentral gyri, superior parietal lobules, and supramarginal gyri (A), as well as increased negative connectivity between the left lateral prefrontal cortex (frontoparietal network seed) and a cluster spanning portions of the right precentral and frontal gyri (B). Analyses were thresholded at a voxel level of $P < .001$ (uncorrected) and at a cluster level of $P < .05$ (corrected for false discovery rate). Colored areas show increases in negative (blue) connectivity between the groups. Values above each image indicate the brain section being displayed.

Figure 2. Causal Mediation by Frontoparietal Network–Frontal Cortex Connectivity

A Connectivity in children exposed vs not exposed to prenatal acetaminophen

B Connectivity in children with below-median vs above-median hyperactivity

C Causal mediation by frontoparietal network-frontal cortex connectivity

Individual values (points), median (horizontal lines), and interquartile range (boxes) depict significant differences in connectivity between the frontoparietal network and right precentral/frontal gyrus in children with vs without prenatal exposure to acetaminophen (A) and connectivity between the frontoparietal network and right precentral/frontal gyrus in children with hyperactivity above vs below the median (B). Mediation by connectivity of the association between acetaminophen detected in meconium and child hyperactivity at ages 9 to 11 years (C) shows the average causal mediation effect (CME) and average direct effect (DE) from causal mediation analysis. Connectivity is expressed as a Pearson correlation between the 2 brain regions with Fisher z transformation. BASC3-PRS indicates Behavioral Assessment System for Children Parent Report Scale.

Among these brain connections associated with meconium acetaminophen, connectivity between the frontoparietal network and right precentral/frontal gyrus was also associated with BASC3-PRS hyperactivity score (eTable 3 in the Supplement). Consistent with the potential for mediation, meconium acetaminophen was associated with decreased connectivity ($\beta = -0.18$ [95% CI, -0.26 to -0.10]) (Figure 2A), and children with decreased connectivity were more hyperactive (OR for a 1-point increase in Fisher z-transformed Pearson correlation between brain regions, 0.04 [95% CI, 0.00-0.68]; $P = .03$) (Figure 2B). Causal mediation analysis revealed no total (15% increase; 95% CI, -6% to 36%) or direct (1% increase; 95% CI, -200% to 26%) effect of meconium acetaminophen levels on hyperactivity, but a significant indirect effect on increased hyperactivity mediated through frontoparietal network and right precentral/frontal gyrus connectivity (14% increase; 95% CI, 1%-26%) (Figure 2C and eTable 4 in the Supplement). A sensitivity analysis for pretreatment confounders revealed that to bias this result to the null, an unobserved confounder would need to induce a correlation between the residuals of the mediator and outcome regressions of $\rho = -0.3$.

Discussion

In this prospective Eastern Canadian cohort, children exposed to acetaminophen prenatally were at increased risk of ADHD at ages 6 to 7 years. Categorical and continuous models suggested that higher levels of meconium acetaminophen increased the risk of ADHD in children in a linear manner. Prenatal acetaminophen exposure was also associated with increased negative connectivity between the left prefrontal cortex (frontoparietal seed) and the right precentral/frontal gyrus, which mediated the association of acetaminophen with hyperactivity. Several prior studies[10,16] have implicated prenatal acetaminophen exposure in the etiology of neurodevelopmental diseases such as ADHD and autism spectrum disorder, yet none have examined brain function after acetaminophen exposure. Further, to the best of our knowledge, our results are just the second report of an association between ADHD in children and prenatal acetaminophen levels measured not via questionnaire, but in a biological sample,[19] and the first study of the association between acetaminophen levels measured in meconium and ADHD. A prior study in this cohort[42] examined the association between meconium acetaminophen levels and the Wechsler Intelligence Scale for Children and found no consistent associations.

