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Prenatal exposure to paracetamol and SSRIs

Paracetamol use during pregnancy and attention and executive function in offspring at age 5 years

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Abstract

Background: Recent studies suggested that *inutero* exposure to paracetamol, the most common pain and fever medication used in pregnancy, may affect neurodevelopment in offspring. We aim to examine whether maternal use of paracetamol during pregnancy affects the attention and executive function of children at age 5 years.

Methods: We studied 1491 mothers and children enrolled in the Danish National Birth Cohort (DNBC; 1996-2002). Prenatal paracetamol use was prospectively recorded in three telephone interviews. Trained psychologists assessed child's attention function using the Test of Everyday Attention for Children at Five (TEACh-5). Parents and preschool teachers completed Behaviour Rating Inventory of Executive Function (BRIEF) to assess executive functions. We estimated the differences of composite mean outcome scores, and odds ratios (OR) for subnormal attention or executive function (defined as 1 standard deviation below the mean), adjusting for maternal IQ, maternal mental health, indications for paracetamol use and other potential confounders.

Results: First trimester use of paracetamol was associated with poorer attention scores in childhood [mean difference -0.34, 95% confidence interval (CI) -0.63, -0.05 for overall attention, and -0.25, 95% CI -0.50, 0.01 for selective attention]. Children prenatally exposed to paracetamol were also at a higher risk for subnormal overall attention (OR = 1.5, 95% CI 1.0, 2.5), selective attention difficulties (OR = 1.5, 95% CI 1.0, 2.4), and parent-rated subnormal executive function (metacognition index, OR = 1.5, 95% CI 0.9, 2.3). The risks for subnormal overall attention or executive function were elevated with longer duration of paracetamol use in pregnancy.

Conclusions: We found some evidence that maternal paracetamol use during pregnancy was associated with poorer attention and executive function in 5-year-olds.

Key words: Paracetamol (acetaminophen), neurodevelopment, attention, executive function, prenatal exposure

Introduction

Paracetamol has been considered safe and remains the first-line medical treatment for pain and fever during pregnancy.[1,2] However, recent research raised concerns that paracetamol may interfere with optimal fetal brain development, resulting in a higher risk of neurobehavioural disorders in childhood.[3–5] In particular, among 64 322 mothers and children enrolled in the Danish National Birth Cohort (DNBC) with 11 years of follow-up, paracetamol use during pregnancy was associated with an increased risk of diagnosis and treatment for attention-deficit/hyperactivity disorder (ADHD) in childhood.[4] In the DNBC, prenatal use of paracetamol was also linked to autism spectrum disorders (ASD) accompanied by hyperkinetic symptoms, but not to other subtypes of ASD, suggesting that paracetamol exposure may specifically affect the hyperactive behavioural phenotype.[6] The Norwegian Mother and Child Cohort study (MOBA), which followed 48 631 children up to 3 years of age, used a sibling-controlled design and reported that prenatal exposure to paracetamol for more than 28 days was associated with adverse psychomotor development.[5] In addition, the Auckland Birthweight Collaborative Study suggested, in support of the DNBC findings, that prenatal paracetamol use is positively associated with ADHD-like symptoms in children at 7 to 11 years of age, measured by the parent-rated Conners' behavioural rating scale and the strength-and-difficulty questionnaire,[3] the latter screening for emotional symptoms, conduct problems, hyperactivity, peer relationship and pro-social behaviour in children.[7] These findings appeared to be specific to paracetamol since the MOBA and the Auckland cohort reported that other painkillers such as ibuprofen were not associated with neurodevelopmental endpoints, and the DNBC studies adjusted for maternal use of aspirin or ibuprofen in all analyses.

Paracetamol is accessible over the counter, and more than half of pregnant women in North America and Western/Northern European countries reported using paracetamol during pregnancy.[1,8] Thus, even small adverse effects of paracetamol on the neuropsychological function in children may have important public health consequences. Additional research concerning the safety of paracetamol use during pregnancy is urgently needed. In this study, we use a sub-sample from the DNBC to estimate the effects of prenatal paracetamol use on two critical functional domains of brain development, including attention and executive function in the offspring assessed at age 5 years.

