



Original article

Acetaminophen use in pregnancy and neurodevelopment: attention function and autism spectrum symptoms

Claudia B. Avella-Garcia,^{1,2,3,4,5} Jordi Julvez,^{1,3,6,*} Joan Fortuny,⁷ Cristina Rebordosa,⁷ Raquel García-Esteban,^{1,3,6} Isolina Riaño Galán,⁸ Adonina Tardón,^{6,9} Clara L. Rodríguez-Bernal,¹⁰ Carmen Iñiguez,¹⁰ Ainara Andiarena,^{11,12} Loreto Santa-Marina^{6,12,13} and Jordi Sunyer^{1,3,4,5}

¹Center for Research in Environmental Epidemiology (CREAL), ²Unitat Docent de Medicina Preventiva i Salut Pública H. Mar-UPF-ASPB, ³IMIM (Hospital del Mar Medical Research Institute), ⁴Universitat Pompeu Fabra (UPF), ⁵Universitat Autònoma de Barcelona, Barcelona, Spain, ⁶CIBER Epidemiología y Salud Pública (CIBERESP), Spain, ⁷RTI Health Solutions, Barcelona, Spain, ⁸Servicio de Pediatría, Hospital San Agustín, Aviles Asturias, Spain, ⁹Public Health Department, University of Oviedo, Oviedo, Spain, ¹⁰Environment and Health Area, CSISP-FISABIO-REDISSEC, Valencia, Spain, ¹¹Basic Psychological Processes and Development Department, Faculty of Psychology, University of the Basque Country, Gipuzkoa, ¹²Health Research Institute, Biodonostia, San Sebastián, Spain and ¹³Public Health Division of Gipuzkoa, Gipuzkoa, Basque Government, Spain

*Corresponding author. Centre for Research in Environmental Epidemiology-PRBB, C. Doctor Aiguader 88, 08003 Barcelona, Spain. E-mail: jjulvez@creal.cat

Accepted 13 April 2016

Abstract

Background: Acetaminophen is extensively used during pregnancy. But there is a lack of population-representative cohort studies evaluating its effects on a range of neuropsychological and behavioural endpoints. We aimed to assess whether prenatal exposure to acetaminophen is adversely associated with neurodevelopmental outcomes at 1 and 5 years of age.

Methods: This Spanish birth cohort study included 2644 mother-child pairs recruited during pregnancy. The proportion of liveborn participants evaluated at 1 and 5 years was 88.8% and 79.9%, respectively. Use of acetaminophen was evaluated prospectively in two structured interviews. Ever/never use and frequency of use (never, sporadic, persistent) were measured. Main neurodevelopment outcomes were assessed using Childhood Autism Spectrum Test (CAST), Conner's Kiddie Continuous Performance Test (K-CPT) and ADHD-DSM-IV form list. Regression models were adjusted for social determinants and co-morbidities.

Results: Over 40% of mothers reported using acetaminophen. Ever-exposed offspring had higher risks of presenting more hyperactivity/impulsivity symptoms [incidence rate ratio (IRR) = 1.41, 95% confidence interval (CI) 1.01–1.98], K-CPT commission errors (IRR = 1.10,

1.03–1.17), and lower detectability scores (coefficient $\beta = -0.75$, -0.13 – -0.02). CAST scores were increased in ever-exposed males ($\beta = 0.63$, 0.09 – 1.18). Increased effect sizes of risks by frequency of use were observed for hyperactivity/impulsivity symptoms (IRR = 2.01, 0.95 – 4.24) in all children, K-CPT commission errors (IRR = 1.32, 1.05 – 1.66) and detectability ($\beta = -0.18$, -0.36 – 0.00) in females, and CAST scores in males ($\beta = 1.91$, 0.44 – 3.38).

Conclusions: Prenatal acetaminophen exposure was associated with a greater number of autism spectrum symptoms in males and showed adverse effects on attention-related outcomes for both genders. These associations seem to be dependent on the frequency of exposure.

Key words: Acetaminophen, paracetamol, pregnancy, attention function, neurodevelopment, autism spectrum symptoms

Key Messages

- Prenatal exposure to acetaminophen may increase autism spectrum symptoms in male children.
- Maternal use of acetaminophen during pregnancy may be harmful to attention function and may be associated with a higher risk of hyperactivity/impulsivity symptoms in the offspring.
- Prenatal acetaminophen exposure was not found to be associated with effects on cognitive, motor or social development.

Introduction

Acetaminophen (paracetamol) is an over-the-counter medication that is widely used by pregnant women as an antipyretic and analgesic.¹ Nevertheless, there is evidence linking prenatal and early life acetaminophen use with alterations of neurodevelopment.^{2–5}

The prevalence of attention-deficit/hyperactivity disorder (ADHD) and autism spectrum conditions (ASC) has increased during recent decades,⁶ reaching around 5% and 1% of children in Western countries, respectively.^{7,8} The factors influencing this trend are unclear, but environmental factors, such as acetaminophen use, may be potential contributors.⁶ An ecological correlation between early life acetaminophen use and ASC prevalence⁴ suggests an association. Recent cohort studies also report a harmful action of acetaminophen on neurodevelopment, especially regarding ADHD-related outcomes.^{2,3,5}

Further assessment of the effects of prenatal exposure to acetaminophen on child development is warranted using prospective in-person neuropsychological evaluation of children. Continuous measures of neurological outcomes are also needed to better explore milder dysfunctions that may not be severe enough to reach diagnostic thresholds or require the use of medication. Therefore, the aim of this study is to evaluate whether maternal use of acetaminophen during pregnancy is adversely associated with child

neurodevelopment at 1 and 5 years of age, by using data from the INfancia y Medio Ambiente (INMA) project to address these issues.

