ERJ Express. Published on April 16, 2008 as doi: 10.1183/09031936.00132607

Smoke exposure, airway symptoms and exhaled nitric oxide in infants: the Generation R Study

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Short title: Smoke exposure, respiratory symptoms and FE_{NO}

Abstract

We evaluated the effect of pre- and postnatal smoke exposure on exhaled nitric oxide (FE_{NO}) in infants and investigated the association between respiratory symptoms and FE_{NO} in the first 2 months of life. The Generation R Study is a population-based prenatally recruited birth cohort. Exposures were assessed by means of questionnaires prospectively administered during pregnancy and after birth. Successful off-line FE_{NO} measurements during tidal breathing were obtained in 187 infants (median age 6.9 weeks). The association between possible determinants and logFE_{NO} was investigated with multiple linear regression analysis. Infants exposed pre- and postnatally to smoke showed lower FE_{NO} than infants exposed only after birth (difference: 1.5 [1.0-2.1] ppb; p=0.042) and than never exposed infants (difference: 1.4 [1.0-1.8] ppb; p=0.052). FE_{NO} was reduced in infants with severe upper respiratory symptoms compared to infants with non-severe symptoms (difference: 1.6 [1.0-2.4] ppb; p=0.047). Infants with symptoms of the lower respiratory tract had lower FE_{NO} than asymptomatic infants (difference: 1.2 [1.0-1.5] ppb; p=0.046). We conclude that the nature of the association between smoke exposure and FE_{NO} is dependent on timing and intensity of exposure. The occurrence and the severity of respiratory symptoms in the first 2 months of life are associated with lower FE_{NO}.

Key words: early respiratory morbidity, exhaled nitric oxide, prenatal and postnatal exposures, prospective birth cohort.

Introduction

Fractional exhaled nitric oxide (FE_{NO}) is increased in asthmatic adults [1], children [2], and infants with eczema [3] and recurrent wheezing[4], and has been proposed as a noninvasive marker of eosinophilic airway inflammation. Compared to healthy infants, lower FE_{NO} levels have been found in infants with virus-associated acute wheezy bronchitis [5] and in infants with upper respiratory symptoms such as rhinorrhea [6]. Several pre- and postnatal factors have been shown to influence the levels of FE_{NO} in infants, such as tobacco smoke exposure [7-9], coffee consumption during pregnancy [8], maternal atopic disease [8, 10], birth weight [11], gestational age [11, 12], gender [8] and infections [13]. However, the influence of risk factors for respiratory morbidity on FE_{NO} in infancy is not clear. Previous studies investigating the association between smoke exposure, one of the best known risk factors for respiratory morbidity in infants, and FE_{NO} have given conflicting results. Hall et al found lower FE_{NO} in infants exposed to smoking during pregnancy than in unexposed infants [9]. In a subsequent report of the same authors, this difference was only significant in infants of mothers without atopic disease [8]. Also, the role of postnatal exposure to tobacco smoke in infants has been investigated, but the results are not consistent. In a recent paper, Franklin et al [7] reported higher FE_{NO} in infants exposed to postnatal tobacco smoking, whereas previous studies did not show such an effect, or a lower FE_{NO} in exposed asthmatics and healthy subjects [14, 15].

Previous studies that sought to investigate the effect of different determinants of FE_{NO} levels in early infancy retrospectively assessed prenatal exposure variables after birth, rather than prospectively during pregnancy. Therefore, the temporality or succession of

events is not documented and the exposure assessment is more prone to recall bias. The aim of this prospective birth cohort study was to evaluate whether and to what extent pre- and early postnatal exposures influence FE_{NO} in early infancy. We also investigated the effect of upper and lower respiratory symptoms (URS and LRS) in the first 2 months of life on FE_{NO}.

Subjects and methods

The Generation R Study is a prospective population-based prenatally recruited birth cohort study in Rotterdam. A randomly selected group of 1,232 Dutch pregnant women and their children have been enrolled in the Generation R focus study. In the focus study more detailed assessments of fetal and postnatal growth and development are performed [16, 17]. Women were enrolled during pregnancy. Sociodemographic factors and exposure to risk factors for respiratory diseases were assessed by means of questionnaires, administered to the mother in early (gestational age < 18 weeks), mid- (gestational age 18 - 25 weeks) and late (gestational age ≥ 25 weeks) pregnancy and to the partner at 20 weeks. Information was gathered on the following exposure variables for both the mother and the partner: sociodemographic factors, smoking habits, atopy and siblings. A questionnaire was administered to the parents when the children were 2 months old and exposure variables were again assessed together with onset, occurrence and severity of URS and LRS.