Materials And Methods

Study design and participants

We used data from the Lifestyle During Pregnancy Study (LDPS), a sub-cohort nested within the DNBC. The DNBC is a longitudinal cohort established during 1996-2002, in which 101 041 pregnancies were enrolled at the first general practitioner antenatal visit (during weeks 6 to 12).[9] The LDPS was designed to study the relations between prenatal lifestyle factors, primarily maternal alcohol intake, and neuropsychological outcomes in children. The design and sampling scheme of the LDPS have been described elsewhere.[10] Briefly, 3478 mothers and children from the DNBC were invited to participate in a 3-hours neuropsychological assessment conducted by trained psychologists when the children reached 5 years of age (age range: 60-64 months). The neuropsychological tests were conducted in a controlled setting at four regional sites (Copenhagen, Odense, Aarhus and Aalborg). The psychological tests were administered by 10 psychologists who were blinded to exposure status. The LDPS standardized all testing procedures and performed regular inter-rater comparisons for examiners. Exclusion criteria for the LDPS were non-singleton birth, women and children who could not speak sufficient Danish to participate, children with impaired hearing or vision to the extent that the neuropsychological tests could not be performed, and severe disabilities due to congenital defects. Among those invited 1782 (51%) participated, but 291 women did not complete all three telephone interviews at baseline when exposure information was collected; thus the final sample included 1491 mothers and children.

Exposure assessment

Information about paracetamol use was collected in three computer-assisted telephone interviews conducted at gestational weeks 12 and 30 and 6 months postpartum. During each interview, mothers were asked whether they had taken any painkillers during the previous pregnancy period. Those who answered 'yes' were provided with a

list of the 44 most common types of analgesics including paracetamol as a single or combination drug, either over the counter or via prescription. Mothers who indicated using paracetamol were asked to report use on a week-by-week basis, allowing us to examine trimester-specific use. We also compute duration of use by summing the total weeks of use throughout the entire gestation.

Outcome assessment and computation of test scores

Attention

Child's attention was measured by the Test of Everyday Attention for Children at Five (TEACh-5). TEACh-5 is a recent development in a series of comprehensive attention test batteries.[11] An introduction to TEACh-5 including the instruments, validation and psychometric properties can be found elsewhere.[11,12] Four subtests were administered: two assessed sustained attention including the 'Barking' and 'Draw-a-line', and two examined selective attention including 'The Great Balloon Hunt' and 'Hide and Seek II'. In the Barking test, the children were asked to listen to six slowly presented soundclips and to count how many dog barks occurred in each. The Draw-a-line test recorded the time used to trace a line as slowly as possible without stopping and lifting the pen. In the Great Balloon Hunt test, the child was given 15 seconds to mark as many of 48 balloons as possible, first on a sheet with nothing but balloons, and later on a sheet with the target balloons distributed among visual distractors. Children were asked to listen to 14 soundclips each lasting 10 seconds in the Hide and Seek II test, and then to report whether the target element (a bark from a dog) was absent or present. The level of performance was scored based on the mean reaction time (in seconds) to give a correct answer.

Each of the subtest scores were first standardized to a mean of 0 and a standard deviation (SD) of 1 based on the full LDPS sample. A composite measure of overall attention was then computed based on the mean of the four standardized sub-scores, and the sustained and selective attention scores were obtained by taking the mean of the two standardized sub-scores. Finally, the composite scores for overall, sustained and selective attention were re-standardized to a mean of 0 and an SD of 1. For TEACh-5 measures, lower scores indicate poorer attention function.

Executive function

The children's executive function was measured using the Behaviour Rating Inventory of Executive Function (BRIEF). The BRIEF questionnaire was completed independently by the parent and by the preschool teacher.[13,14] The questionnaire contains 86 statements measuring eight clinical scales that tap multiple domains of executive functions. A translated version of BRIEF was used for Danish preschool children. A normalizing T-score transformation for each subclinical scale based on the distribution from the full LDPS sample was computed, with higher scores indicating more executive function difficulties.