Methods

Study population

The INMA Project's main objective is to examine the health effects of early life exposures⁹ in a birth cohort including participants from different regions of Spain. Participants were recruited from four different regions during the time periods that follow: Asturias (2004–07), Gipuzkoa (2006–08), Sabadell (2004–07) and Valencia (2004–05). Mothers were considered eligible for inclusion if they were residents in the cohort area, at least 16 years old, were carrying a singleton pregnancy and were planning to give birth at the reference hospital. Mothers who had participated in an assisted fertility programme and those with communication difficulties were excluded (Appendix 1, available as Supplementary data at *IJE* online). For the regional cohorts, the proportion of participants in INMA out of the women identified as eligible was 60% for Sabadell, 54% for Valencia, 45% for Asturias and 68% for Gipuzkoa. For Sabadell, there was a higher educational level among participants compared with

non-participants, for Gipuzkoa a higher proportion of working mothers participated and for Valencia there was also a higher proportion of older women and working mothers among participants. There were no differences between participants and non-participants in Valencia. At 1 and 5 years of age, 88.8% and 79.9% of all children live-born to recruited mothers were included in the study. [Appendix 2](#) (available as [Supplementary data](#) at *IJE* online) describes participant disposition throughout the study. Written consent was obtained from all participants at recruitment and at each follow-up. Approval was given by the Institut Municipal d'Investigació Mèdica, Barcelona and the ethics committees of each participating institution.

Acetaminophen exposure

Data were collected prospectively by interviewing the expectant mothers twice, at weeks 12 and 32 of pregnancy, using standardized questionnaires completed by trained evaluators. Exposure information was obtained by asking the question 'Have you taken any medication (sporadically or continuously) since 1 month before becoming pregnant or during this pregnancy?' If the answer was positive, the name of the medication, dose, duration, gestational age at use and the indication as reported by the mother were enquired using open questions. All medications taken during pregnancy or 1 month before pregnancy were documented in order to consider the uncertainty of the date of conception. At week 32, mothers were asked about use of medication after week 12. Data were coded by a pharmacologist. Acetaminophen could have been used as a single drug or as a fixed-dose combination. Mothers were classified as users of acetaminophen during pregnancy if they had taken any dose of acetaminophen at any time up to week 32 of pregnancy or the month before becoming pregnant. Otherwise, they were considered non-exposed. Gestational age at acetaminophen use was used to identify and determine the number of trimesters of exposure. If mothers had taken acetaminophen in the month previous to pregnancy, exposure was considered to have occurred during the first trimester. Indication for use of acetaminophen was classified as analgesia, infection or other indications. Frequency of acetaminophen use was defined as never, sporadic (use of any dose in one or two trimesters) or persistent (use of any dose in all three trimesters).

Measures of neurodevelopmental outcomes

Neuropsychological development was assessed at a mean child age of 14.84 [standard deviation (SD): 2.69] months using the Bayley Scales of Infant Development (BSID).¹⁰ Children were tested again at a mean age of 4.8 (SD: 0.62)

years with a battery of tests: McCarthy Scales of Children's Abilities (MCSA)¹¹ to evaluate cognitive and psychomotor development, California Preschool Social Competence Scale (CPSCS) for assessment of social competence,¹² Childhood Autism Spectrum Test (CAST)¹³ which quantifies autism spectrum symptoms in children (each point represents one symptom of ASC with a cut-off of 15 or more points, having a 100% sensitivity and 97% specificity for ASC¹⁴), Attention-Deficit/Hyperactivity Disorder Criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition form list (ADHD-DSM-IV)¹⁵ for identification of inattention and hyperactivity/impulsivity symptoms (which are as valid for ADHD diagnosis in children from 2 to 5 years of age as they are in older children¹⁶) and Conner's Kiddie Continuous Performance Test (K-CPT)¹⁷, a computerized test that evaluates attention function, reaction time, accuracy and impulse control. Measures on K-CPT include: omission errors in which a target stimulus is presented but the child fails to respond to it; commission errors in which the child responds to a non-target stimulus; HRT-SE (Hit Reaction Time Standard Error) which corresponds to the variation in time of latency before a response; and detectability which shows the capacity to distinguish target from non-target stimuli. These K-CPT variables have all been correlated to ADHD symptoms and have been repeatedly used for ADHD research.^{18,19}

A common protocol was followed for evaluation. BSID and MSCA were performed by a trained psychologist who also administered the CAST questionnaire to the child's parents in order to increase CAST score accuracy. ADHD-DSM-IV and CPSCS were teacher-rated (for details on all tests see [Appendix 3](#), available as [Supplementary data](#) at *IJE* online).

Other variables

Standardized questionnaires were used to collect data on other variables of interest such as socio-demographic characteristics, mothers' medical history, and child health-related events. Data on other variables such as anthropometric measures or parental mental health and cognitive scores were obtained through manual review of clinical records, tests performed by a trained psychologist or self-completed rating scales ([Appendix 3](#)).

