

Maternal Atopic Disease Modifies Effects of Prenatal Risk Factors on Exhaled Nitric Oxide in Infants

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In a prospective healthy birth cohort, we determined whether levels of exhaled nitric oxide (eNO) in healthy unselected infants at the age of 1 month were associated with maternal atopic disease and prenatal and early postnatal environmental exposures. Tidal eNO was measured in 98 healthy, unsedated infants (35 from mothers with atopy) (mean age $\pm$ SD, 36.0 ± 6.2 days) and was compared with histories taken in standardized interviews. eNO was higher in males compared with females (17.7 vs. 14.6 ppb, $p = 0.042$) and infants exposed to postnatal maternal smoking (+4.4 ppb, $p = 0.027$), adjusting for weight and tidal breathing parameters. Prenatal tobacco exposure was associated with higher eNO (+12.0 ppb, $p = 0.01$) in infants of mothers with asthma and lower eNO (−5.7 ppb) in infants of mothers without asthma (p for interaction < 0.0001). Coffee consumption in pregnancy decreased eNO (−6.0 ppb, $p = 0.008$) only in children of mothers with atopy (p for interaction = 0.015). Paternal atopy had no influence. In the early phase of immunologic development, before the onset of infections and allergic disease, the effect of prenatal or early postnatal environmental factors on eNO was modified by the presence of maternal atopic disease. This underlines the complex interaction of maternal and environmental factors in the development of airway disease.

Keywords: nitric oxide; infants; atopy; smoking; caffeine

Exhaled nitric oxide (eNO) is increased in adults with asthma (1) and in adults, children (2–4), and infants (5) with allergies. Elevated levels of eNO are strongly associated with conditions known to increase allergic inflammation in children with asthma (6). It has been hypothesized that elevated eNO may be associated with an increased risk for developing asthma (7). eNO is also influenced by genetic (8–11) and environmental factors, such as infections (12), tobacco smoking (13, 14), coffee drinking (15), and steroid medication (16) in adults and older children. All three NO synthase isoforms (NOS1–NOS3) are expressed in epithelial cells and have been implicated as the primary source of levels of NO in expired air. Genetic factors related to NOS1 and NOS3 have been associated with asthma (8–10, 16), but the increase in eNO in the expired air of individuals with asthma has mainly been attributed to an activation of the inducible form of NOS mediated through proinflammatory cytokines (17). However, it is unclear whether eNO is already influenced at

birth by risk factors of asthma and atopic disease or whether eNO is only elevated in individuals with chronic airway inflammation and recurrent symptoms. Furthermore, it is unclear whether environmental factors (e.g., tobacco smoke, caffeine) can influence NO synthases prenatally or during early infancy. Preliminary evidence showed that eNO in infants of smoking mothers can be decreased (14).

These questions are important because altered immune maturation leading to asthma and other atopic diseases is likely to be primed very early in life, and an expanding body of data suggests that initial priming of the T cell system to environmental allergens may occur before birth (18, 19). There is increasing recognition of the role of gene–environment interactions in the pathogenesis of asthma (18–21), and little is known about whether sex or parental factors (family history of atopic disease), exposure to environmental factors, or the interaction between the two affect eNO in young infants.

To address these questions, we measured eNO in healthy 1-month-old infants in a prospective birth cohort study, conducted before development of respiratory symptoms. We examined whether eNO is influenced by important risk factors for childhood asthma (22), including prenatal and early postnatal environmental factors. We were particularly interested in whether the effects of environmental factors on eNO in newborns are modified by the presence of atopic disease in the parents.

METHODS

Subjects and Study Design

In a prospective cohort study, 105 white infants were recruited prenatally from two maternity hospitals in the region of Berne, Switzerland. Exclusion criteria for the study were: ethnicity other than white, significant preterm delivery (< 35 weeks), major birth defects, respiratory distress with need for oxygen for more than 30 minutes after birth, or other significant perinatal disease. The 20 infants reported in our technical article (14) were part of this larger sample.

