Urinary prostanoids in preschool wheeze

To the Editor:

Acute episodes of wheeze in children of preschool age are frequently triggered by viral upper respiratory tract infections and result in a significant burden to health services [1]. However, to date, the inflammatory mechanisms underlying preschool wheeze remain unclear. Mediators that have not been studied in preschool wheeze, but are implicated in the pathogenesis of wheeze in adults with asthma, include the pro-inflammatory prostanoid prostaglandin D_2 (PGD_2) [2] and the anti-inflammatory prostanoid PGE_2 [3, 4]. In this study, we sought evidence for either increased PGD_2 biosynthesis or reduced PGE_2 biosynthesis, or a combination of both in children with preschool wheeze. To achieve this, we measured the major metabolites of PGD_2 and PGE_2 in the urine: 9α-hydroxy-11,15-dioxo-2,3,4,5-tetranor-prostan-1,20-dioic acid (tetranor-PGDM) and 9,15-dioxo-11α-hydroxy-13,14-dihydro-2,3,4,5-tetranor-prostan-1,20-dioic acid (tetranor-PGEM), respectively [5, 6].

Preschool children with a history suggestive of ongoing wheeze were recruited from the Wheeze and Intermittent Treatment trial. Urine samples for prostanoid analysis were obtained from children while asymptomatic, after informed parental consent, and before the issue of trial medication (UK National Health Service Multicenter Research Ethics Committee reference 09/H1102/110). Children were aged between 10 months and 5 years, with a history of two or more episodes of wheeze, at least one of which was physician-confirmed, and at least one of which had occurred within the preceding 3 months [7]. Urine was obtained between 09:00 h and 16:00 h. Healthy controls were preschool siblings of children attending the outpatient clinics of the Royal London Hospital (London, UK), and preschool children with atopic disease were recruited from a paediatric allergy clinic with a clinical diagnosis of food allergy, but with no history of wheeze (atopic disease controls).

Urine was collected and stored at −80°C within 1 h of collection. Urinary tetranor-PGDM and tetranor-PGEM were analysed using high-performance liquid chromatography (HPLC) separation and mass spectrometry measurements. After thawing on ice, samples were centrifuged for 10 min at 10 000×*g* at 4°C and 0.5 mL of supernatant was used for extraction and analysis of tetranor-PGDM and tetranor-PGEM. Chemically identical internal deuterated standards were added to each sample: 10 ng tetranor-prostaglandin E metabolite-d_6 (tetranor-PGEM-d_6 or 11R-hydroxy-9,15-dioxo-13,14-dihydro-2,3,4,5-tetranor-prostan-17,17′,18,18′,19,19′-d_6-1,20-dioic acid) and 10 ng tetranor-prostaglandin D metabolite-d_6 (tetranor-PGDM-d_6 or 9R-hydroxy-11,15-dioxo-13,14-dihydro-2,3,4,5-tetranor-prostan-17,17′,18,18′,19,19′-d_6-1,20-dioic acid) (Cayman Chemical Co., Ann Arbor, MI, USA). Samples were then acidified (pH 3.5) using acetic acid and mixed with 0.5 mL tert-butyl-ether:methanol (80:20 v/v). The organic phase of the resulting mixture was then separated by a short centrifugation step and then dried under nitrogen at 37°C. The dried solid extract was redissolved in methanol (60 µL). 10 µL of this mixture was used for HPLC separation and mass spectrometry measurements (Shimadzu Sil-2-AC; Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with Phenomenex Synergy Fusion RP-100A 100×2 mm column (Phenomenex International, Torance, CA, USA). The retention times for tetranor-PGEM and tetranor-PGDM were 13.2 and 13.4 min, respectively. Analytes were measured using multiple reaction monitoring mode (MRM) tandem mass spectrometry (Qtrap 4000; AB Sciex, Concord, ON, Canada) equipped with electrospray ion source and operating in negative ionisation mode. Both tetranor-PGEM and tetranor-PGDM had the same pseudomolecular ions 327 Mz and monitored ions 309 Mz (333 and 315 Mz for deuterated standards). Quantification was performed using a stable isotope dilution method from the area under the peak. Urinary creatinine was assessed using a standard analytical assay and Vitros 350 (Ortho Diagnostics, Raritan, NJ, USA) and prostanoids were indexed to urinary creatinine ($pg \cdot mg^{-1}$ creatinine). Urinary cotinine was determined using a commercial microplate enzyme immunoassay (Cozart Forensic Microplate; Concateno, Abingdon, UK) and exposure to environmental tobacco smoke was classified as present if the creatinine corrected cotinine concentration was >30 $ng \cdot mg^{-1}$ [8, 9].