Between November 2004 and September 2005, FE_{NO} measurements were attempted in 225 infants participating in the focus study at a median [range] age of 6.7 weeks [3.7-16.9]. Mixed oral/nasal FE_{NO} was measured off-line during tidal breathing with a

facemask covering nose and mouth without the use of sedation as previously described [18]. A FE_{NO} measurement was considered successful if exhaled air was sampled during quiet tidal breathing, if the facemask was tightly fitted to nose and mouth during the whole procedure and if at least five breaths were obtained. All FE_{NO} measurements were conducted with the infants awake. Ambient NO was measured before each FE_{NO} measurement (Sievers 280 B, Boulder, CO, USA) and in case of ambient NO concentration above 10 parts per billion (ppb), the infant inhaled at least 2 tidal breaths of NO-free air from an NO-inert 750 ml balloon in order to permit the washout of the dead space of the lungs [19]. However, as FE_{NO} showed a positive significant association with ambient NO levels, we always included ambient NO in the multivariable models.
All infants were free of respiratory symptoms and had no clinical evidence of airways infection at the time of the measurement. The Medical Ethical Committee of the Erasmus Medical Center, Rotterdam, approved the study. Mothers and their partners received written and verbal information about the study and gave written informed consent.

Definition of variables considered in the analysis

Educational level of the mother was divided into 3 categories (lower, intermediate, higher vocational training) according to the classification of Statistics Netherlands [20]. Parental atopy was defined as self-reported or doctor diagnosed allergy or atopic disease (allergic asthma, hay fever, eczema). Prenatal maternal smoking was assessed in the first questionnaire by asking the mother whether she smoked during the pregnancy (no, smoked until the pregnancy was known, or continued smoking after the pregnancy was known). In the second and third questionnaires, the mother was asked whether she had

smoked in the past 2 months (no, yes). If the answer was positive at least at 2 time points, the infant was classified as prenatally exposed. Exposure to passive tobacco smoking was also assessed by asking whether people smoked regularly in the house or in the working environment of the mother during pregnancy. Gestational age, birth weight and length were obtained from midwife and hospital registries.

Postnatal exposure to tobacco smoke was assessed by asking whether the infant had been exposed to smoke by the mother or by any of the members of the household at least once a week. Also, parents were asked whether their child had had runny and/or blocked nose (URS), breathlessness, a whistling noise when breathing, wheezing, panting, difficult breathing and/or cough (LRS) in the past 2 months. Symptoms were considered severe if they required a visit to a physician, as reported by the parents.

Statistical Analysis

FE_{NO} values were log-normally distributed. Univariable analyses using Student *t* test and simple linear regression were used to determine associations between $logFE_{NO}$ and the following explanatory variables: ambient NO, birth weight, gestational age, gender, breastfeeding, maternal educational level, maternal and paternal atopy, prenatal and postnatal tobacco smoke exposure, siblings, weight, length and age at the study date, upper and lower respiratory symptoms. Factors that had a significance level ≤ 0.1 from univariate analyses were included in the multiple linear regression models in order to evaluate the relation between $logFE_{NO}$ (dependent variable) and pre- and postnatal exposures while controlling for other relevant factors. Although not directly associated to FE_{NO} in our study population, atopic status of the mother, birth weight, gender and age

at the study date were included in all regression models as covariates, since these have been shown to influence FE_{NO} or the occurrence of respiratory symptoms [4, 8]. Effect modification by maternal atopy and gender was investigated by adding interaction terms in the final models.

FE_{NO} values were backtransformed after the analysis and are reported as geometric mean and 95% confidence interval [95% CI] in ppb. Comparisons of FE_{NO} between groups are presented as geometric mean of the difference [95% CI]. Two-tailed p value < 0.05 was considered significant. Data analyses were performed using the Statistical Package of Social Sciences version 11 for Windows (SPSS Inc, Chicago, IL, USA).

Due to the paucity of data in the literature, no power calculation could be performed in order to evaluate the size of the study needed to detect a difference in FE_{NO} values between groups of infants.

Results

FE_{NO} measurements were attempted in 225 infants and succeeded in 187 infants (success rate 83%). Thirty-eight measurements were excluded because a quiet tidal breathing pattern was not maintained during the whole procedure (n=31) or because less than 5 breaths could be collected in the sampling balloon (n=7).