A few days after birth, an extensive questionnaire was applied by standardized interview. Included were questions pertaining to sociodemographic factors, prenatal and postnatal exposure to putative risk factors for respiratory disease, and eNO modification, such as environmental tobacco smoke (ETS), exposure to pets in pregnancy, maternal consumption of coffee, and exposure to infections (e.g., nursery, siblings). History of parental atopic disease, defined as self-reported, doctor-diagnosed asthma, hay fever or eczema, was verified by a standardized interview by a pediatric pulmonologist (U.F.). Lung function studies were performed at a median age of 36 days (range, 23–58 days) before the occurrence of recurrent respiratory infections. The Ethics Committee of the University Children's Hospital of Berne approved the study.

eNO Measurements

The infants were studied unsedated, during quiet sleep in a supine position with the head in the midline position as previously described and validated (14). Tidal flow (V_T), volume (V), eNO, and CO_2 were measured via an infant mask (Size 1; Homedica AG, Cham, Switzerland) using a prototype infant lung function apparatus (Exhalys;

(Received in original form July 22, 2003; accepted in final form March 26, 2004)

Supported by Swiss National Foundation SCORE grants 3200-052197.97/1 and 32-68025.02, the National Health and Medical Research Council, Australia, Astra-Zeneca (Switzerland), MSD Switzerland, and the Swiss CF Foundation. G.L.H. is a Neil Hamilton Fairley Fellow, Australia. C.K. had a research scholarship from Swiss National Science PROSPER, grants 3233-069348 and 3200-069349.

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Am J Respir Crit Care Med Vol 170, pp 260–265, 2004

DOI: 10.1164/rccm.200307-1002OC

Internet address: www.atsjournals.org

EcoMedics, Dürnten, Switzerland) according to the standards of infant lung function testing (23). A bias flow of NO-free air was used to ensure that no contamination of exhaled eNO by ambient NO occurred. Flow was determined using an ultrasonic flow meter (Spiroson Model M30.8001; EcoMedics) and NO was measured on line with a rapid response chemiluminescence analyser (CLD 77 AM; EcoMedics) in the range 0–100 ppb and an error rate of < 1 ppb. To adjust for the $\dot{V}_T$ dependence of tidal eNO (14), the third quartile of the eNO (14) and $\dot{V}_T$ were measured. eNO, $\dot{V}_T$, and $\dot{V}_T$ values of individual breaths were averaged to produce a median value of each parameter for the analyzed epoch of 100 breaths. As previously validated, the time-based third quartile eNO value was chosen because intraindividual variability of 100 breaths was lowest (group mean coefficient of variation, 9.8 [range 6–14] %) (14).

Statistical Analysis

Data analyses were performed using STATA version 8.2 for Windows (STATA Corporation, College Station, TX). All continuous variables were reasonably normally distributed. Multiple linear regression analyses were performed to examine the effect of different environmental exposures on eNO while controlling for the other relevant factors. The following variables were entered one by one in the model: sex, age, weight, length, gestational age, birth weight, $\dot{V}_T$ parameters (14) (breathing frequency, expiratory time, minute ventilation [$\dot{V}_E$], PTEF, and $\dot{V}_T$), family history of atopic diseases, prenatal and postnatal parental smoking, coffee consumption in pregnancy, pets, spilling (regurgitation), number of siblings, and other environmental exposures associated with atopic disease (22) or NO. For the regression models, continuous variables ($\dot{V}_T$ parameters, age, and weight) were centered (as described in the footnote of the corresponding tables) and categorical variables were entered as indicator variables. *t* Tests were used to test for statistical significance. All regression models were systematically tested for effect modification by maternal and paternal doctor-diagnosed asthma and atopic disease (asthma, hay fever, or eczema) and sex of the child by including interaction terms and performing stratified analyses. Sex, weight, and $\dot{V}_E$ were included in all models. As all $\dot{V}_T$ parameters and all anthropometric measures were highly correlated, adjustment for $\dot{V}_E$ and current weight was sufficient.