Three composite measures were generated from the subscales: the Global Executive Composite (GEC), the Behavioural Regulation Index (BRI) and the Metacognition Index (MI). The GEC is an overarching summary score that incorporates all BRIEF clinical scales. The BRI includes following subscales: inhibit (controls impulses), shift (transitions and solves problems flexibly and appropriately as the circumstances demand) and emotional control (monitors emotional responses appropriately). The MI includes: initiates (begins tasks independently), working memory (holds information in mind in order to complete task), plans/organizes (plans future events, sets goals and carries out steps in a systematic manner), organization of materials (keeps possessions and work/play spaces orderly), and monitors (self-monitors work or behaviour during and after tasks).

Statistical analysis

We used multivariable linear regression to estimate the mean differences of the TEACh-5 and BRIEF scores, comparing children born to mothers who used paracetamol during pregnancy with never users as the reference group. In addition, we dichotomized the TEACh-5 measures using 1 SD below the mean as a cut-off to indicate subnormal attention function, and the BRIEF measures 1 SD above the mean (*T*-score of approximately 58 for each of the composite measures) indicating subnormal executive function. We used logistic regression to estimate odds ratios (ORs) for subnormal attention or executive function following paracetamol exposure in pregnancy. We examined ever/never, trimester-specific (only used in the first, second or third trimesters) and total weeks of paracetamol use during pregnancy (1, 2-5, $>$ 5 weeks). We also calculated P-values for trend using linear trend testing by fitting total weeks of exposure as a continuous variable. For the analysis of weeks of use, 1275 mother-child pairs provided complete information and 1292 participants had teacher-rated BRIEF measures available. Potential confounders were first selected a priori considering factors that affect child neurobehavioural development and may also be associated with paracetamol use. In all models we adjusted for mother's age at child birth (continuous), parity (1, $>$ 1), parental education index (continuous; total years of education averaged for both parents), maternal IQ (continuous), maternal

mental health (illness reported yes/no), maternal pre-pregnancy body mass index (BMI) (< 18.5, 18.5-25, > 25), maternal smoking during pregnancy (yes/no) and maternal alcohol intake during pregnancy (0, 1-4, > 5 drinks per week). Regarding maternal mental health, mothers were asked if they had seen a doctor or psychologist during or before pregnancy because of depression or anxiety and whether they had suffered from childhood psychiatric disorders or other mental health problems. Child's sex is expected to be a rather strong predictor on neurobehavioural test scores at age 5, so we included sex in the models to reduce variance.[15] We also included an indicator for each tester to address potential variations in neuropsychological assessments. In addition, we adjusted for three important indications for paracetamol use in pregnancy that may also be associated with neurodevelopment, including fever, inflammation or infection, and pain or musculoskeletal diseases. We also performed subgroup analyses restricted to mothers who did not experience these conditions in pregnancy. In addition, in all models we adjusted for prenatal use of aspirin and ibuprofen to address potential confounding from other commonly used pain or fever medications. Additional potential confounders we evaluated, such as paternal age, prenatal use of antidepressants, folic acid supplementary intake and maternal marital status at the time of the interviews, were not included in the final models because changes in the effect estimate size were minimal (< 5%). Furthermore, we conducted analyses separately for boys and girls to examine potential effect measure modification by sex. We used multiple imputations to address missing covariate values in all analyses (< 4% with at least one missing value).

We used inverse probability weights to account for subject selection because the LDPS over-sampled mothers with high alcohol intake during pregnancy, and among those invited only ~ 51% participated. The sampling of the LDPS from all DNBC women was random within each alcohol intake category, and the sampling probabilities were available for adjustment and have been used in previous studies.[10,15] In addition, we also estimated the probability of selective non-participation in the LDPS using measured factors for all women in the DNBC collected at baseline. Among a wide range of evaluated factors, we found that preterm birth was negatively associated with participation, whereas maternal higher socioeconomic status and organic eating habits during pregnancy were positively related to participation. We also include some factors that were weakly associated with LDPS participation such as maternal age, season of conception, pre-pregnancy BMI, home size, planned pregnancy, location of birth and missing a telephone interview at baseline. Prenatal use of paracetamol was not related to participation in the LDPS (OR = 0.99, 95% CI 0.86, 1.14). We created inverse probability weights combining the probabilities of sampling and participation, and then performed weighted regressions throughout. We computed 95% confidence intervals (CIs) using robust variance estimators in all weighted analyses. Statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Characteristics of the study participants are presented in Table 1. More than half of the women (59%) in the LDPS indicated using paracetamol during pregnancy.