Excluded infants had younger mothers (median age [range] 30.3 [18.5 – 40] yrs) and fathers (32.8 [25.3 – 39.6] yrs) than infants with successful FE_{NO} measurements (p=0.003 and p=0.017, respectively; t-test), but the other baseline characteristics and anthropometrics at the study date did not differ between the two groups (table 1).

Pre- and postnatal exposures and FE_{NO}

On univariable analysis, anthropometrics were not related to FE_{NO} (table 2), whereas prenatal maternal smoking affected FE_{NO}, with lower levels in exposed infants (p=0.047) (table 3). Paternal smoking and maternal passive tobacco smoke exposure during pregnancy did not affect FE_{NO}, therefore an infant was considered prenatally exposed if the mother smoked during pregnancy, independent of other sources of smoke. With regard to prenatal smoke, 44 infants were exposed (23 only prenatally and 21 pre- and postnatally), whereas 51 infants were exposed to environmental smoke after birth by the mother or by other members of the household (30 only postnatally and 21 pre- and postnatally). Gender, parental atopic status and maternal asthma were not related to FE_{NO} (table 3). In order to compare the different smoke exposure categories directly, we created one variable resulting from the combination of prenatal and postnatal smoke exposure, with four mutually exclusive categories: never exposed, exposed only prenatally, exposed only postnatally and continuously exposed both pre- and postnatally. The association between the combined variable 'smoke exposure' and FE_{NO} was significant in the univariable analysis (table 3) and also in the multivariable regression model (table 4). Infants exposed pre- and postnatally to smoke showed lower FE_{NO} than infants exposed only after birth (difference: 1.5 [1.0-2.1] ppb; p=0.042) and than never exposed infants (1.4 [1.0-1.8] ppb; p=0.052) (figure 1). This association was independent of respiratory symptoms and not modified by gender (p for interaction = 0.69) or by maternal atopy (p for interaction = 0.46). However, among the 60 infants of atopic mothers, only 22 were exposed to smoke (6 only prenatally, 10 only postnatally and 6

pre- and postnatally). The association between ambient NO and FE_{NO} remained significant ($p<0.001$) also in the multivariable model.

Respiratory symptoms and FE_{NO}

LRS were reported for 83 infants (63 non-severe and 20 severe), whereas 130 infants had URS (120 non-severe and 10 severe symptoms). In the multivariable analysis, FE_{NO} values were lower in infants with LRS (n=83) than in asymptomatic (n=104) infants (difference: 1.2 [1.0-1.5] ppb; p=0.046) (table 4), but no association was found between the severity of LRS and FE_{NO}. Gender and atopy did not modify this association (p for interaction=0.68 and p=0.88, respectively). Such difference was not found for URS in univariate analysis (p=0.3), nor when URS was added into the multivariable model as a binary outcome (p=0.9). However, considering the severity of the symptoms (coded as 0=no symptoms; 1=non-severe symptoms; 2=severe symptoms), infants with URS that required a visit by a doctor (n=10) had lower FE_{NO} compared to infants with non-severe symptoms (difference: 1.6 [1.0-2.4] ppb; p=0.047) and tended to have lower FE_{NO} than asymptomatic infants (difference 1.5 [0.9-1.2] ppb; p=0.075) (figure 2). This effect was independent of smoke exposure and LRS, still significantly associated to FE_{NO} (table 4). Tobacco smoke exposure or parental atopy, as well as the other investigated determinants, were not associated with occurrence or severity of respiratory symptoms.

Discussion

In the present birth cohort study we found an association between tobacco smoke exposure and FE_{NO} values in infants. Infants continuously exposed to smoke both in utero and after birth had lower FE_{NO} than never exposed infants and than infants exposed only postnatally. None of the other investigated risk factors for respiratory morbidity affected FE_{NO}. The association between respiratory symptoms and lower FE_{NO} was significant in infants with severe upper and lower respiratory symptoms.