Strengths and Limitations

A strength of this study was the unbiased, biological measure of fetal acetaminophen exposure. All but 1 of the prior studies of the association between prenatal acetaminophen exposure and child ADHD[43-49] have relied on questionnaires requiring mothers to recall drug use at intervals greater than 3 months. Difficulty recalling drug use during pregnancy may result in nondifferential misclassification bias toward the null. This source of bias may explain the smaller pooled risk ratio of 1.34 for ADHD from past cohort studies[10] compared with the nearly 2.5-fold increased odds reported herein. Supporting this hypothesis, the only other study not relying on maternal self-report,[19] which measured acetaminophen levels in cord plasma, reported an OR of 2.26 for the second tertile and 2.86 for the third tertile compared with the first tertile of exposure. However, it is possible that results in this population, which is highly educated and genetically homogeneous,[50] are not generalizable to other populations with different characteristics.

This is the first study, to our knowledge, to examine associations of prenatal acetaminophen exposure with functional connectivity in childhood. Alterations in connectivity between the default mode and frontoparietal networks to the sensorimotor cortices have been previously documented in both children[51] and adults[52] with ADHD and have been linked to symptom severity. Herein we offer a putative mechanistic insight into the association between prenatal acetaminophen exposure and ADHD in offspring. Causal mediation analysis revealed that altered frontoparietal network connectivity may link prenatal acetaminophen exposure with increased child hyperactivity at ages 9 to 11 years. Although this result suggests that brain connectivity may also mediate an indirect effect on

ADHD, we were unable to explore this possibility, because ADHD diagnosis information was obtained when children were aged 6 to 7 years. Studies have previously associated altered functional brain connectivity with environmental exposures, including air pollution,[53] social stress,[54] and prenatal use of selective serotonin reuptake inhibitors,[55] although this is the first neuroimaging study of prenatal acetaminophen exposure, to our knowledge. Taken with the wide confidence interval of the mediation analysis indirect effect and the small MRI sample size, studies in larger and more diverse cohorts are needed to replicate these novel findings.

Confounding by unmeasured or unknown factors is always a possibility. Although we did not control for indications for acetaminophen use in this study, prior cohort studies controlling for maternal fevers, infections, and other indications for acetaminophen use[19,44-47,49] have reported lack of confounding by these factors. However, lack of confounding by indicators in prior cohort studies does not necessarily apply to the cohort studied herein. We also considered the possibility that meconium acetaminophen concentrations were a reflection of acetaminophen administered during labor rather than throughout pregnancy. However, excluding women who were administered acetaminophen at delivery did not change our results. Although meconium is known to accumulate drugs and drug metabolites throughout the last two-thirds of pregnancy, we did not explicitly correlate maternal acetaminophen use with acetaminophen concentrations in meconium, a potential limitation that should be the subject of future work. Another possibility is confounding by unknown genetic, social, and familial factors associated with acetaminophen use. This concern has been recently addressed with negative control exposure analysis: maternal acetaminophen use before and after pregnancy and a partner's acetaminophen use were not associated with child ADHD in populations in which maternal acetaminophen use during pregnancy increased the risk.[28,29] Furthermore, our study population has high genetic and sociodemographic homogeneity.[50] Hence, confounding by unknown or unmeasured factors is unlikely. Finally, although children did not take ADHD medications on the day of the scan, we could not rule out prior medication use.

Conclusions

By using a direct measurement of prenatal acetaminophen exposure that is unbiased by maternal recall, these results add evidence in support of the association between prenatal acetaminophen use and child ADHD. Taken together with the large ORs reported in the Boston Birth Cohort study,[19] these results suggest that prior studies may have been biased toward the null by inaccurate maternal recall. Thus, the association between prenatal acetaminophen and ADHD may be even stronger than previously estimated. This study additionally supports altered resting-state brain connectivity as a potential underlying mechanism linking prenatal acetaminophen use with child hyperactivity. Along with the multitude of other cohort studies drawing similar conclusions, this work joins the Boston Birth Cohort study as the second study

addressing the concerns of the US Food and Drug Administration and Society for Maternal-Fetal Medicine about maternal self-report and lack of quantification of prenatal acetamino-phen dose. These institutions should therefore consider re-evaluating the evidence regarding the safety of fetal acet-aminophen exposure.

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