Statistical analysis

All outcome scores were treated as continuous variables, rather than using cut-off points to evaluate the outcomes. Multivariable linear regression models were used to estimate the effects of acetaminophen exposure on BSID,

MSCA, CPSCS, K-CPT HRT-SE and detectability scores. The remaining K-CPT outcomes and ADHD-DSM-IV symptom scores were evaluated using negative binomial regression models to account for over-dispersion of the data, and results are shown as IRR (incidence rate ratios) which should be interpreted as relative risks. CAST scores were analysed with both linear regression and negative binomial regression models obtaining consistent results with similar *P*-values and are given as linear regression coefficients which show the difference between groups in terms of the number of ASC symptoms. Pooled analyses of data from the four study regions were performed as there was no interaction ($P = 0.658$ at 1 year and $P = 0.393$ at 5 years of age) between the exposure and the regional cohorts in relation to the outcome.

To address confounding, we adjusted for a series of predefined variables that were forced to remain in the models. Predefined variables were selected based on previous literature^{2,3,12,19} (i.e. region, child gender, age at testing, gestational age at birth, quality of test as rated by the performing psychologist-only for BSID and MSCA, maternal social class, IQ, education and whether the mother reported having any chronic illness, fever or urinary tract infection-not necessarily related to acetaminophen use-during pregnancy; for outcomes at 1 year of age, child age at testing was adjusted for prematurity). Other covariates were included in the models only when they showed a crude association with both the exposure and the outcome (P -values < 0.20) and caused a change $> 5\%$ in the regression coefficient of acetaminophen when they were introduced one by one in the basic model (Appendix 4, available as Supplementary data at *IJE* online). See result table footnotes for the covariates retained in each model (Tables 2 and 3).

Confounding by indication was addressed by including reported maternal chronic illness, fever or urinary tract infections at any time during pregnancy in all models. Sensitivity analyses were performed by: (i) excluding mothers with each of these conditions; (ii) excluding mothers with any of these; and (iii) including exposed mothers by indication (analgesia/infection) to evaluate possible 'within exposure group' variations by indication.

We assessed outcome-association differences between never and ever exposed groups, exposure in specific trimesters (or combinations of these) and frequency of acetaminophen use. Finally, because previous literature^{20,21} suggests gender differences in the prevalence and clinical manifestations of ASC and ADHD, the exposure interaction by gender for the related scales used here (ADHD-DSM-IV, K-CPT, CAST) was assessed. An interaction was found in relation to K-CPT and CAST outcomes, so we stratified these results by gender. The relationship between

ADHD-DSM-IV and CAST scores was assessed by Spearman correlation. Sensitivity analyses were done evaluating the association between prenatal acetaminophen exposure and CAST scores after excluding children who met ADHD-DSM-IV form list diagnostic criteria for ADHD. Further analyses examined the association between prenatal acetaminophen use and ADHD-DSM-IV symptoms after excluding children with CAST scores compatible with a possible diagnosis of ASC (score > 15).

Statistical analyses were performed using STATA Special Edition 12.1 (Stata Corp., College Station, TX, USA).

Results

In all, 43% of children evaluated at age 1 ($n = 2195$) and 41% of those assessed at age 5 ($n = 2001$) were exposed to acetaminophen up to gestational week 32. Analgesia was the main indication for use (66% of reported indications). Overall, mothers had a mean age of 31 years; 37% had a university degree, 5% reported fever, 11% reported urinary tract infection and 32% reported having chronic illness during pregnancy. Table 1 shows the characteristics of the study population for both follow-ups. Exposed mothers were younger and had a lower education level than non-exposed mothers. Compared with the mothers of study participants, the mothers of children who did not participate in the follow-ups were younger, more likely to come from countries other than Spain, presented a lower education level and social class stratum, were more likely to have smoked during pregnancy and were less likely to have breastfed their children (results not shown).

Hyperactivity/impulsivity symptoms and attention function (K-CPT)

In Table 2, we present the associations between prenatal exposure to acetaminophen and child neurodevelopment outcomes at 5 years of age. Exposed children showed a higher risk of presenting hyperactivity/impulsivity symptoms than non-exposed children (IRR = 1.41, 95% CI 1.01–1.98), a greater risk of K-CPT commission errors (IRR = 1.10, 95% CI 1.03–1.17) and lower detectability scores ($\beta = -0.75$, 95% CI -0.13 – -0.02), than non exposed children after full multivariate adjustment.

Table 3 presents exposure-outcome associations taking into account the frequency of acetaminophen use. Persistently exposed children showed poorer attention function as evidenced by greater variability of K-CPT HRT-SE, and more K-CPT omission errors (IRR = 1.29, 95% CI 1.02–1.64) compared with non-exposed children. Indeed, such outcomes presented association trends (Table 3 footnotes). In addition, the association pattern with attention

Table 1. Characteristics of study participants according to any acetaminophen exposure at each follow-up period