Table 2 presents the adjusted mean differences in the attention and executive function test scores according to paracetamol use during pregnancy. Children born to mothers who used paracetamol during pregnancy in general performed poorer in the tests for overall attention (mean difference -0.10, 95% CI -0.26, 0.05) and selective attention function (mean difference -0.12, 95% CI -0.28, 0.04) compared with the unexposed. These effect estimates were larger when paracetamol was used in the first trimester (mean difference -0.34, 95% CI -0.65, -0.05 for overall attention, and -0.25, 95% CI -0.50, 0.01 for selective attention). Children born to mothers who used paracetamol during pregnancy also performed more poorly (i.e. had higher scores) regarding both parent- and teacher-rated executive function, but these estimates were imprecise and there was no apparent pattern for trimester-specific effects.

Table 3 presents associations for subnormal attention or executive function in children with prenatal paracetamol use. Again, use of paracetamol in pregnancy was associated with increased risk of overall (OR = 1.5, 95% CI 1.0, 2.5) and selective attention difficulties (OR = 1.5, 95% CI 1.0, 2.4) and parent-rated executive difficulties based on the metacognition index (OR = 1.5, 95% CI 0.9, 2.3). First trimester paracetamol use was also associated with higher risks for overall (OR = 1.9, 95% CI 0.9, 4.1) and sustained attention difficulties (OR = 2.8, 95% CI 1.5, 5.5), but trimester-specific effects were not apparent for executive function. We also found that increasing number of gestational weeks of use associated with higher risks for overall attention difficulties and for subnormal executive function in all three parent-rated scales (Table 4).

In stratified analyses, results for abnormal attention function were similar for boys and girls, but effect estimates for parent-rated executive difficulties (GEC and BRI) appeared to be larger for boys (Table 5). Effects estimates for both subnormal attention and executive function changed minimally when the analyses were restricted to mothers who did not experience fever, infection or

Table 1. Characteristics of study participants

	Maternal use of paracetamol during pregnancy			
	Yes ($n = 881$)		No ($n = 610$)	
	n	%	*n*	%
Mother's age at child birth (years; mean ± SD)	30.8 ± 4.4		30.8 ± 4.2	
Parental education[a] (years; mean ± SD)	13.1 ± 1.9		13.3 ± 1.9	
Maternal IQ (mean ± SD)	99.6 ± 14.9		100.8 ± 14.8	
Parity				
1	401	45.5	348	57.0
> 1	480	54.5	262	43.0
Maternal drinking during pregnancy				
Never	398	45.2	321	52.6
1-4 glasses per week	382	43.4	232	38.0
More than 4 glasses per week	101	11.5	57	9.3
Maternal smoking during pregnancy				
No	579	65.7	445	73.0
Yes	302	34.3	165	27.0
Maternal pre-pregnancy body mass index (BMI)				
< 25	629	71.3	449	73.6
25 to < 30	164	18.6	106	17.4
≥ 30	69	7.8	44	7.2
Maternal psychiatric illness	107	12.2	49	8.0
Maternal fever during pregnancy	328	37.2	141	23.1
Pain or musculoskeletal diseases during pregnancy	105	11.9	48	7.9
Infection or inflammation during pregnancy	115	13.1	47	7.7
Maternal use of aspirin during pregnancy	89	10.1	53	8.7
Maternal use of ibuprofen during pregnancy	70	7.9	29	4.8

[a]Average years of education for both parents.
[b]The missing values for parental education, maternal IQ and pre-pregnancy BMI are 0.3%, 0.5% and 2%, respectively.

inflammation, or diseases or pain in muscles/joints during pregnancy (Table 5).

Discussion

In this subcohort nested within the Danish National Birth Cohort, we found that children born to mothers who used paracetamol during pregnancy performed relatively poorly on the TEACH overall and selective attention indices, and on the parent-rated BRIEF they had poorer metacognitive skills at age 5 compared with their unexposed counterparts. Associations between paracetamol and childhood subnormal attention function appear to be stronger when paracetamol was used in the first trimester. We also found a dose-response-like relation between increasing weeks of use and executive difficulties rated by parents. There were no apparent associations between paracetamol use and scores on executive scales rated by preschool teachers; however, this outcome measure is expected to have large variability at age 5. Preschool teachers might not have sufficient specific contact with the child to detect a potentially subtle effect of paracetamol on child's executive function.

