

Prenatal paracetamol exposure and risk of asthma and elevated immunoglobulin E in childhood

S. O. Shaheen*, R. B. Newson*, A. J. Henderson†, J. E. Headley†, F. D. Stratton†, R. W. Jones†, D. P. Strachan‡ and the ALSPAC Study Team†

*Department of Public Health Sciences, Guy's, King's and St Thomas' School of Medicine, King's College London, London, UK, †Unit of Paediatric and Perinatal Epidemiology, Institute of Child Health, University of Bristol, Bristol, UK and ‡Department of Community Health Sciences, St George's Hospital Medical School, London, UK

Summary

Background We recently found that paracetamol (acetaminophen) use in late pregnancy was associated with an increased risk of early wheezing in the offspring.

Objective To see whether use of paracetamol in late pregnancy is associated with an increased risk of asthma, wheezing and other atopic outcomes in the child at school age.

Methods In the population-based Avon Longitudinal Study of Parents and Children, we measured associations of paracetamol and aspirin use in late pregnancy (20–32 weeks) with asthma, hayfever, eczema ($n = 8511$) and wheezing (8381) in the offspring at 69–81 months, and with atopy (positive skin prick test to *Dermatophagoides pteronyssinus*, cat or grass, $n = 6527$) and blood total IgE ($n = 5148$) at 7 years. We used logistic and linear regression to analyse binary outcomes and log-transformed IgE, respectively, controlling for potential confounders.

Results Use of paracetamol, but not aspirin, in late pregnancy was positively associated with asthma (odds ratios (ORs), comparing children whose mothers took paracetamol 'sometimes' and 'most days/daily' with those whose mothers never took it, 1.22 (95% confidence interval (CI): 1.06–1.41) and 1.62 (95% CI: 0.86–3.04), respectively; P trend = 0.0037), wheezing (ORs 1.20 (95% CI: 1.02–1.40) and 1.86 (95% CI: 0.98–3.55), respectively; P trend = 0.011), and total IgE (geometric mean ratios 1.14 (95% CI: 1.03–1.26) and 1.52 (95% CI: 0.98–2.38), respectively; P trend = 0.0034), but not hayfever, eczema or skin test positivity. The proportion of asthma attributable to paracetamol use in late pregnancy, assuming a causal relation, was 7%.

Conclusion: Paracetamol exposure in late gestation may cause asthma, wheezing and elevated IgE in children of school age.

Keywords asthma, birth cohort, fetal, IgE, paracetamol (acetaminophen), pregnancy, prenatal, wheezing

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Introduction

A number of maternal exposures during pregnancy have been linked to the risk of wheezing, asthma, and other atopic conditions in the offspring. Risk factors include maternal smoking, infections and other complications, and use of antibiotics [1–6]. Following our observations linking frequent paracetamol (acetaminophen) use to asthma and wheeze in adults and children [7, 8], we recently reported a positive association between frequent paracetamol use in pregnancy and risk of early childhood wheezing in the offspring [9]. In the Avon Longitudinal Study of Parents and Children (ALSPAC), a population-based birth cohort, we found that children whose mothers took paracetamol frequently ('on most days or daily') during late pregnancy, but not early

pregnancy, were twice as likely as children whose mothers never took paracetamol to wheeze at 3.5 years of age, particularly if the wheezing started in the first 6 months after birth. Evidence for an increased risk associated with moderate use ('sometimes') was weak, the children were too young to explore possible relations with asthma, and skin test reactivity and IgE had not yet been measured on the whole cohort.

Here we report the results of further analyses in this cohort to examine whether prenatal paracetamol exposure is associated with an increased risk of asthma, wheezing and other reported and objective atopic outcomes when the child was 6–7 years old.