Characteristics	15 months of age			5 years of age		
	Exposed (n = 955)	Non-exposed (n = 1240)	P-value	Exposed (n = 828)	Non-exposed (n = 1173)	P-value
Male gender, n (%)	486 (51)	634 (51)	0.881	428 (52)	599 (51)	0.783
Premature, n (%)	38 (4)	52 (4)	0.802	33 (4)	52 (4)	0.631
Breastfeeding, n (%)						
None	131 (14)	185 (15)		115 (14)	176 (15)	
0–16 wk	250 (26)	288 (23)		212 (26)	263 (22)	
16–24 wk	131 (14)	206 (17)	0.114	115 (14)	197 (17)	0.112
> 24 wk	427 (45)	527 (43)		370 (45)	484 (41)	
Maternal social class, n (%)						
Low	192 (20)	299 (24)		183 (22)	293 (25)	
Middle	244 (26)	342 (28)	0.015	271 (26)	327 (28)	0.127
High	518 (54)	599 (48)		427 (52)	553 (47)	
Maternal education, n (%)						
Primary or less	262 (27)	237 (19)		204 (25)	213 (18)	
Secondary	420 (44)	491 (40)	< 0.001	372 (45)	460 (39)	< 0.001
University	271 (28)	509 (41)		248 (30)	497 (42)	
Any maternal smoking in pregnancy, n (%)	348 (36)	318 (26)	< 0.001	288 (35)	288 (25)	< 0.001
Maternal chronic illness, n (%)	344 (36)	365 (29)	0.001	311 (38)	350 (30)	< 0.001
Maternal fever, n (%)	86 (9)	25 (2)	< 0.001	68 (8)	24 (2)	< 0.001
Maternal UTI, n (%)	126 (13)	111 (9)	0.001	100 (12)	110 (9)	0.051
Maternal age, median (IQR), years	30 (27–33)	31 (29–34)	< 0.001	30 (28–33)	31 (29–34)	< 0.001
Maternal verbal IQ, Median (IQR)	9.76 (8.29–19)	9.76 (7.56–19)	0.231	9.76 (8.29–19)	9.76 (8.29–19)	0.25

Percentages are rounded to nearest whole number. Maternal chronic illness included: diabetes, heart conditions, alterations of blood coagulation, renal or suprenal disease, thyroid alterations, tuberculosis, chronic intestinal inflammatory disease, reproductive apparatus tumours and other chronic illnesses diagnosed by a physician and reported by the mother. It did not include psychiatric diagnoses, as maternal mental health measures were included as separate covariates.

IQ, Intelligence Quotient; IQR, interquartile range; UTI, urinary tract infection; wk, weeks.

Table 2. Crude^a and adjusted^b associations between exposure to any acetaminophen *in utero* and child attention-related and autism spectrum outcomes at 5 years of age

Outcome	N ^c	Crude IRR (95% CI)	Adjusted IRR (95% CI)
ADHD-DSM-IV total symptoms (score range 0–18)	1382	1.08 (0.84–1.39)	1.25 (0.93–1.69)
Inattention symptoms (0–9)	1382	0.95 (0.71–1.29)	1.12 (0.79–1.58)
Hyperactivity/impulsivity symptoms (0–9)	1382	1.23 (0.92–1.63)	1.41 (1.01–1.98)*
K-CPT omission errors (0–129)	1255	0.76 (0.70–0.84)*	0.98 (0.89–1.08)
K-CPT commission errors (0–49)	1255	1.12 (1.05–1.19)*	1.10 (1.03–1.17)*
		β (95% CI)	β (95% CI)
K-CPT HRT-SE (9–123)	1255	–0.07 (–0.12–0.02)*	0.02 (–0.03–0.07)
K-CPT detectability (0–2)	1255	–0.09 (–0.14–0.04)*	–0.08 (–0.13–0.02)*
CAST all children (0–31)	1467	–0.21 (–0.55–0.13)	0.08 (–0.28–0.44)
CAST males	751	0.18 (–0.32–0.69)	0.63 (0.09–1.18)*
CAST females	716	–0.63 (–1.06–0.20)*	–0.51 (–0.98–0.05)*

Higher scores in the outcomes mean adverse neurodevelopment endpoints, with the exception of K-CPT (detectability). For CAST, each score point represents one autism spectrum symptom.

ADHD, Attention-Deficit/Hyperactivity Disorder DSM-IV form list of symptoms; CAST, Childhood Autism Spectrum Test; K-CPT, Conner's Kiddie Continuous Performance Test; HRT-SE, Hit Reaction Time Standard Error.

^aExposure and outcome only.

^bAdjusted by cohort, child gender, age at testing, gestational age at birth, and maternal social class, education, IQ, chronic illness, fever during pregnancy, urinary infection during pregnancy, use of any other medication. K-CPT and CAST outcome models were not adjusted for use of any other medication.

^cNumber of subjects with acetaminophen exposure, neurodevelopment outcome and potential confounders available.

*P-value < 0.05.

Table 3. Dose-response analysis^a of acetaminophen *in utero* and child K-CPT and CAST outcomes at the age of 5 years