As a sensitivity analysis, robustness of the findings was investigated by adjusting the models for other biometric data and other tidal breathing parameters. We also repeated the whole analysis using NO output as the dependent variable instead of eNO. Results obtained using these alternative models were very similar to those of the main model.

TABLE 1. ANTHROPOMETRIC AND LUNG FUNCTION DATA OF THE STUDY CHILDREN

	Mean	SD	Range
Anthropometric data			
Age at study date, d	36.00	6.17	23–58
Weight at study date, kg	4.50	0.58	3.30–6.30
Length at study date, cm	55.69	2.31	49–61
Gestational age, wk	39.89	1.32	35–42
Birth length, cm	49.44	1.93	44–54
Birth weight, kg	3.46	0.45	2.17–4.82
Lung function data			
f, breaths/min	43.5	12.5	23.5–80.9
Te, s	0.86	0.28	0.35–1.69
$\dot{V}_T$, ml	30.2	5.5	18–43
PEF, ml/s	57.1	17.4	21–133
$\dot{V}_E$, ml/min	1,280	330	590–2,776
eNO, ppb	16.60	7.17	3–34
NO output, nl/s	0.643	0.248	0.10–1.31

Definition of abbreviations: CI = confidence interval; eNO = tidal exhaled nitric oxide; f = breathing frequency; Te = expiratory time.

n = 98.

Data did not differ between children of mothers with and without atopic disease, except for birth length (49.9 and 48.8 cm, respectively; $p = 0.029$) and length at study date (56.6 and 55.2 cm, respectively; $p = 0.024$).

RESULTS

In 98 of 103 recruited infants (95%), valid eNO measurements were obtained and included in the analysis (Table 1). In the other five infants, sleep time was insufficient for the measurements. In a subgroup of 10 infants, short-term repeated measurements (in the same sleeping session) were performed and showed good repeatability (mean $\pm$ SD eNO differences, -0.49 ± 1.26 ppb, p [paired *t* test] = 0.25). Thirty-five mothers and 42 fathers had a doctor-diagnosed, atopic disease (asthma, hay fever, or eczema) (Table 2). Thirteen mothers smoked both during pregnancy and postpartum (all less than 20 cigarettes per day), whereas 10 other women resumed smoking after delivery. Coffee was consumed by 65 mothers (1–4 cups per day) during late pregnancy. All infants were breast-fed.

Association between $\dot{V}_T$ Pattern and eNO Measurements

As previously shown (14), NO clearance from the lung depends on $\dot{V}_T$ patterns in infants and, thus, on anthropometric data. It is therefore mandatory to adjust all subsequent multivariate regression analyses for $\dot{V}_T$ parameters (Table 3). Because all of them were highly correlated, adjustment for $\dot{V}_E$ was sufficient, with no additional effect of other tidal values on eNO in a multivariable equation.

Association between Sex, Parental Atopy, Environmental Factors, and eNO

The group mean eNO was 16.6 ppb (95% confidence interval [CI], 15.2–18.0 ppb), and boys (17.7 ppb; CI, 15.7–19.6 ppb) had significantly ($p = 0.042$) higher values than girls (14.6 ppb; CI, 12.7–16.5 ppb). This difference remained significant after adjusting for weight and $\dot{V}_E$ (standing for anthropometric and $\dot{V}_T$ differences) with 14.7 ppb for a girl with a weight of 4.5 kg and a $\dot{V}_E$ of 1,280 ml/minute and 17.6 ppb for a similar boy ($p = 0.049$). Group means were similar in infants of mothers with asthma (18.5 ppb; CI, 13.3–23.8 ppb), infants of mothers