Methods

ALSPAC [10, 11] is a prospective study of 14 541 pregnancies. Women were enrolled as early in pregnancy as possible, on the basis of an expected date of delivery between 1 April 1991

Correspondence: Dr Seif Shaheen, Department of Public Health Sciences, Capital House, 42 Weston Street, London SE1 3QD, UK.
E-mail: seif.shaheen@kcl.ac.uk

and 31 December 1992, and place of residence within the three Bristol-based health districts of the former county of Avon, UK. It was estimated that 85–90% of eligible pregnant women were enrolled. There were 14 062 live births (January 1991 to February 1993), and 13 971 infants survived to 1 year. The Study is closely monitored by its own Ethics and Law Committee [12, 13] and by four local research ethics committees.

Exposures

Mothers were asked at 18–20 weeks how often they had taken paracetamol and aspirin ('not at all, sometimes, most days, every day') during their pregnancy. At 32 weeks, they were asked the same question about use in the previous 3 months. As very few mothers reported daily use, we combined this category with use on 'most days'. Primary exposures of interest were use of paracetamol in early (<18–20 weeks) and late (20–32 weeks) pregnancy, and secondary exposures were use of aspirin in early and late pregnancy (never, sometimes, most days/daily, unknown).

Although the mothers had also been asked about use of paracetamol in the infant (first 6 months after birth), we did not analyse this exposure, for two reasons. First, most infants had been given paracetamol more than once during that period, and information on more frequent use was not available. Second, any associations with the outcomes of interest might occur through persistence of infant symptoms and 'reverse causation'.

Outcomes

When the child was 81 months old, mothers were asked: 'Has he/she had any of the following in the past 12 months: *Asthma*; *Eczema*; *Hayfever*?'. [Preliminary data collected at 91 months suggest that, of mothers who responded positively to the question about asthma at 81 months, and subsequently gave a clear answer to the following question at 91 months: 'Has a doctor ever actually said that your study child has asthma?', 95% responded positively to this later question too.] Mothers were also asked: 'In the past year has he/she had any periods when there was wheezing with whistling on his/her chest when he/she breathed?' and 'If yes, how many separate times has this happened in the past 12 months?' We used information on number of episodes to derive 'wheezing frequency' (never, once/twice, three or more times), and 'new wheezing', defined as wheezing with whistling at 69–81 months (yes or no) only in children whose mothers said that they did not suffer from wheezing with whistling at 30–42 months.

At 7 years of age, the child underwent allergen skin prick testing and venepuncture. After applying the allergen solution to the skin, a prick was made using a sterile lancet, and the solution was blotted. After 20 min any visible weal was measured in two planes, the longest diameter and perpendicular to this. The maximum weal diameter was used. Atopy was defined as any positive reaction to *Dermatophagoides pteronyssinus*, cat or grass (after subtracting positive saline reactions from the size of the histamine and allergen weals and excluding children unreactive to histamine), and serum total IgE (kU/L) was measured using the Pharmacia UNICAP system (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden).

Confounders

Information on potential confounding factors was collected from questionnaires completed by the mother at intervals during pregnancy, infancy and childhood, and from anthropometric measurements of the child at birth and at 7 years of age. Potential confounders are listed in Table 1, and are categorized in order of importance as 'primary' (sex of child, maternal, prenatal and perinatal factors), 'secondary' (post-natal factors), and 'tertiary' (infant paracetamol and antibiotic use). We reasoned that 'primary' confounders were more likely than 'secondary' confounders to be associated with prenatal paracetamol use, but that it might not be appropriate to control for use of paracetamol and antibiotics in infancy, as these factors might be associated with wheezing in infancy (and hence with wheezing that persisted later in childhood) through 'reverse causation'.