Exposure	K-CPT					CAST	
	HRT-SE		Omission errors	Commission errors	Detectability	Total score	
	N ^d	β (95% CI)	IRR (95% CI)	IRR (95% CI)	β (95% CI)	n	β 95% CI
All participants							
Never	676	0 (Ref)	1 (Ref)	1 (Ref) [†]	0 (Ref) [†]	823	0 (Ref)
Sporadic ^b	531	0.01 (−0.04; 0.06)	0.96 (0.87; 1.06)	1.10 (1.02; 1.17)*	−0.08 (−0.13; −0.03)*	595	0.03 (−0.33; 0.40)
Persistent ^c	48	0.18 (0.05; 0.30)*	1.29 (1.02; 1.64)*	1.09 (0.93; 1.28)	−0.01 (−0.14; 0.12)	49	0.81 (−0.13; 1.73)
Males							
Never	330	0 (Ref)	1 (Ref)	1 (Ref)	0 (Ref)	421	0 (Ref) [†]
Sporadic ^b	272	0.02 (−0.05; 0.09)	1.00 (0.87; 1.15)	1.05 (0.97; 1.15)	−0.06 (−0.13; 0.01)	308	0.56 (0.001; 1.11)*
Persistent ^c	21	0.22 (0.03; 0.41)*	1.56 (1.09; 2.24)*	0.83 (0.67; 1.04)	0.21 (0.03; 0.39)*	22	1.91 (0.44; 3.38)*
Females							
Never	346	0 (Ref)	1 (Ref)	1 (Ref) [†]	0 (Ref) [†]	402	0 (Ref)
Sporadic ^b	259	−0.01 (−0.08; 0.07)	0.93 (0.81; 1.07)	1.14 (1.03; 1.26)*	−0.10 (−0.18; −0.02)*	287	−0.54 (−1.01; −0.06)*
Persistent ^c	27	0.16 (−0.01; 0.32)	1.07 (0.78; 1.45)	1.32 (1.05; 1.66)*	−0.18 (−0.36; 0.00)	27	−0.22 (−1.34; 0.90)

CAST: Childhood Autism Spectrum Test, HRT-SE: Hit Reaction Time Standard Error, K-CPT: Conner's Kiddie Continuous Performance Test, Ref: Reference value.

Higher scores in the outcomes mean adverse neurodevelopment endpoints, with the exception of K-CPT (Detectability). For CAST, each score point represents 1 autism spectrum symptom. All results are adjusted for: regional cohort, age at testing, gestational age at birth, child gender in the case of all participants, and maternal social class, IQ, education, chronic illness, fever during pregnancy and urinary tract infection during pregnancy.

^bSporadic: use of acetaminophen in 1 or 2 trimesters of pregnancy.

^cPersistent: use of acetaminophen during all 3 trimesters of pregnancy.

^dNumber of subjects with acetaminophen exposure, neurodevelopment outcome and potential confounders available.

*P-value < 0.05.

[†]indicates P trend < 0.05 (Commission errors all children: 0.012, Commission errors females: 0.002, Detectability all children: 0.026, Detectability females: 0.007, CAST males: 0.006). P for interaction by gender: HRT-SE (0.41), Omission errors (0.22), Commission errors (0.04), Detectability (0.02), CAST (0.02).

function (commission errors and detectability) was modified by gender (P for interaction in Table 3 footnotes). In females, persistent exposure was associated with a higher risk of commission errors (IRR = 1.32, 95% CI 1.05–1.66) and poorer detectability scores (β = −0.18, 95% CI −0.36–0.00). eTable 1 (available as Supplementary data at IJE online) presents the results for ADHD-DSM-IV symptom scores by frequency of acetaminophen use, in which we observed a significant association trend (P trend = 0.03) for hyperactivity/impulsivity symptoms, but no modification effect by child gender.

ASC symptoms

In exposed male children, there was an increase in CAST symptom scores (β = 0.63, 95% CI 0.09–1.18), that differed significantly from the result found in female peers, who showed a certain decrease in CAST scores (P interaction = 0.01) (Table 2). Furthermore, persistently exposed male children presented more autism spectrum symptoms (β = 1.91, 0.44–3.38), and this association showed a P for trend = 0.006 by increasing frequency of the exposure (Table 3). In females there was no clear trend by frequency of exposure.

Sensitivity analyses

Sensitivity analyses were performed excluding mothers reporting: (i) fever (eTable 2); (ii) chronic illness (eTables 3 and 4, available as Supplementary data at IJE online); (iii) urinary tract infection (data not shown); or (iv) any of these three conditions (data not shown), during pregnancy; as well as analyses including only mothers whose indication for acetaminophen use was analgesia (eTables 5 and 6, available as Supplementary data at IJE online) or infection (eTables 7 and 8, available as Supplementary data at IJE online). The results of these analyses were similar, following the same direction of the effect and dose-response trends for acetaminophen.

CAST and ADHD-DSM-IV scores showed a Spearman correlation of 0.21 (P -value < 0.001). The association by co-occurrence of ADHD and ASC cases (according to ADHD-DSM-IV form list and CAST criteria), was moderate (eTable 9, available as Supplementary data at IJE online). Sensitivity analyses evaluating the association between prenatal acetaminophen exposure and CAST scores only in children not fulfilling ADHD-DSM-IV form list criteria for ADHD (eTable 10, available as Supplementary data at IJE online); and examining the association with ADHD-DSM-IV scores only in children

with CAST scores < 15 (that do not reach the diagnostic threshold for suspecting ASC according to the CAST); showed virtually no change (eTable 11, available as Supplementary data at IJE online), with similar dose-response trends.

No changes were observed after adjustment for other maternal medication use during pregnancy (data not shown). No different association patterns emerged from analysis by exposure in specific trimesters or their combinations (data not shown).