Few studies addressed the separate effects of pre-and postnatal tobacco smoke exposure in infants [7-9, 14]. Frey et al [8] measured FE_{NO} in a selected group of healthy infants and assessed pre-and postnatal environmental tobacco smoke exposure after birth. They found that maternal smoking in pregnancy was associated with lower FE_{NO}, but only in infants of mothers without asthma, whereas the same exposure in mothers with atopic disease was associated with higher levels. In univariable analysis, we also found that FE_{NO} was lower in infants of mothers who smoked during pregnancy, whereas in the multivariable analysis infants exposed to smoke prenatally had lower FE_{NO} than never exposed only if smoke exposure was protracted also after birth. In our study neither parental atopy nor gender modified this association, confirming earlier findings of Hall et al [9]. However, due to the small numbers of infants per group, there was insufficient power to adequately investigate such interactions. Our study is embedded in a larger population-based birth cohort, and infants were not selected depending on their health status. Furthermore, the repeated assessment of exposure variables during pregnancy gave us the opportunity to study prenatal smoke exposures in greater detail and reduced the likelihood of recall bias, strengthening the validity of our findings. In a recent study,

Franklin et al [7], found increased FE_{NO} in infants exposed to parental smoking with evidence of a dose-response relationship. We could not demonstrate a clear effect of postnatal smoke exposure on FE_{NO} as we found that FE_{NO} was higher in infants exposed to smoke only postnatally than in infants exposed both pre- and postnatally, but not different from never exposed infants. The mechanisms for increased FE_{NO} in infants exposed to postnatal maternal smoking, as observed in this and previous studies are not clear. A possible explanation may be a direct irritant effect of smoke on the airways [7]. The lower FE_{NO} found in infants exposed to prenatal tobacco smoking would support the hypothesis that smoke exposure during pregnancy inhibits inducible NO synthase [21]. Possible implications of such suppression is hypothetical, but as NO may serve important functions in local defense and in maintenance of normal vaso- and bronchomotor tone, any factor that modifies baseline NO generation in the airways of young infants should be reason for concern and further study.

The occurrence and the severity of respiratory symptoms were associated with lower FE_{NO} in infants. Franklin et al also found low FE_{NO} in infants with ongoing rhinorrhea, but FE_{NO} increased 4-12 weeks after the initial assessment, when symptoms had resolved [6]. Although children in our study were free of respiratory symptoms and had no evidence of respiratory infection at the time of testing, they might have had symptoms in the weeks preceding the FE_{NO} measurement. Therefore, our results should be interpreted with caution, as the age at which symptoms occurred and the timing of symptoms in relation to the FE_{NO} measurements might have influenced the findings. We cannot exclude that a reduced FE_{NO} in infants with respiratory symptoms is related to a delayed effect of acute symptoms on the NO generation or diffusion through the airways. Another

reason for caution when interpreting our results is that we showed this association when comparing FE_{NO} between the relatively small group of infants with severe upper respiratory symptoms and infants with non-severe symptoms. A child may be taken to the doctor for many reasons, symptom severity being one of those, but also anxiety of the parents could have influenced the decision, and this might have led to misclassification. However, if such misclassification occurred, this is not likely to bias the direction of our results as this would mean that we underestimated the effect size.

We found lower FE_{NO} in infants who had had lower respiratory symptoms, but no association was found between wheezing and FE_{NO}. Previous studies found an association between wheezing and FE_{NO} in selected populations of infants with high risk of developing atopic disease or with recurrent wheeze [4, 22]. Also, in a prospective study [10], higher FE_{NO} at 1 month was predictive of the development of respiratory symptoms in the first year only in infants of atopic mothers, who are at higher risk of developing asthma. In contrast, in the same study, a trend toward a negative association between FE_{NO} and severe respiratory symptoms in infants of nonatopic mothers was found. Infants in our study came from an unselected population and maternal atopy did not modify any of the associations that we found, and this may explain the discrepancies. Although there is evidence that most asthma starts in early life [23], respiratory morbidity in the preschool child is mostly related to neutrophilic airway inflammation [24] and there is very little evidence of chronic eosinophilic bronchial inflammation in the first months [25].

One could argue that measuring mixed oral/nasal FE_{NO} without controlling for expiratory flow might introduce variability [26], especially in infants exposed to tobacco smoke that

may have abnormal airway mechanics. We previously demonstrated that FE_{NO} measured with variable flow was reproducible [18] and able to differentiate between infants with different respiratory diseases in a similar way as more sophisticated techniques taking into account also lung function parameters and breathing pattern [4]. However, differences between groups may still be due to differences in tidal flows, particularly when comparing groups with potentially different tidal breathing patterns. Indeed, differences in tidal flows might introduce variability and explain some of the overlap and the relatively small differences between groups in our study.