Statistical methods

Analyses were carried out using the Stata statistical package [14]. We used logistic regression to estimate odds ratios (ORs) for asthma, wheezing, new wheezing, eczema, hayfever and atopy associated with the different categories of paracetamol or aspirin use compared with never-use. For each combination of exposure and outcome, we estimated unadjusted ORs, and ORs controlling first for primary confounders alone, then for primary and secondary confounders (presented in the Tables), and finally for primary, secondary and tertiary confounders. We used multinomial logistic regression to estimate corresponding ORs for the two wheezing frequency outcomes (compared with a baseline category of no wheezing in the past year). After log transforming total IgE we used linear regression to estimate geometric mean (GM) ratios for IgE. Confidence limits were calculated using Huber variances [14]. For each combination of exposure, outcome and confounders in the regression analyses, we fitted two models, one containing a separate odds or GM ratio for each of the three non-baseline exposure categories ('sometimes', 'most days/daily' and 'unknown'), and one containing an odds or GM ratio for the unknown category and a common ordinal per-category odds or GM ratio for the other two non-baseline categories, to measure overall exponential trend of increasing outcome with increasing exposure. Adjusted ORs were preferred to adjusted relative risks because, when a complicated model is fitted, a logistic regression model is less likely to fail to converge.

Population-attributable fractions were calculated using the method of Greenland and Drescher [15], with the models containing a separate OR for each exposure level and controlling for primary and secondary confounders. For each combination of exposure and outcome, the attributable fraction was calculated as the decrease in prevalence expected if the 'most days/daily' users, the 'sometimes' users and the 'unknown frequency' users had been 'never-users'.

Results

The frequency of paracetamol and aspirin use during early and late pregnancy is shown in Table 2. Moderate use

Table 1. List of potential confounders included in the adjusted models

Potential confounder	Levels
<i>Primary confounders</i>	
Child's sex	Female*, male
Mother's age (years)	<20, 20–24, 25–29*, 30–34, 35+
Maternal smoking in pregnancy (cigarettes/day)†	Not exposed*, passive only, 1–9/day, 10–19/day, 20+/day, unknown
Mother's education level	Below O-level, O-level only*, A-level or higher, unknown
Mother's housing tenure	Owned/mortgaged*, council rented, non-council rented, unknown/other
Mother's parity	0*, 1, 2+, unknown
Mother's anxiety score in pregnancy (CCEI units)‡	0–4*, 5–9, 10+, unknown
Mother's ethnic origin	White*, Non-White, unknown
Multiple pregnancy	No*, Yes
Child's birthweight (grams)	<2500, 2500–2999, 3000–3499*, 3500–3999, 4000+, unknown
Child's gestational age at birth (weeks)	<37, 37–40*, 41+
Child's head circumference (cm)	<33, 33–34.99*, 35–36.99, 37+, unknown,
Mother ever had asthma	No*, yes
Mother ever had eczema	No*, yes
Mother ever had rhinitis	No*, yes
Mother ever had migraine	No*, yes
Mother's history of these conditions unknown	No*, yes
Mother had cold/flu in late pregnancy	No*, yes
Mother had urinary infection in late pregnancy	No*, yes
Mother had other infections in late pregnancy	No*, yes
Mother's history of infections unknown	No*, yes
Mother's use of antibiotics in late pregnancy	No*, yes
<i>Secondary confounders</i>	
Number of younger siblings at 7 years	None*, 1, 2+
Pets in first year	No pets*, cat and/or dog, other pets only, unknown
Breast fed in first 6 months	No, yes*, unknown
Day care use in first 8 months	No*, yes, unknown
Damp and/or mould in home	No*, yes, unknown
Weekend tobacco exposure in first 6 months	Not exposed*, exposed, unknown
Child's body mass index at 7 years (kg/m ²)	<15.00, 15.00–17.49*, 17.50–20.49, 20.50+, unknown
<i>Tertiary confounders</i>	
Paracetamol use in first 6 months	Never*, once, more than once, unknown
Antibiotic use in first 6 months	Never*, once, more than once, unknown

*Reference category.

†This is the maximum level of exposure reported by the mother in the questionnaires completed at 8 weeks, 18–20 weeks and/or 32 weeks of pregnancy.

‡This is the sub-scale of the Crown–Crisp Experiential Index (CCEI) relating to free-floating anxiety.