The results of this study are in line with our previous findings of higher risk of ADHD following prenatal exposure to paracetamol.[4] The previous study, however, was solely based on hospital diagnosis and treatment records for ADHD and did not include any functional measures. Here, we provide additional evidence that paracetamol exposure may affect children's attention and executive function measured as early as age 5, before the age of 7-9 at which clinical diagnosis and treatment of ADHD are more common. The deficits seen in children with ADHD typically include impulsivity and inattention, and these salient features are also part of the executive function measures used here.[16] Decades of research have shown that children with better attention and executive function do better in every aspect of life, not only in school but also regarding income earning potential and adult physical health.[16,17] Given the high frequency of paracetamol use in pregnancy, even an on average relatively small negative effect on attention or executive function in children may be important in societies that place a high value on such abilities in its citizens, and may have more severe consequences in those children who are already scoring low on these abilities for other reasons.

Table 2. Mean differences for attention and executive function in 5-year-old children according to maternal prenatal use of paracetamol

Neuropsychological measures[b]	Never use ($n = 610$)	Use of paracetamol during pregnancy			
		Ever use ($n = 881$)	1st trimester only ($n = 159$)	2nd trimester only ($n = 76$)	3rd trimester only ($n = 165$)
	Mean (reference)	**Mean difference[a] (95% CI)**	**Mean difference[a] (95% CI)**	**Mean difference[a] (95% CI)**	**Mean difference[a] (95% CI)**
Attention (TEACh-5)					
Overall attention	0.01	−0.10 (-0.26, 0.05)	−0.34 (-0.63, -0.05)	−0.16 (-0.46, 0.15)	0.05 (-0.21, 0.30)
Selective attention	0.02	−0.12 (-0.28, 0.04)	−0.25 (-0.50, 0.01)	−0.21 (-0.57, 0.16)	−0.03 (-0.29, 0.24)
Sustained attention	0.01	−0.04 (-0.19, 0.11)	−0.24 (-0.52, 0.04)	0.00 (-0.30, 0.30)	0.03 (-0.21, 0.27)
Executive function (BRIEF), parent-rated					
General Executive Composite (GEC)	49.24	0.78 (-0.44, 2.00)	0.05 (-1.80, 1.91)	1.07 (-1.41, 3.55)	0.46 (-1.78, 2.69)
Behavioral Regulation Index (BRI)	49.38	0.43 (-0.89, 1.75)	−0.66 (-2.55, 1.22)	1.49 (-1.27, 4.24)	−0.45 (-2.86, 1.96)
Metacognition Index (MI)	49.16	0.99 (-0.29, 2.28)	0.48 (-1.58, 2.54)	0.82 (-1.68, 3.32)	1.01 (-1.29, 3.31)
Executive function (BRIEF), teacher-rated[c]					
General Executive Composite (GEC)	49.89	0.41 (-0.82, 1.65)	0.58 (-1.52, 2.68)	1.92 (-0.98, 4.82)	−1.24 (-3.52, 1.04)
Behavioral Regulation Index (BRI)	50.03	0.15 (-1.23, 1.52)	0.85 (-1.54, 3.24)	2.20 (-0.91, 5.30)	−1.40 (-3.99, 1.19)
Metacognition Index (MI)	49.80	0.57 (-0.72, 1.87)	0.38 (-1.78, 2.54)	1.75 (-1.16, 4.66)	−1.15 (-3.46, 1.17)

[a]Adjusted for parental education, maternal IQ, maternal mental health status, prenatal smoking, prenatal drinking, parity, maternal age at child birth, child's sex, maternal pre-pregnancy BMI, maternal musculoskeletal diseases, fever or infection/inflammation during pregnancy, and maternal use of ibuprofen or aspirin. Estimates for TEACh-5 were additionally adjusted for tester.

[b]For TEACh-5, lower scores indicate poorer attention function, and for BRIEF higher scores indicate poorer executive function.

[c]For preschool teacher-rated BRIEF outcomes, we analysed 520 children with mothers who never used paracetamol during pregnancy, 772 who ever used paracetamol and 136, 66 and 141 who only used during the first, second or third trimester, respectively.