Other neurodevelopmental outcomes

No differences between acetaminophen exposure groups (ever exposed vs non-exposed) were found in relation to the other developmental outcomes, for: BSID evaluating mental and psychomotor development at 1 year of age ($\beta = 0.75$, 95% CI -0.75 – 2.25), MSCA assessing cognitive and motor development at 5 years of age ($\beta = -0.21$, 95% CI -1.70 – 1.28) or CPSCS examining social competence at 5 years of age ($\beta = -1.15$, 95% CI -3.16 – 0.87) in the fully adjusted models (eTable 12, available as Supplementary data at IJE online).

Discussion

In this study, over 40% of children were exposed to acetaminophen *in utero*. Exposure was associated with lower attention function development as measured by K-CPT parameters and with presenting greater risk for more ADHD-DSM-IV hyperactivity/impulsivity symptoms. Exposure was also related to a greater number of CAST autism spectrum symptoms in male children. Similar findings were observed in relation to the frequency of acetaminophen use, suggesting a dose-response effect. No changes in general cognitive or social development were observed.

To our knowledge, this is the first prospective study to report an independent association between the use of acetaminophen during pregnancy and autism spectrum symptomatology in exposed children. It is also the first paper to report differential gender effects of prenatal acetaminophen exposure on neurodevelopment.

In our study, there was a weak positive correlation between ADHD-DSM-IV and CAST scores, which was expected because ADHD and ASC features frequently co-occur in the same individuals. Indeed, both share similar neuropsychological substrates despite being considered two distinct disorders and have similar epidemiological characteristics such as being more prevalent in males and having increased in prevalence in recent decades.²² Therefore, it was important to analyse whether the associations between acetaminophen exposure and ADHD and ASC symptoms

were independent from each other. The results of our sensitivity analyses suggest separate adverse effects of this exposure on each of the outcomes. Different mechanisms may explain acetaminophen's harmful influence on neurodevelopment. These include the stimulation of the endocannabinoid system which could affect neuronal differentiation, axonal migration, synapse positioning or immune modulation,^{23,24,25} toxicity due to deficits in sulphation capacity (reduced during pregnancy and in some autistic children)^{1,4,26} and oxidative stress.²⁷ Finally, acetaminophen may act as an endocrine disruptor affecting testicular function and the production of androgens, which could interfere with fetal brain development.^{28,29}

The contrasting effects of acetaminophen exposure by gender in this study could be linked to sex differences in the metabolism of acetaminophen. Animal studies have suggested that male mice undergo greater toxicity than female mice after being administered a similar dose of acetaminophen.³⁰ Furthermore, the male brain may be more vulnerable to early life stressors²⁰ and this could explain why neuropsychiatric disorders of childhood, such as ASC and ADHD, are more prevalent in male children.^{8,31} Our dissimilar results by gender suggest that androgenic endocrine disruption (to which male brains could be more sensitive) may be a strong candidate as an explanatory mechanism for the association between acetaminophen use and ASC symptoms. Studies on other endocrine disruptors such as bisphenol A have also documented contrasting effects on neurodevelopment according to gender,³² but to our knowledge this is the first study to report this regarding fetal exposure to acetaminophen.

Our findings agree with reports of an association between prenatal exposure to acetaminophen and ADHD behaviours, diagnosis or medication use in childhood^{2,3,5} and with an ecological study that reported an association with ASC prevalence.⁴ Since we did not use cut-off points to evaluate the outcomes, a strength of our study is that it links prenatal exposure to acetaminophen to ADHD and ASC symptoms in a manner that goes beyond examining only disorders, to include milder dysfunctions that are more widespread in the population. A novelty was the use of standardized in-person evaluation of the children by trained psychologists, computer-based measures, teacher-rated scales and specific symptom diagnostic tools for ADHD and ASC symptoms. These proceedings are more objective for neurobehavioural assessment than the self-reported questionnaires used previously.^{2,3,5,12} Another strength is the use of a prospective non-clinical birth cohort, with increased potential for generalizability of the results. Finally, the use of multiple endpoints provides a comprehensive evaluation of different areas of child neurodevelopment.

We were unable to evaluate the effects of dosage because of mothers' difficulties in recalling the dose taken. For this reason, we used a proxy of the frequency of acetaminophen exposure (never, sporadic, persistent), which suggested a trend with growing exposure but which does not allow for differentiating how many doses of acetaminophen were taken in each trimester. Since ADHD and ASC have been associated with maternal infection and inflammation,^{33,34} despite adjustment for reported maternal chronic illness, urinary tract infection and fever, residual confounding by indication could still be a limitation. However, this is unlikely to be a major concern after sensitivity analyses regarding maternal illness and the indication for acetaminophen use hardly changed the results. Reduced sample sizes limited our possibility of running further sensitivity analyses (such as including only: mothers with fever or chronic illness during pregnancy), which could have further confirmed that indication bias was not present. Other limitations include unmeasured genetic confounding, as ADHD and ASC may have genetic components^{35,36} (although a previous study that adjusted for this using data from siblings also documented deleterious effects of prenatal paracetamol exposure on neurodevelopment³), residual confounding from other sources and reliance on maternal report (which can be imprecise) for exposure measurement. Finally, since we have no information on acetaminophen use after week 32, there may be some misclassification of the exposure.

In our study, there was no differential loss to follow-up related to exposure or socioeconomic status, since prevalence was similar at both follow-ups (1 and 5 years), reducing the likelihood of loss to follow-up bias. However, about 50% of the women who were approached for recruitment participated, with a slight tendency for higher socioeconomic status among refusals. Because of this, extrapolation of the results to the general population requires caution. Also, residual confounding due to higher exposure prevalence in mothers with a lower educational level cannot be ruled out. Nevertheless, adjustment for socioeconomic status and education (as well as other covariates), makes this unlikely.