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For analysis, we divided children with preschool wheeze into two groups; those recruited at the same site as the healthy controls (group 1) and those recruited at other sites (group 2). Urinary prostanoid concentrations were $\log_{10}$ transformed before analysis. Groups were compared using either ANOVA with *post hoc* Tukey's multiple comparisons test or by t-test using GraphPad Prism (version 6.00; GraphPad Software, La Jolla, California, USA). Correlations (Pearson correlation coefficient (r)) and multiple regression analyses were performed using R (version 3.2; R Core Team, Vienna, Austria). Data are summarised as mean±SEM. A p-value of <0.05 was considered significant.

We recruited 24 healthy controls, five nonwheezy children with atopic disease, 149 children with preschool wheeze recruited at the same site as controls (group 1) and 810 children with preschool wheeze recruited from other sites (group 2). No child had received nonsteroidal anti-inflammatory therapy in the 2 weeks prior to urine sampling. There was no difference in age between controls and both preschool wheeze groups (healthy controls 3.0±0.27 years, atopic disease 2.9±0.81 years, group 1 3.0±0.08 years and group 2 2.6±0.04 years).

There was no difference in tetranor-PGDM between healthy controls and children with atopic disease and no history of wheeze (3.8±0.09 pg·mg^{-1} creatinine, n=24 *versus* 3.8±0.06 pg·mg^{-1} creatinine, n=5). Tetranor-PGDM was increased in both preschool wheeze groups compared with healthy controls (group 1 4.3±0.04 pg·mg^{-1} creatinine, group 2 4.3±0.01 pg·mg^{-1} creatinine, healthy controls 3.8±0.09 pg·mg^{-1} creatinine, n=24; p<0.0001) (figure 1a). In a multiple regression for PGDM including age and preschool wheeze status (group 1 and 2 combined and controls), both age and preschool wheeze status remained statistically significant (R^2 0.13) (coefficient±SE of age −0.096±0.01, p<0.0001; coefficient±SE of no wheeze (wheeze set as reference level) −0.37±0.75, p<0.0001).

In 959 children with preschool wheeze (*i.e.* groups 1 and 2 combined), the correlation between tetranor-PGDM and age was negative (r −0.30, p<0.0001), and PGDM was reduced in those receiving inhaled corticosteroids (ICS) (p<0.05). Sex, clinical pattern of wheeze (exclusive viral wheeze *versus* multiple trigger wheeze), exposure to environmental tobacco smoke (either parent-reported or measured by urinary cotinine) and eczema were not associated with tetranor-PGDM. In a multiple regression analysis limited to children with preschool wheeze, and including age and ICS, only age remained statistically significant (R^2 0.11) (coefficient±SE for age −0.049±0.005, p<0.0001; coefficient±SE for ICS 0.020±0.025, p=0.42).

There was no difference in tetranor-PGEM between healthy controls and children with atopic disease (4.4±0.07 pg·mg^{-1} creatinine, n=24 *versus* 4.1±0.12 pg·mg^{-1} creatinine, n=5). There was no difference in tetranor-PGEM between controls (4.4±0.07 pg·mg^{-1} creatinine), and preschool wheeze groups 1 (4.4±0.03 pg·mg^{-1} creatinine) and 2 (4±0.01 pg·mg^{-1} creatinine) (figure 1b). In 959 children with preschool wheeze, tetranor-PGEM was inversely associated with age (r −0.33, p<0.0001).