Table 3. Odds ratios for subnormal attention or executive function in 5-year-old children according to maternal prenatal use of paracetamol

Neuropsychological measures[b]	Never use ($n = 610$)	Use of paracetamol during pregnancy							
		Ever use ($n = 881$)		1st trimester only ($n = 159$)		2nd trimester only ($n = 76$)		3rd trimester only ($n = 165$)	
	n (reference)	*n*	**OR[a] (95% CI)**	n	**OR[a] (95% CI)**	n	**OR[a] (95% CI)**	n	**OR[a] (95% CI)**
Subnormal attention (TEACh-5)									
Overall attention	89	146	1.5 (1.0, 2.5)	26	1.9 (0.9, 4.1)	14	1.7 (0.7, 4.1)	33	1.7 (0.8, 3.6)
Selective attention	93	137	1.5 (1.0, 2.4)	28	1.6 (0.8, 3.2)	13	2.1 (0.8, 5.3)	27	1.7 (0.8, 3.9)
Sustained attention	103	134	1.3 (0.8, 2.1)	27	2.8 (1.5, 5.5)	15	1.6 (0.7, 4.0)	24	0.9 (0.4, 2.0)
Subnormal executive function (BRIEF), Parent-rated									
General Executive Composite (GEC)	86	148	1.3 (0.8, 2.1)	15	1.0 (0.5, 2.3)	13	1.6 (0.6, 4.3)	30	1.3 (0.6, 2.9)
Behavioral Regulation Index (BRI)	81	141	1.3 (0.8, 2.1)	14	0.7 (0.3, 1.7)	11	1.6 (0.6, 4.5)	30	1.2 (0.6, 2.7)
Metacognition Index (MI)	87	161	1.5 (0.9, 2.3)	19	1.3 (0.6, 2.6)	15	1.2 (0.4, 3.4)	31	1.6 (0.8, 3.2)
Subnormal executive function (BRIEF), Teacher-rated[c]									
General Executive Composite (GEC)	89	128	1.2 (0.7, 2.0)	19	1.3 (0.6, 2.8)	9	1.4 (0.4, 4.2)	19	0.9 (0.4, 2.2)
Behavioral Regulation Index (BRI)	81	126	1.0 (0.6, 1.6)	24	1.4 (0.6, 3.0)	14	2.3 (0.9, 5.7)	19	0.8 (0.3, 1.9)
Metacognition Index (MI)	89	132	1.3 (0.8, 2.2)	19	1.2 (0.5, 2.9)	9	1.3 (0.4, 4.2)	18	0.9 (0.4, 2.2)

[a]Adjusted for parental education, maternal IQ, maternal mental health status, prenatal smoking, prenatal drinking, parity, maternal age at child birth, child's sex, maternal pre-pregnancy BMI, maternal musculoskeletal diseases, fever or infection/inflammation during pregnancy, and maternal use of ibuprofen or aspirin. Estimates for TEACh-5 were additionally adjusted for tester.

[b]Subnormal attention (TEACh-5) was defined as 1 SD below the mean, and subnormal executive function (BRIEF) was defined as 1 SD above the mean.

[c]For preschool teacher-rated BRIEF outcomes, we analysed 520 children with mothers who never used paracetamol during pregnancy, 772 who ever used paracetamol and 136, 66, and 141 who only used during the first, second or third trimester, respectively

Table 4. Odds ratios for subnormal attention or executive function in 5-year-old children according to total gestational weeks of paracetamol use