(‘sometimes’) of paracetamol was common throughout pregnancy, whereas moderate aspirin use was very uncommon. Use of paracetamol and aspirin was lower in late vs. early pregnancy. Frequent use (‘most days/daily’) of both analgesics was very uncommon throughout pregnancy.

Among 8511 children with available information, 1062 (12.48%) were reported as having asthma at 69–81 months. Table 3 shows the association between asthma and use of paracetamol and aspirin in pregnancy. Before controlling for confounders, a significant positive association was seen between paracetamol use in early pregnancy and asthma in the offspring, but this effect was attenuated and became non-significant after controlling for confounders. A stronger and highly significant crude positive association was seen for paracetamol use in late pregnancy, which, although attenuated after controlling for primary and secondary confounders, remained significant (*P* for overall trend, 0.0037). Moderate (‘sometimes’) use was associated with a small, but significant, increase in risk of asthma compared

with never-use (adjusted OR 1.22 (95% confidence interval (CI): 1.06–1.41)). Frequent (‘most days/daily’) use was associated with a greater increase in risk (OR 1.62 (95% CI: 0.86–3.04)). The wide confidence limits around the latter estimate reflect the very small number of children in this exposure group.

The prevalence of wheezing with whistling at 69–81 months, among 8381 children with data, was 9.87% (*n* = 827). Table 4 shows the unadjusted and adjusted ORs for this outcome associated with use of analgesics in pregnancy. After controlling for primary and secondary confounders, frequent use of paracetamol in early pregnancy was associated with a significantly increased risk of wheezing with whistling (OR 1.81 (95% CI: 1.05–3.13)), although the overall trend with increasing frequency of use was not significant. A significant positive association was seen between paracetamol use in late pregnancy and risk of wheezing with whistling (*P* for trend, 0.011). The adjusted ORs associated with moderate and frequent use, compared

with never-use, were 1.20 (95% CI: 1.02–1.40) and 1.86 (95% CI: 0.98–3.55), respectively.

The proportions of asthma and wheezing with whistling at 69–81 months that were attributable to paracetamol use in late pregnancy (population-attributable fraction), assuming a causal relation, were 7.00% (95% CI: 1.82–11.91%) and 3.89% (95% CI: –2.89% to 10.22%), respectively.

When we controlled additionally for use of paracetamol and antibiotics in infancy, positive associations remained between paracetamol use in late pregnancy and asthma and wheezing (*P* for trend, 0.016 and 0.050, respectively), with

negligible reductions in the ORs compared with those after controlling only for primary and secondary confounders.

Of 8351 children with information on the number of episodes of wheezing at 69–81 months, 7554 (90.46%) never wheezed, 356 (4.26%) wheezed infrequently (one to two times over the year), and 441 (5.28%) wheezed frequently (three or more times over the year). When we compared the prevalence of wheezing with whistling at 30–42 months and at 69–81 months we found that, of 6621 children who were not wheezing in the earlier period, 356 (5%) developed 'new wheezing' subsequently. Paracetamol use in late pregnancy was positively associated with frequent wheezing (adjusted ORs associated with moderate and frequent use, compared with never-use, were 1.24 (1.00–1.53) and 2.31 (1.05–5.06), respectively; *P* for trend, 0.018), but not with infrequent wheezing. There was weak evidence for a positive association between paracetamol use in late pregnancy and new wheezing (*P* for trend, 0.11) (Table available on request).

Of 8511 children with data, 1363 (16.01%) had eczema and 535 (6.29%) had hayfever at 69–81 months. There was no detectable association between paracetamol use in pregnancy and eczema (*P* for trend, 0.25). There was weak evidence for a positive association between paracetamol use in late pregnancy and hayfever, but no convincing trend (*P* for trend, 0.079) (Table available on request).