ADHD is the most prevalent neuropsychiatric disorder of childhood and ASC, although less prevalent, can produce important functional compromise. Comparing persistently exposed to non-exposed children, we have detected an increase of around 30% in the risk of detriment of some attention function measures, and an increase of almost two clinical symptoms of ASC in exposed male children, compared with non-exposed males. Acetaminophen use has been documented to be present in 46–56% of pregnancies in several developed countries.^{2,3,5} This widespread exposure could increase the number of children with sub-diagnostic

threshold symptomatology of ADHD and ASC and could worsen the severity of cases in the general population.

Future studies should use large cohorts with more precise exposure measurement, examine the potentially harmful effects of acetaminophen use in neonates, assess the risk/benefit relationship of use in pregnant women and infants undergoing fever or pain-related distress and evaluate therapeutic alternatives as well as the mechanisms through which acetaminophen could produce neurotoxicity.

Conclusions

This is the first cohort study to show that gestational exposure to acetaminophen may increase symptoms of ASC in male children. Our results also suggest that prenatal exposure to this medication can affect attention function at 5 years of age, affecting males and females differently. Further, our results suggest an association with hyperactivity/impulsivity behaviours for all children. Finally, these associations would appear to be dependent on the frequency of exposure, but further dosage assessments are warranted.

Supplementary Data

Supplementary data are available at *IJE* online.

Acknowledgments

We particularly thank all the participants of the INMA PROJECT for their generous collaboration. We thank Silvia Fochs, Anna Sánchez, Maribel López, Muriel Ferrer and Nuria Pey for their support in the fieldwork for Sabadell; Ana Sabater, Belén Plaza, Lucía Fernández, Sara Martínez and Vanesa Gallent for their support in the fieldwork for Valencia; Cristina Arias, Isolina Riaño, José Ignacio Suárez Tomás and Cristina Rodríguez Delhi for their support in the fieldwork for Asturias; and Haizea Begiristain, María Jesús Arroyo, Lourdes Arteche and Mercedes Maiztegi from the Hospital of Zumarraga. A full roster of the INMA PROJECT Investigators can be found at [<http://www.PROYECTOINMA.org/>].

Funding

This work was supported by grants from Instituto de Salud Carlos III (Red INMA G03/176 and CB06/02/0041, CP14/00108, FIS-FEDER 03/1615, 04/1509, 04/1112, 04/1931, 05/1079, 05/1052, 06/1213, 07/0314 and 09/02647), Spanish Ministry of Health (FIS-PI041436, FIS-PI081151, FIS-PI06/0867 and FIS-PS09/00090), Generalitat de Catalunya-CIRIT (1999SGR 0024)1, Miguel Servet (MS) Fellowship (MS14/00108) awarded by the Spanish Institute of Health Carlos III (Ministry of Economy and Competitiveness), Comissionat per a Universitats i Recerca del Departament d'Innovació, Universitats i Empresa de la Generalitat de Catalunya, the Conselleria de Sanitat Generalitat Valenciana (FISS-PI042018, FISS-PI09/02311), Obra Social Cajastur, Universidad de Oviedo, Department of Health of the Basque Government (2005111093 and 2009111069), the Provincial Government of Gipuzkoa (DFG06/004 and DFG08/001) and Fundació Roger Torné.

Conflict of interest: The authors have no conflicts of interest relevant to this article to disclose.