Neuropsychological measures[b]	Never use ($n = 610$)	Gestational weeks of paracetamol use in pregnancy							
		1 week ($n = 212$)		2-5 weeks ($n = 242$)		> 5 weeks ($n = 211$)		Per 1-week increase in use	
	n (reference)	*n*	OR[a] (95% CI)	*n*	OR[a] (95% CI)	*n*	OR[a] (95% CI)	OR[a] (95% CI)	*P*-trend
Subnormal attention (TEACh-5)									
Overall attention	89	29	1.0 (0.5, 2.1)	51	2.1 (1.1, 3.8)	36	1.6 (0.8, 3.1)	1.06 (1.00, 1.13)	0.05
Selective attention	93	32	1.5 (0.8, 3.0)	44	1.5 (0.8, 2.9)	30	1.3 (0.6, 2.5)	1.04 (0.97, 1.11)	0.28
Sustained attention	103	31	1.7 (0.9, 3.2)	50	1.6 (0.9, 2.9)	31	0.8 (0.3, 1.8)	0.98 (0.92, 1.04)	0.48
Subnormal executive function (BRIEF), Parent-rated									
General Executive Composite (GEC)	86	30	0.9 (0.4, 1.9)	45	1.6 (0.9, 2.9)	39	1.4 (0.7, 2.8)	1.06 (0.99, 1.12)	0.09
Behavioral Regulation Index (BRI)	82	28	0.8 (0.4, 1.9)	42	1.6 (0.9, 2.9)	38	1.3 (0.7, 2.6)	1.05 (1.00, 1.11)	0.06
Metacognition Index (MI)	87	34	1.0 (0.5, 2.0)	44	1.7 (0.9, 3.2)	42	1.7 (0.9, 3.2)	1.07 (1.01, 1.14)	0.02
Subnormal executive function (BRIEF), Teacher-rated[c]									
General Executive Composite (GEC)	89	27	1.5 (0.7, 3.1)	42	1.7 (0.9, 3.4)	27	0.8 (0.4, 1.8)	0.99 (0.92, 1.06)	0.69
Behavioral Regulation Index (BRI)	81	25	0.9 (0.4, 2.0)	45	1.6 (0.8, 2.9)	26	0.6 (0.3, 1.4)	0.94 (0.87, 1.02)	0.12
Metacognition Index (MI)	89	28	1.3 (0.6, 2.9)	42	2.4 (1.0, 4.4)	31	1.2 (0.6, 2.4)	1.02 (0.97, 1.08)	0.46

[a]Adjusted for parental education, maternal IQ, maternal mental health status, prenatal smoking, prenatal drinking, parity, maternal age at child birth, child's sex, maternal pre-pregnancy BMI, maternal musculoskeletal diseases, fever or infection/inflammation during pregnancy, and maternal use of ibuprofen or aspirin. Estimates for TEACh-5 were additionally adjusted for tester.

[b]Subnormal attention (TEACh-5) was defined as 1 SD below the mean, and subnormal executive function (BRIEF) was defined as 1 SD above the mean.

[c]For preschool teacher-rated BRIEF outcomes, we analysed 520 children with mothers who never used paracetamol during pregnancy, and 185, 212 and 183 who used paracetamol for 1, 2-5 or > 5 weeks in pregnancy, respectively

Table 5. Odds ratios for subnormal attention or executive function in 5-year-old children according to maternal prenatal use of paracetamol, by child's sex or indications of use

Use of paracetamol during pregnancy	Adjusted OR (95% CI)[a]					
	Subnormal attention (TEACh-5)			Subnormal executive function (BRIEF; parent-rated)		
	Overall	Selective	Sustained	General Executive (GEC)	Behavioral Regulation (BRI)	Metacognition (MI)
Ever use versus never use						
By sex of the child						
Boys only ($n = 769$)	1.5 (0.8, 3.0)	1.6 (0.9, 3.0)	1.1 (0.6, 2.1)	1.5 (0.8, 2.9)	1.8 (0.9, 3.5)	1.5 (0.8, 2.8)
Girls only ($n = 722$)	1.7 (0.8, 3.9)	1.6 (0.8, 3.3)	1.4 (0.6, 3.2)	1.0 (0.5, 2.1)	0.9 (0.4, 1.8)	1.5 (0.7, 3.0)
By indication of use in pregnancy						
Among mothers with no fever ($n = 1021$)	1.9 (1.1, 3.4)	1.6 (1.0, 2.8)	1.0 (0.6, 1.8)	1.5 (0.8, 2.5)	1.4 (0.8, 2.5)	1.7 (1.0, 2.9)
Among mothers with no infection or inflammation ($n = 1329$)	1.6 (1.0, 2.7)	1.5 (0.9, 2.5)	1.3 (0.8, 2.2)	1.5 (0.9, 2.4)	1.4 (0.8, 2.3)	1.6 (1.0, 2.5)
Among mothers with no musculoskeletal diseases ($n = 1338$)	1.6 (1.0, 2.7)	1.5 (0.9, 2.5)	1.2 (0.8, 2.0)	1.4 (0.9, 2.4)	1.3 (0.8, 2.2)	1.7 (1.0, 2.7)

[a]Adjusted for parental education, maternal IQ, maternal mental health status, prenatal smoking, prenatal drinking, parity, maternal age at child birth, child's sex, maternal pre-pregnancy BMI, maternal musculoskeletal diseases, fever or infection/inflammation during pregnancy, and maternal use of ibuprofen or aspirin. Estimates for TEACh-5 were additionally adjusted for tester.