Of 6527 children with skin prick test data, 1404 (21.5%) were atopic. Table 5 shows the unadjusted and adjusted ORs for atopy associated with use of analgesics in pregnancy. There was no association between paracetamol use in pregnancy and atopy. Information on total IgE was available

Table 2. Frequency of use of paracetamol and aspirin in early and late pregnancy by mothers of 8511 children with data on asthma, eczema and hayfever at 69–81 months

Frequency of use	Paracetamol		Aspirin	
	Number	Per cent	Number	Per cent
0–20 weeks				
Never	3908	45.92	7937	93.26
Sometimes	4326	50.83	346	4.07
Most days/daily	92	1.08	54	0.63
Unknown	185	2.17	174	2.04
20–32 weeks				
Never	4687	55.07	7827	91.96
Sometimes	3318	39.98	189	2.22
Most days/daily	66	0.78	55	0.65
Unknown	440	5.17	440	5.17

Table 3. Association between asthma at 69–81 months and use of paracetamol and aspirin in pregnancy (*n* = 8511)

Analgesic use	Unadjusted		Adjusted for primary confounders*		Adjusted for primary+secondary confounders*	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
<i>Paracetamol</i>						
0–20 weeks						
Never	1.00		1.00		1.00	
Sometimes	1.16	(1.01, 1.32)	1.10	(0.96, 1.27)	1.09	(0.95, 1.26)
Most days/daily	1.61	(0.93, 2.78)	1.42	(0.81, 2.50)	1.29	(0.74, 2.27)
<i>P</i> for trend	0.013		0.11		0.16	
20–32 weeks						
Never	1.00		1.00		1.00	
Sometimes	1.31	(1.15, 1.50)	1.24	(1.07, 1.43)	1.22	(1.06, 1.41)
Most days/daily	2.15	(1.18, 3.90)	1.74	(0.93, 3.27)	1.62	(0.86, 3.04)
<i>P</i> for trend	0.00001		0.0017		0.0037	
<i>Aspirin</i>						
0–20 weeks						
Never	1.00		1.00		1.00	
Sometimes	1.20	(0.88, 1.63)	1.11	(0.81, 1.52)	1.10	(0.80, 1.52)
Most days/daily	1.05	(0.48, 2.34)	0.94	(0.41, 2.15)	0.95	(0.43, 2.13)
<i>P</i> for trend	0.33		0.68		0.69	
20–32 weeks						
Never	1.00		1.00		1.00	
Sometimes	1.13	(0.74, 1.72)	0.99	(0.64, 1.53)	0.98	(0.63, 1.53)
Most days/daily	1.38	(0.67, 2.83)	1.32	(0.61, 2.85)	1.34	(0.64, 2.82)
<i>P</i> for trend	0.31		0.64		0.63	

OR, odds ratio; CI, confidence interval.

*See Table 1 for list of primary and secondary confounders.

Table 4. Association between wheezing with whistling at 69–81 months and use of paracetamol and aspirin in pregnancy ($n = 8381$)

	Unadjusted		Adjusted for primary confounders*		Adjusted for primary + secondary confounders*	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
<i>Paracetamol</i>						
0–20 weeks						
Never	1.00		1.00		1.00	
Sometimes	1.15	(0.99, 1.34)	1.07	(0.92, 1.25)	1.06	(0.91, 1.24)
Most days/daily	2.38	(1.39, 4.08)	1.96	(1.13, 3.43)	1.81	(1.05, 3.13)
<i>P</i> for trend	0.0092		0.13		0.21	
20–32 weeks						
Never	1.00		1.00		1.00	
Sometimes	1.34	(1.15, 1.55)	1.21	(1.03, 1.42)	1.20	(1.02, 1.40)
Most days/daily	2.63	(1.42, 4.88)	1.94	(1.02, 3.70)	1.86	(0.98, 3.55)
<i>P</i> for trend	0.000013		0.0062		0.011	
<i>Aspirin</i>						
0–20 weeks						
Never	1.00		1.00		1.00	
Sometimes	1.20	(0.86, 1.69)	1.10	(0.78, 1.56)	1.10	(0.78, 1.56)
Most days/daily	0.76	(0.27, 2.11)	0.62	(0.22, 1.73)	0.64	(0.23, 1.73)
<i>P</i> for trend	0.61		0.88		0.91	
20–32 weeks						
Never	1.00		1.00		1.00	
Sometimes	1.30	(0.84, 2.03)	1.13	(0.72, 1.78)	1.13	(0.72, 1.79)
Most days/daily	0.74	(0.27, 2.05)	0.64	(0.22, 1.84)	0.65	(0.23, 1.84)
<i>P</i> for trend	0.71		0.79		0.82	

OR, odds ratio; CI, confidence interval.