References

- Thiele K, Kessler T, Arck P, Erhardt A, Tiegs G. Acetaminophen and pregnancy: short- and long-term consequences for mother and child. *J Reprod Immunol* 2013;**97**:128–39.
- Liew Z, Ritz B, Rebordosa C, Lee P-C, Olsen J. Acetaminophen use during pregnancy, behavioral problems, and hyperkinetic disorders. *JAMA Pediatr*. 2014;**168**:313–20.
- Brandlistuen RE, Ystrom E, Nulman I, Koren G, Nordeng H. Prenatal paracetamol exposure and child neurodevelopment: a sibling-controlled cohort study. *Int J Epidemiol* 2013;**42**:1702–13.
- Bauer AZ, Kriebel D. Prenatal and perinatal analgesic exposure and autism: an ecological link. *Environ Health* 2013;**12**:41.
- Thompson JMD, Waldie KE, Wall CR, Murphy R, Mitchell EA. Associations between acetaminophen use during pregnancy and ADHD symptoms measured at ages 7 and 11 years. *PLoS One* 2014;**9**:e108210.
- Good P. Did acetaminophen provoke the autism epidemic? *Altern Med Rev* 2009;**14**:364–72.
- Polanczyk G, de Lima MS, Horta BL, Biederman J, Rohde LA. The worldwide prevalence of ADHD: a systematic review and meta-regression analysis. *Am J Psychiatry* 2007;**164**:942–48.
- Wingate M, Kirby R, Pettygrove S, Cunniff C, Schulz E, Ghosh T. Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. *MMWR Surveill Summ* 2014;**63**:1–21.
- Guxens M, Ballester F, Espada M *et al*. Cohort Profile: The INMA—Infancia y Medio Ambiente—(Environment and Childhood) Project. *Int J Epidemiol* 2012;**41**:930–40.
- Bayley N. *Escalas Bayley de Desarrollo Infantil*. (In English : Bayley Scales of Infant Development) Madrid: TEA Ediciones, 1977.
- McCarthy D. *MSCA. Escalas McCarthy de Aptitudes y Psicomotricidad para Niños*. Madrid: TEA Ediciones, 2009.
- Julvez J, Forns M, Ribas-Fito N, Mazon C, Torrent M, Garcia-Esteban R. Psychometric characteristics of the California preschool social competence scale in a Spanish population sample. *Early Educ Dev* 2008;**19**:795–815.
- Scott F, Baron-Cohen S, Bolton P, Brayne C. The CAST (Childhood Asperger Syndrome Test): Preliminary development of UK screen for mainstream primary-school children. *Autism* 2002;**6**:9–31.
- Williams J, Scott F, Stott C *et al*. The CAST (Childhood Asperger Syndrome Test): test accuracy. *Autism* 2005;**9**:45–68.
- American Psychiatric Association. *Manual Diagnóstico y Estadístico de los Trastornos Mentales. IV*. Barcelona: Masson, 2002.
- Sterba S, Egger HL, Angold A. Diagnostic specificity and nonspecificity in the dimensions of preschool psychopathology. *J Child Psychol Psychiatry* 2007;**48**:1005–13.
- Conners C, Staff M. *Conners' Kiddie Continuous Performance Test (K-CPT): Computer Program for Windows Technical Guide and Software Manual*. Toronto, ON: Multi-Health Systems, 2001.
- Epstein JN, Erkanli A, Conners CK, Klaric J, Costello JE, Angold A. Relations between Continuous Performance Test performance measures and ADHD behaviors. *J Abnorm Child Psychol* 2003;**31**:543–54.
- Conners CK, Epstein JN, Angold A, Klaric J. Continuous performance test performance in a normative epidemiological sample. *J Abnorm Child Psychol* 2003;**31**:555–62.
- Rutter M, Bishop D, Pine D *et al*. *Rutter's Child and Adolescent Psychiatry*. 5th edn. Oxford, UK: Blackwell Publishing, 2008.
- Quintero Gutiérrez del Alamo FJ, Correas Lauffer J, Quintero Lumberras F. *Trastorno por Déficit de Atención e Hiperactividad (TDAH) a lo Largo de la Vida*. 3rd edn, Masson, Barcelona; 2009.
- Leitner Y. The co-occurrence of autism and attention deficit hyperactivity disorder in children - what do we know? *Front Hum Neurosci* 2014;**8**:268.
- Schultz ST. Can autism be triggered by acetaminophen activation of the endocannabinoid system? *Acta Neurobiol Exp (Wars)* 2010;**70**:227–31.
- Posadas I, Santos P, Blanco A, Muñoz-Fernández M, Ceña V. Acetaminophen induces apoptosis in rat cortical neurons. *PLoS One* 2010 2014;**5**:e15360.
- Torres AR. Is fever suppression involved in the etiology of autism and neurodevelopmental disorders? *BMC Pediatr* 2003;**3**:9.
- Davies MH, Ngong JM, Yucesoy M *et al*. The adverse influence of pregnancy upon sulphation: a clue to the pathogenesis of intrahepatic cholestasis of pregnancy? *J Hepatol* 1994;**21**:1127–34.
- Ghanizadeh A. Acetaminophen may mediate oxidative stress and neurotoxicity in autism. *Med Hypotheses* 2012;**78**:351.
- Jensen MS, Rebordosa C, Thulstrup AM *et al*. Maternal use of acetaminophen, ibuprofen, and acetylsalicylic acid during pregnancy and risk of cryptorchidism. *Epidemiology* 2010;**21**:779–85.
- Mazaud-Guittot S, Nicolas Nicolaz C, Desdoits-Lethimonier C *et al*. Paracetamol, aspirin, and indomethacin induce endocrine disturbances in the human fetal testis capable of interfering with testicular descent. *J Clin Endocrinol Metab* 2013;**98**:E1757–67.
- McConnachie LA, Mohar I, Hudson FN *et al*. Glutamate cysteine ligase modifier subunit deficiency and gender as determinants of acetaminophen-induced hepatotoxicity in mice. *Toxicol Sci* 2007;**99**:628–36.
- Skounti M, Philalithis A, Galanakis E. Variations in prevalence of attention deficit hyperactivity disorder worldwide. *Eur J Pediatr* 2007;**166**:117–23.
- Evans SF, Kobrosly RW, Barrett ES *et al*. Prenatal bisphenol A exposure and maternally reported behavior in boys and girls. *Neurotoxicology* 2014;**45**:91–99.
- Lee BK, Magnusson C, Gardner RM *et al*. Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. *Brain Behav Immun* 2015;**44**:100–05.
- Silva D, Colvin L, Hagemann E, Bower C. Environmental risk factors by gender associated with attention-deficit/hyperactivity disorder. *Pediatrics*. 2014;**133**:e14–22.
- Talkowski ME, Minikel EV, Gusella JF. Autism spectrum disorder genetics: diverse genes with diverse clinical outcomes. *Harv Rev Psychiatry* 2014;**22**:65–75.
- Valbonesi S, Magri C, Traversa M *et al*. Copy number variants in attention-deficit hyperactive disorder: identification of the 15q13 deletion and its functional role. *Psychiatr Genet* 2015;**25**:59–70.