[b]Subnormal attention (TEACh-5) was defined as 1 SD below the mean, and subnormal executive function (BRIEF) was defined as 1 SD above the mean.

The underlying biological mechanisms explaining the potential link between paracetamol and neurodevelopment are still unknown, but some have been proposed. Paracetamol can cross the placenta and the fetal brain barrier during critical periods of development.[18,19] Research data are accumulating that paracetamol exhibits endocrine-disruptive properties and is capable of altering animal and human reproductive function.[20–22] Hormone signalling is tightly regulated during pregnancy, and disruptions of its balance may affect neurodevelopment of the fetus.[23,24] Furthermore, recent animal data suggested that cognition and behaviours can be affected by exposure to therapeutic doses of paracetamol.[25–27] A study in mice showed that paracetamol (2 x 30 mg/kg) administered to neonates during brain development resulted in altered locomotor activity and failure to acquire spatial learning in adulthood.[26] Moreover, in the same study, levels of the brain-derived neurotrophic factor (BDNF) in the neonatal brain were also affected. Another study reported that paracetamol causes direct neurotoxicity and induces concentration-dependent neuronal death of rat cortical neurons both in vitro and in vivo.[28] Nevertheless, more research is still needed to further elucidate potential mechanisms of neurodevelopmental toxicity of paracetamol, with particular emphasis on in utero exposure.

Our study has several strengths. First, data on paracetamol use were ascertained from mothers in three interviews before the outcome assessment in children. Denmark has a register recording redeemed pharmaceutical prescriptions, but prescription databases do not capture the use of over the counter medications such as paracetamol during pregnancy. Second, the attention function tests were administered by trained psychologists blinded to the exposure status and with rigorous quality control procedures. Measures of executive function were obtained from both parents and preschool teachers independently. Third, we were able to control for a wide range of potential confounders, including maternal IQ, maternal mental health, parental education and some important indications of paracetamol use. Finally, participants were selected from a well-designed longitudinal cohort and we accounted for sampling and non-participation using weighted regression analyses to minimize the potential influence of selection bias on our estimates.

Some limitations of the study should also be acknowledged. Given the observational nature of our study, we cannot rule out the possibility of uncontrolled confounding, in particular by unmeasured indications of drug use and other lifestyle factors. The exact reason for paracetamol use was unknown, but we were able to adjust for some conditions associated with paracetamol use such as fever, infection/inflammation and musculoskeletal diseases. The results were also robust in subgroup analysis restricted to the mothers who did not experience these conditions during pregnancy. Furthermore, our results did not change after controlling for the use of aspirin and ibuprofen, i.e. medications also commonly used to treat fever and pain, but with a much lower frequency of use by pregnant women in Denmark possibly due to contraindications. Uncontrolled genetic confounding is also possible if genes that affect ADHD-like behavioural traits are associated with maternal medication use behaviours in pregnancy.[29] Although differential recall bias is unlikely because mothers were interviewed several years before outcomes assessment, non-differential exposure misclassification due to flawed recall of drug names and frequency and timing of use could potentially bias the effect estimates towards the null. Many women (> 80%) did not recall the exact number or doses of paracetamol, limiting our dose-response analysis. Findings based on parent-reported BRIEF are susceptible to correlated errors in exposure and outcome assessment, but this is not expected to affect TEACh-5 measures. The attention and executive scales measured in children as young as 5 years may have large variability leading to an underestimation of effect sizes.

In conclusion, we found some evidence that paracetamol use during pregnancy was moderately associated with subnormal attention and executive function in the offspring at age 5. Our findings add to the growing body of evidence in the literature suggesting that paracetamol exposure *in utero* may alter neurodevelopment in the offspring.

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