*See Table 1 for list of primary and secondary confounders.

for 5148 children. The unadjusted and adjusted GM ratios for total IgE associated with use of analgesics in pregnancy are shown in Table 6. After controlling for primary and secondary confounders, paracetamol use in late pregnancy was positively associated with total IgE (P for trend, 0.0034). The adjusted GM ratios associated with moderate and frequent use, compared with never-use, were 1.14 (95% CI: 1.03–1.26) and 1.52 (95% CI: 0.98–2.38), respectively. These effects estimates were unchanged when we controlled additionally for paracetamol and antibiotic use in infancy.

There were no significant trends in the associations of aspirin use in pregnancy with the outcomes presented in Tables 3–6, nor with wheezing frequency, new wheezing, eczema or hayfever.

Discussion

We have shown that paracetamol use in late pregnancy was positively associated with asthma, wheezing and total IgE in the offspring at 6–7 years of age. These findings extend our previous observation, in the same population-based birth cohort, of an association with wheezing earlier in childhood [9], before asthma could be formally diagnosed, and provide further evidence to implicate the prenatal environment in the aetiology of asthma. A small number of children developed 'new wheezing' after 42 months of age and, although the effect estimate associated with moderate paracetamol use was

not significant, it was very similar to that for wheezing as a whole. This would suggest that our findings for wheezing at 81 months, while partly reflecting persistence of effects seen at 42 months [9], also indicate independent effects in a different set of individuals.

Possible causal link and underlying mechanisms

The significant trend in the associations between paracetamol use in late pregnancy and asthma, wheezing and IgE suggests a dose–response relationship, and is in keeping with a causal interpretation. We propose that prenatal paracetamol exposure may have permanent effects on the developing lung and immune system, and the findings for 'new wheezing' would suggest that these effects may remain latent and subclinical in some individuals during the first few years of life, and not manifest clinically until the child is older. We speculate that regular exposure of the fetus to paracetamol after 20 weeks' gestation leads to production of the toxic metabolite *N*-acetyl-*p*-benzoquinoneimine (NAPQI) and depletion of glutathione. This, in turn, may cause oxidative stress and damage to fetal airway epithelium and increase vulnerability of the airways to more profound oxidant damage after birth, leading to postnatal bronchial hyper-responsiveness (BHR). In adult mice, high doses of paracetamol can deplete pulmonary glutathione and cause necrosis of bronchiolar epithelium [16–20], although whether this might occur with therapeutic doses early in life is unknown. We also postulate that paracetamol exposure in

Table 5. Association between skin test positivity at 7 years and use of paracetamol and aspirin in pregnancy (*n* = 6527)