In the present study we found a positive correlation between FE_{NO} and ambient NO, in agreement with a previous study by Pijnenburg et al [27]. Although significant also in the multivariable model, we showed that such correlation did not affect the results of our study as the associations that we found were independent of ambient NO concentrations. Using NO-free air always when measuring FE_{NO} in infants could reduce the influence of ambient NO on FE_{NO}, as suggested by recently published guidelines [28]. However, such recommendations were not yet available when we started the study, and we used NO-free air only if room concentrations were above 10 ppb, in agreement with previously published guidelines for infants [19]. Our findings suggest that infants should inhale more than 2 tidal breaths of NO-free air, as this would reduce the contamination by ambient NO, but on the other hand this might reduce the success rate of the measurements, as awake infants might not tolerate the facemask for a longer time.

A possible limitation to our study is that smoke exposure was assessed by means of questionnaires and was not confirmed with the measurement of specific biomarkers. Although a good agreement between parental report of smoking and air nicotine

concentration has been shown [29], some misclassification might have occurred, as parents would underreport smoking. If this is the case, we would underestimate the effect of smoke on FE_{NO}, as smoking parents would be classified in the group of non-smokers. Therefore, we hypothesize that the size of this effect could be greater than reported in our study.

We conclude that pre- and postnatal tobacco smoke exposure are associated with mixed oral/nasal FE_{NO} in early infancy, with lower FE_{NO} in prenatally exposed infants and higher FE_{NO} in case of postnatal exposure. Reported airway symptoms, depending on their frequency and severity, were associated with lower FE_{NO} already in the first 2 months of life. The meaning of changes in FE_{NO} for respiratory health in infancy needs to be further elucidated.

Acknowledgments

Authors are thankful to Christi Wagemakers, Katja van Willigen-Broekhuize and Miranda van Leeuwen for assistance in sampling FE_{NO} in infants. The authors also thank Dr. Wim C. Hop for statistical advice.

The Generation R Study is conducted by the Erasmus Medical Center in close collaboration with the Erasmus University Rotterdam, School of Law and Faculty of Social Sciences, the Municipal Health Service Rotterdam area, Rotterdam, the Rotterdam Homecare Foundation, Rotterdam, and the Stichting Trombosedienst & Artsenlaboratorium Rijnmond (STAR), Rotterdam. We gratefully acknowledge the contribution of general practitioners, hospitals, midwives and pharmacies in Rotterdam. The first phase of the Generation R Study is made possible by financial support from: Erasmus Medical Centre, Rotterdam, Erasmus University Rotterdam and the Netherlands Organization for Health Research and Development (ZonMw). The present study was supported by an additional grant from the Netherlands Asthma Foundation (Project number 3.2.02.41).

References

1. Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. Lancet. 1994 Jan 15;343(8890):133-5.

2. Brussee JE, Smit HA, Kerkhof M, Koopman LP, Wijga AH, Postma DS, et al. Exhaled nitric oxide in 4-year-old children: relationship with asthma and atopy. Eur Respir J. 2005 Mar;25(3):455-61.

3. Dinakar C, Craff M, Laskowski D. Infants and toddlers without asthma with eczema have elevated exhaled nitric oxide levels. J Allergy Clin Immunol. 2006 Jan;117(1):212-3.

4. Gabriele C, Nieuwhof EM, Van Der Wiel EC, Hofhuis W, Moll HA, Merkus PJ, et al. Exhaled nitric oxide differentiates airway diseases in the first two years of life. Pediatr Res. 2006 Oct;60(4):461-5.

5. Ratjen F, Kavuk I, Gartig S, Wiesemann HG, Grasemann H. Airway nitric oxide in infants with acute wheezy bronchitis. Pediatr Allergy Immunol. 2000 Nov;11(4):230-5.

6. Franklin PJ, Turner SW, Hall GL, Moeller A, Stick SM. Exhaled nitric oxide is reduced in infants with rhinorrhea. Pediatr Pulmonol. 2005 Feb;39(2):117-9.

7. Franklin PJ, Turner S, Mutch R, Stick SM. Parental smoking increases exhaled nitric oxide in young children. Eur Respir J. 2006 Oct;28(4):730-3.

8. Frey U, Kuehni C, Roiha H, Cernelc M, Reinmann B, Wildhaber JH, et al. Maternal atopic disease modifies effects of prenatal risk factors on exhaled nitric oxide in infants. Am J Respir Crit Care Med. 2004 Aug 1;170(3):260-5.

9. Hall GL, Reinmann B, Wildhaber JH, Frey U. Tidal exhaled nitric oxide in healthy, unsedated newborn infants with prenatal tobacco exposure. J Appl Physiol. 2002 Jan;92(1):59-66.