These results suggest that in children with a history of severe preschool wheeze but with no active wheeze on the day of sampling, PGD_2 biosynthesis, but not PGE_2 biosynthesis, is increased. The mechanism whereby

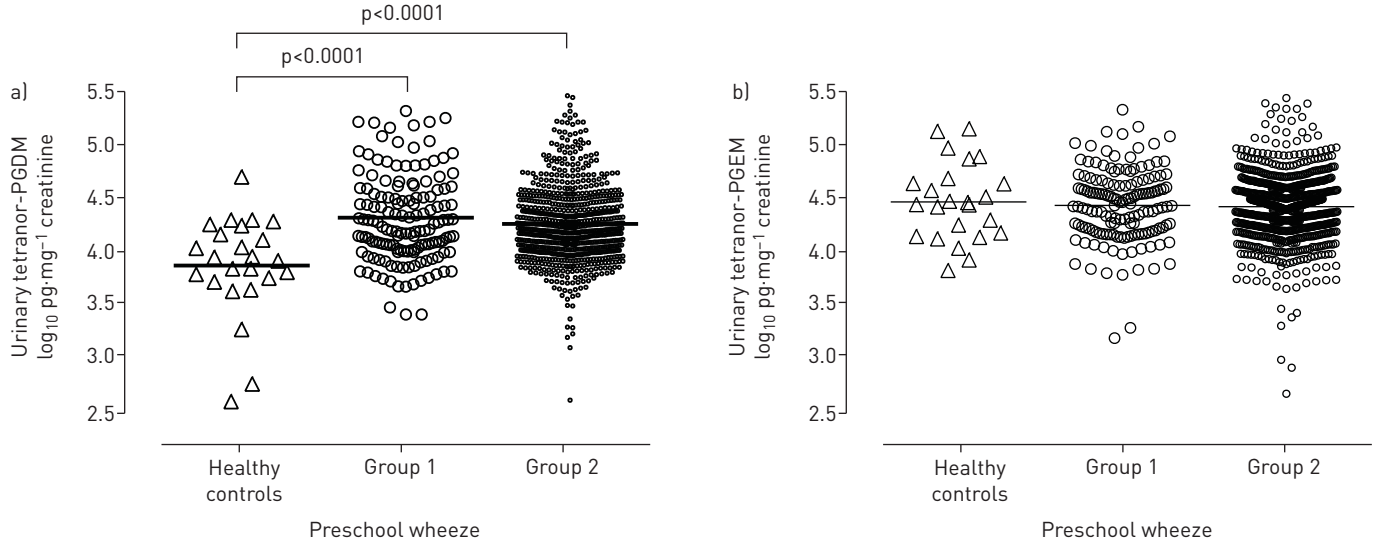

FIGURE 1 a) Dotplot of urinary tetranor-PGDM (9α-hydrox-11,15-dioxo-2,3,4,5-tetranor-prostan-1,20-dioic acid) ($\log_{10}$) in healthy controls and children with preschool wheeze recruited at the same site as controls (group 1), and those recruited at other sites (group 2). Urinary tetranor-PGDM is increased in group 1 and group 2 compared with controls (ANOVA and *post hoc* Tukey's multiple comparisons test). b) Dotplot of urinary tetranor PGEM (9,15-dioxo-11α-hydroxy-13,14-dihydro-2,3,4,5-tetranor-prostan-1,20-dioic acid) in healthy controls and group 1 and group 2 children. There is no difference between groups by ANOVA.

airway PGD_2 contributes to the pathogenesis of preschool wheeze is unclear. One potential mechanism is that increased airway PGD_2, rather than directly causing bronchoconstriction, primes the airway for an exaggerated inflammatory response during viral colds, an interaction observed in an animal model [10].

There are important limitations to this study. First, although the pattern of urinary prostanoids in preschool wheeze is similar to that reported for adults with mild intermittent wheeze [11], whether increased tetranor-PGDM in the urine reflects either increased levels of PGD_2 in the airway, or increased biosynthesis in other organs is unclear. Second, we did not assess several important potential confounders of PGDM in children with preschool wheeze. For example, atopic status (measured either using skin prick testing, or specific serum IgE) was not assessed. While the nonsignificant difference in PGDM between healthy controls and controls with atopic disease is compatible with a lack of effect of atopy, the very small number of children with atopic disease means that a confounding effect of atopy remains possible. However, we are able to exclude an effect of differences in urine sampling handling since there was no difference in the urinary prostanoid profile between children with preschool wheeze recruited at the same site as controls (group 1) and those recruited at other sites (group 2).

We conclude that PGDM is a marker of potential interest in preschool wheeze, but further studies are required in better defined populations. If airway PGD_2 is indeed increased in preschool wheeze, trials of new therapeutic options for this common condition would be suggested, for example of the new oral CHTR2 antagonists, which block the action of PGD_2 on airway cells [12].

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