Analgesic use	Unadjusted		Adjusted for primary confounders*		Adjusted for primary+secondary confounders*	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
<i>Paracetamol</i>						
0–20 weeks						
Never	1.00	(Ref.)	1.00	(Ref.)	1.00	(Ref.)
Sometimes	0.92	(0.81, 1.03)	0.96	(0.85, 1.09)	0.98	(0.86, 1.11)
Most days/daily	0.65	(0.35, 1.21)	0.77	(0.40, 1.49)	0.81	(0.42, 1.57)
<i>P</i> for trend	0.077		0.44		0.63	
20–32 weeks						
Never	1.00	(Ref.)	1.00	(Ref.)	1.00	(Ref.)
Sometimes	0.94	(0.83, 1.06)	0.98	(0.86, 1.12)	1.00	(0.88, 1.15)
Most days/daily	0.75	(0.35, 1.61)	0.81	(0.36, 1.83)	0.84	(0.38, 1.89)
<i>P</i> for trend	0.25		0.7		0.97	
<i>Aspirin</i>						
0–20 weeks						
Never	1.00	(Ref.)	1.00	(Ref.)	1.00	(Ref.)
Sometimes	1.40	(1.05, 1.86)	1.39	(1.04, 1.86)	1.43	(1.07, 1.92)
Most days/daily	0.67	(0.28, 1.60)	0.74	(0.30, 1.79)	0.76	(0.32, 1.85)
<i>P</i> for trend	0.21		0.17		0.12	
20–32 weeks						
Never	1.00	(Ref.)	1.00	(Ref.)	1.00	(Ref.)
Sometimes	1.29	(0.88, 1.90)	1.27	(0.87, 1.87)	1.30	(0.88, 1.91)
Most days/daily	0.59	(0.23, 1.52)	0.67	(0.25, 1.81)	0.68	(0.25, 1.83)
<i>P</i> for trend	0.9		0.77		0.71	

OR, odds ratio; CI, confidence interval.

*See Table 1 for list of primary and secondary confounders.

Table 6. Association between total IgE at 7 years and use of paracetamol and aspirin in pregnancy (*n* = 5148)

Analgesic use	Unadjusted		Adjusted for primary confounders*		Adjusted for primary+secondary confounders*	
	GMR	(95% CI)	GMR	(95% CI)	GMR	(95% CI)
<i>Paracetamol</i>						
0–20 weeks						
Never	1.00	(Ref.)	1.00	(Ref.)	1.00	(Ref.)
Sometimes	1.06	(0.97, 1.17)	1.02	(0.93, 1.12)	1.02	(0.93, 1.12)
Most days/daily	1.06	(0.71, 1.58)	0.98	(0.66, 1.47)	1.01	(0.68, 1.52)
<i>P</i> for trend	0.2		0.73		0.65	
20–32 weeks						
Never	1.00	(Ref.)	1.00	(Ref.)	1.00	(Ref.)
Sometimes	1.17	(1.06, 1.29)	1.13	(1.03, 1.25)	1.14	(1.03, 1.26)
Most days/daily	1.59	(1.01, 2.50)	1.53	(0.98, 2.37)	1.52	(0.98, 2.38)
<i>P</i> for trend	0.00037		0.0052		0.0034	
<i>Aspirin</i>						
0–20 weeks						
Never	1.00	(Ref.)	1.00	(Ref.)	1.00	(Ref.)
Sometimes	1.03	(0.81, 1.30)	1.02	(0.81, 1.29)	1.03	(0.82, 1.31)
Most days/daily	0.78	(0.45, 1.34)	0.76	(0.44, 1.32)	0.77	(0.44, 1.35)
<i>P</i> for trend	0.7		0.65		0.73	
20–32 weeks						
Never	1.00	(Ref.)	1.00	(Ref.)	1.00	(Ref.)
Sometimes	1.25	(0.90, 1.74)	1.19	(0.86, 1.66)	1.19	(0.86, 1.66)
Most days/daily	0.64	(0.39, 1.06)	0.62	(0.37, 1.04)	0.63	(0.37, 1.06)
<i>P</i> for trend	0.76		0.58		0.6	

GMR, geometric mean ratio; CI, confidence interval.