10. Latzin P, Kuehni CE, Baldwin DN, Roiha HL, Casaulta C, Frey U. Elevated exhaled nitric oxide in newborns of atopic mothers precedes respiratory symptoms. Am J Respir Crit Care Med. 2006 Dec 15;174(12):1292-8.

11. Biban P, Zangardi T, Baraldi E, Dussini N, Chiandetti L, Zacchello F. Mixed exhaled nitric oxide and plasma nitrites and nitrates in newborn infants. Life Sci. 2001 May 11;68(25):2789-97.

12. Roiha HL, Kuehni CE, Zanolari M, Zwahlen M, Baldwin DN, Casaulta C, et al. Alterations of exhaled nitric oxide in preterm infants with chronic lung disease. Eur Respir J. 2006 Oct 18.

13. Kharitonov SA, Yates D, Barnes PJ. Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. Eur Respir J. 1995 Feb;8(2):295-7.

14. Dinakar C, Lapuente M, Barnes C, Garg U. Real-life environmental tobacco exposure does not affect exhaled nitric oxide levels in asthmatic children. J Asthma. 2005 Mar;42(2):113-8.

15. Yates DH, Breen H, Thomas PS. Passive smoke inhalation decreases exhaled nitric oxide in normal subjects. Am J Respir Crit Care Med. 2001 Sep 15;164(6):1043-6.

16. Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol. 2007;22(12):917-23.

17. Jaddoe VW, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Verhulst FC, et al. The Generation R Study: Design and cohort profile. Eur J Epidemiol. 2006;21(6):475-84.

18. Gabriele C, van der Wiel EC, Nieuwhof EM, Moll HA, Merkus PJFM, de Jongste JC. Methodological aspects of exhaled nitric oxide measurements in infants. Pediatr Allergy Immunol. 2007(18):36-41.

19. Baraldi E, de Jongste JC. Measurement of exhaled nitric oxide in children, 2001. Eur Respir J. 2002 Jul;20(1):223-37.

20. Voorburg/Heerlen. Standaard onderwijsindeling 2003. Statistics Netherlands. 2004.

21. Hoyt JC, Robbins RA, Habib M, Springall DR, Buttery LD, Polak JM, et al. Cigarette smoke decreases inducible nitric oxide synthase in lung epithelial cells. Exp Lung Res. 2003 Jan-Feb;29(1):17-28.

22. Wildhaber JH, Hall GL, Stick SM. Measurements of exhaled nitric oxide with the single-breath technique and positive expiratory pressure in infants. Am J Respir Crit Care Med. 1999 Jan;159(1):74-8.

23. Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. N Engl J Med. 2003 Oct 9;349(15):1414-22.

24. Oommen A, Patel R, Browning M, Grigg J. Systemic neutrophil activation in acute preschool viral wheeze. Arch Dis Child. 2003 Jun;88(6):529-31.

25. Saglani S, Malmstrom K, Pelkonen AS, Malmberg LP, Lindahl H, Kajosaari M, et al. Airway remodeling and inflammation in symptomatic infants with reversible airflow obstruction. Am J Respir Crit Care Med. 2005 Apr 1;171(7):722-7.

26. Deykin A, Massaro AF, Drazen JM, Israel E. Exhaled nitric oxide as a diagnostic test for asthma: online versus offline techniques and effect of flow rate. Am J Respir Crit Care Med. 2002 Jun 15;165(12):1597-601.

27. Pijnenburg MW, Lissenberg ET, Hofhuis W, Ghiro L, Ho WC, Holland WP, et al. Exhaled nitric oxide measurements with dynamic flow restriction in children aged 4-8 yrs. Eur Respir J. 2002 Oct;20(4):919-24.

28. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med. 2005 Apr 15;171(8):912-30.

29. Gehring U, Leaderer BP, Heinrich J, Oldenwening M, Giovannangelo MECA, Nordling E, et al. Comparison of parental reports of smoking and resudential air nicotine concentrations in children. Occup Environ Med. 2006(63):766-72.