*See Table 1 for list of primary and secondary confounders.

late gestation may influence immune cell differentiation leading to increased production of IgE after birth. Paracetamol can deplete intracellular glutathione in lung macrophages *in vitro* [21], and depletion of glutathione in antigen-presenting cells can promote T-helper type 2 (Th2) cytokine responses [22]. Also, in animals Th2 responses are promoted by oxidative stress and this effect is inhibited by antioxidants, particularly thiols which increase glutathione synthesis [23–25]. Data will soon be available on BHR at 8–9 years of age in this cohort and, in view of the findings for total IgE, we would expect to see a relation between prenatal paracetamol exposure and BHR, as total IgE and BHR are known to be strongly associated [26]. In contrast, we did not find an association with skin test positivity. However, while we may have had less power to analyse this binary outcome compared with IgE, which was a continuous variable, these apparently contradictory results are not difficult to reconcile, as regulatory pathways and epidemiological risk factors for specific vs. total IgE are different. Positive associations with head circumference at birth suggest that the prenatal or perinatal environment may influence production of total IgE in childhood and adolescence [27, 28], whereas allergen-specific T cell memory is thought to result primarily from a failure of *postnatal* maturation of T-helper type 1 responses [29].

As in our earlier report [9], the effects of paracetamol were most convincing for use in late pregnancy. Paracetamol crosses the placenta [30], and the fetus is capable of generating NAPQI in late gestation, but to a lesser degree before 20 weeks [31]. We do not know to what extent this occurs on exposure to regular 'therapeutic' doses of paracetamol; however, the fetus has limited ability to metabolize paracetamol through glucuronidation [31], therefore it must be metabolized either via sulphation, or via oxidation with consequent production of NAPQI. Potential toxicity from this metabolite would then depend on the efficiency of detoxification by glutathione conjugation, which may be reduced in fetal airway epithelium in late gestation because of a decline in expression of glutathione-S-transferase (GST) [32, 33]. Toxicity is also likely to depend on the presence of variants in fetal genes which increase production of NAPQI by promoting oxidation rather than sulphation of paracetamol, and which decrease its detoxification.

Consideration of bias and confounding

Our analyses were restricted to subsets of the original birth cohort for whom complete data were available on outcomes at 6–7 years of age. We therefore cannot rule out the possibility that exclusion of children without complete information might have biased our findings. However, for an increased risk of asthma, wheezing and elevated IgE to have arisen spuriously, paracetamol use in late pregnancy would have to have been associated with an equally strong *protective* effect on these outcomes in the excluded children. Furthermore, as information on frequency of use of paracetamol was limited, and no data were available on dosage, considerable misclassification of exposure is likely to have occurred, especially among the large group of mothers who took paracetamol 'sometimes', which could have included usage ranging from occasional to 3 days/week. Such mis-

classification is likely to have been random with respect to the outcomes of interest, and this would tend to lead to an underestimation of the magnitude of the paracetamol effects. Demonstration of an association with IgE, an objective outcome, suggests that the findings for asthma and wheezing cannot simply be attributed to biased reporting by mothers who used more paracetamol in pregnancy.

Significant effects of paracetamol use in late pregnancy remained after controlling for a large number of potential confounders, with most of the attenuation of the crude effects being attributable, as expected, to the primary confounders (maternal, prenatal and perinatal factors), rather than post-natal factors. It is important to consider whether our findings might have been confounded by indications for paracetamol use, either directly, or indirectly through associations with asthma or atopic disease in the mother. Although we did not have information on indications, the most likely ones include headache, migraine, musculoskeletal pain, fever and infections. Of these, migraine [34], which may be more common in adults with asthma [35, 36], and infections and antibiotic use in pregnancy [2, 4], were potential confounders, but our findings remained after controlling for these factors and for maternal asthma and atopic disease.

Possible public health implications

While we would emphasize a cautious interpretation of our findings, there are potential implications to consider if the associations we have found are causal. The population-attributable fraction suggests that the proportion of childhood asthma in the population that might be explained by paracetamol use in late pregnancy is not trivial. Although the risks associated with moderate use were small, this exposure was common, with 40% of mothers reporting use of paracetamol 'sometimes' during late pregnancy. Without more detailed information on frequency of use, we could not determine whether there was a threshold within this exposure category, below which there was no associated risk. We would therefore recommend that, although paracetamol should remain the analgesic of choice throughout pregnancy, it should not be taken unnecessarily, especially after 20 weeks.

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