Table 1 Baseline characteristics and anthropometrics in the study population (n=187)

	Median	*Range*
Age mother (yrs)	32.5	18.5 - 42.9
Age father (yrs)	33.8	16-58.2
Gestational age at enrolment (wks)	13	8.5-23.2
Gestational age at birth (wks)	40.3	34.6-43.0
Birth weight (gr)	3520	1958-5170
Age at the study date (wks)	6.9	3.7-16.9
Weight at the study date (gr)	4890	3350-8230
Length at the study date (cm)	56.9	50.6-66.3

Table 2 Univariable analyses: FE_{NO} and ambient NO and anthropometrics in the study population

Variables	Beta coefficient	95% CI
Ambient NO (ppb)	0.0069*	0.004 – 0.01
Gestational age (wks)	0.0145	-0.016 – 0.045
Birth weight (kg)	-0.0388	-0.12 – 0.042
Age at study date (wks)	0.0025	-0.017 – 0.022
Weight at study date (kg)	-0.0054	-0.06 – 0049
Length at study date (cm)	0.0054	-0.011 – 0.021

*$p<0.001$

Beta coefficients were estimated by linear regression analysis and should be judged as the change of $logFE_{NO}$ per unit change in the variables

Table 3 Univariable analyses: pre and postnatal variables and FE_{NO}

Variable	Geometric mean FE_{NO} [95%CI] (ppb)
Gender	
Boys (n=95)	10.6 [9.2 – 12.2]
Girls (n=92)	11.2 [9.7 – 12.9]
Exclusive breastfeeding	
No (n=123)	10.5 [9.2 – 11.8]
Yes (n=64)	11.7 [9.9 – 14.0]
Maternal education	
Low (n=5)	9.8 [5.2 – 18.3]
Intermediate (n=72)	10.9 [9.3 – 12.8]
High (n=110)	10.9 [9.6 – 12.5]
Maternal atopy	
No (n=127)	10.8 [9.6 – 12.2]
Yes (n=60)	11.0 [9.2 – 13.1]
Maternal asthma	
No (n=175)	10.8 [9.7 – 12.0]
Yes (n=12)	12.1 [8.1 – 18.2]
Paternal atopy	
No (n=143)	10.9 [9.7 – 12.2]
Yes (n=44)	10.8 [8.8 – 13.3]
Paternal smoking	

No (n=130)	10.6 [9.4 – 11.9]
Yes (n=57)	11.6 [9.7 – 13.9]
Maternal passive smoke exposure during pregnancy	
No (n=69)	11.1 [9.4 – 13.1]
Yes (n=118)	10.8 [9.5 – 12.2]
Maternal smoking during pregnancy	
No (n=143)	11.5 [10.3 – 12.9]
Yes (n=44)	9.1 [7.4 – 11.1] †
Postnatal environmental smoke exposure	
No (n=136)	11.1 [9.9 – 12.5]
Yes (n=51)	10.4 [8.6-12.6]
Smoke exposure ¶	
Never (n=113)	11.3 [9.9 – 12.8]
Prenatal only (n=23)	10.1 [7.6 – 13.4]
Postnatal only (n=30)	12.3 [9.6 – 15.8]
Pre- and postnatal (n=21)	8.1 [6.0 – 10.9] §
Siblings	
No (n=121)	11.1 [9.8 – 12.6]
Yes (n=66)	10.4 [8.8 – 12.4]
Upper respiratory Symptoms	
No (n=57)	11.7 [9.8 – 14.0]
Yes (n=130)	10.5 [9.4 – 11.9]
Lower Respiratory Symptoms	

No (104)	11.7 [10.3 – 13.4]
Yes (83)	9.9 [8.5 – 11.5] ‡

† p=0.047 compared to unexposed

¶ this variable is the combination of the two variables above

§ p=0.042 compared to never exposed and p=0.033 compared to exposed only postnatally

‡ p=0.09 compared to asymptomatic infants

LogFE_{NO} values were compared by using the Student t test

Table 4 Multivariable linear regression model with logFE_{NO} as dependent variable

Variable	Geometric mean FE_{NO} [95% CI] ppb	
Smoke exposure		
Never (n=113)	9.8	[8.2-11.8]
Prenatal only (n=23)	9.6	[7.2-12.7]
Postnatal only (n=30)	10.5	[8.0-13.9]
Pre- and postnatal (n=21)	7.2	[5.3-9.8] †
Lower Respiratory Symptoms		
No (n=104)	10.2	[8.5-12.2]
Yes (n=83)	8.3	[6.7-10.2] ‡
Upper Respiratory Symptoms		
No (n=57)	10.4	[8.6-12.7]
Yes, no doctor (n=120)	10.8	[9.3-12.5]
Yes, doctor (n=10)	6.9	[4.6-10.4] §

†p=0.052 compared to never exposed and p=0.042 compared to exposed only postnatally

‡p=0.046 compared to asymptomatic infants

§p=0.047 compared to non-severe symptoms

The FE_{NO} values have been adjusted in the regression model for all the listed factors and for gender, birth weight, maternal atopy, age at study date and ambient NO

Figure legends

Figure 1: Pre- and postnatal maternal smoking and FE_{NO} values in infants

Never exposed n=113; exposed only prenatally n=23; exposed only postnatally n=30; exposed pre- and postnatally n=21.

Bars represent geometric means FE_{NO} estimated with multivariable linear regression and adjusted for gender, birth weight, maternal atopy, age at study date, ambient NO, LRS and URS.

Figure 2: Upper respiratory symptoms and FE_{NO} values in infants

No symptoms n=57; yes, did not visit a doctor n=120; yes, did visit a doctor n=10.

Bars represent geometric means FE_{NO} estimated with multivariable linear regression and adjusted for gender, birth weight, maternal atopy, age at study date, ambient NO, smoke exposure and LRS.

Figure 1

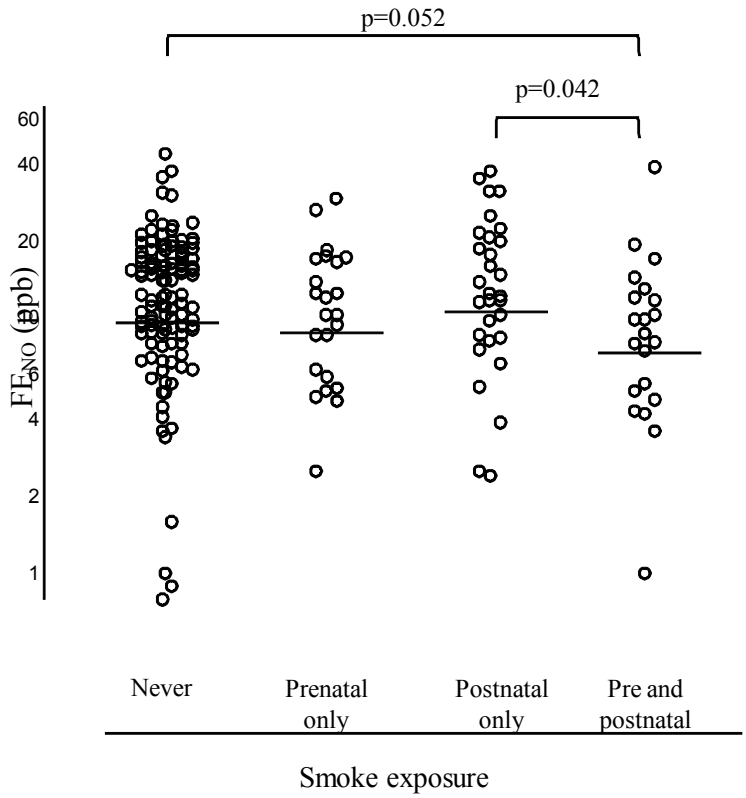
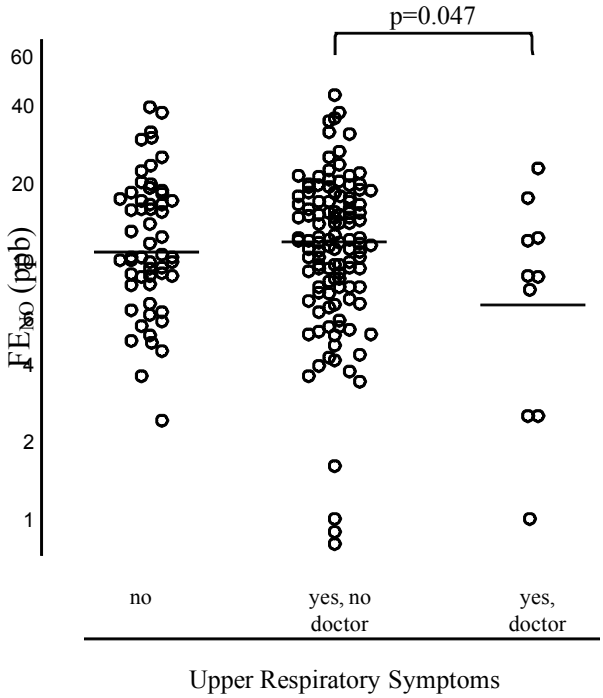

p=0.052
p=0.042
60
40
20
6
4
2
1
FE_{NO} (ppb)
Never
Prenatal only
Postnatal only
Pre and postnatal
Smoke exposure

Figure 2


p=0.047
60
40
20
4
2
1
FE_{NO} (ppb)
no
yes, no doctor
yes, doctor
Upper Respiratory Symptoms