TABLE 2. FAMILY HISTORY AND PRE- AND POSTNATAL ENVIRONMENT OF THE STUDY CHILDREN

	n (%)
Male sex	64 (65)
Family history	
Maternal atopic disease*	35 (36)
Maternal asthma	13 (13)
Paternal atopic disease*	42 (43)
Paternal asthma	10 (10)
Environment	
Maternal smoking postnatally, of which:	23 (23)
Smoking both in pregnancy and postnatally [†]	13 (13)
Smoking only postnatally	10 (10)
Paternal smoking	28 (29)
Pets [‡]	33 (34)
Maternal coffee consumption in pregnancy	65 (66)
Occasional spilling (regurgitation/vomiting) [§]	54 (55)
Respiratory infection [¶]	6 (6)

Environmental exposures did not differ significantly between children of mothers with and without atopic disease, except for coffee consumption in pregnancy (73 and 42%, respectively; $p = 0.02$).

* Atopic disease was defined as the presence of doctor-diagnosed asthma, hay fever or eczema (any of them).

[†] All of these infants were also exposed postnatally.

[‡] All children with pets were exposed both in pregnancy and postnatally.

[§] None of these children had doctor-diagnosed or treated gastro-esophageal reflux.

[¶] Respiratory infection was defined as cough or wheeze together with fever or upper respiratory symptoms lasting more than two consecutive days.

TABLE 3. ASSOCIATION BETWEEN TIDAL FLOW VALUES AND EXHALED NITRIC OXIDE

Model components	eNO (ppb)		
	Parameter Estimate (ppb)	95% CI	p Value
Constant	16.60	+15.3 to +17.9	< 0.0001
$\dot{V}_E$, ml/min	-0.0093	-0.0133 to -0.0053	< 0.0001
T_e , s	9.85	+5.11 to +14.59	0.0001
PEF, ml/s	-0.161	-0.238 to -0.085	0.0001
f , breaths/min	-0.214	-0.322 to -0.106	0.0002
V_T , ml	-0.05	-0.31 to +0.22	0.72

Definition of abbreviations: CI = confidence interval; eNO = tidal exhaled nitric oxide; f = breathing frequency.

The parameter estimates indicate the change of eNO (in ppb) per unit change of the respective tidal flow measure. Predictors were centered when entering into the model. The estimated constant (16.6 ppb) represents the value of an infant with average tidal flow measures: ($\dot{V}_E$ = 1,280 ml/min; T_e = 0.86 sec; PEF = 57 ml/sec; f = 43.5 breaths/min; V_T = 30.2 ml). Except for V_T , log-transformed data were used.

with other atopic disease (15.6 ppb; CI, 12.6–18.5 ppb), and infants of mothers without atopic disease (16.6 ppb; CI, 14.8–18.3 ppb).

Similarly, neither paternal atopic disease nor any of the environmental or nutritional factors (tobacco smoke, caffeine) were associated with eNO in the whole group. The only exception was exposure to maternal smoking after birth, but not during pregnancy (i.e., infants whose mothers recommenced smoking after delivery), which was associated with increased eNO levels compared with infants of nonsmoking mothers (+4.45 ppb; CI, 0.51–8.38 ppb; p = 0.027).

Modification of the Association between Environmental Exposures (Smoking, Coffee Consumption) and eNO by Maternal Atopy and Asthma

We found that the effect of sex, maternal smoking, and coffee consumption during pregnancy on eNO in the infants was significantly modified by maternal atopic disease (Table 4). The Δ eNO term in Table 4 describes the effect of male sex, postnatal maternal smoking, and prenatal maternal coffee consumption on eNO levels in different subgroups: infants of mothers with atopy (n = 35), with asthma (n = 13), and without atopic disease (n = 63).

Male sex. In infants of mothers with atopic disease, eNO was higher in males compared with females (+7.34 ppb; p = 0.005). In contrast, no such sex difference was seen in offspring of mothers without atopic disease (-0.23 ppb, p = 0.9; p for interaction = 0.046).

The sex difference was even more striking in the subgroup (n = 13) of children of mothers with asthma (+11.91 ppb; p = 0.011). In infants of mothers with atopic disease other than asthma (hay fever or eczema, n = 22), a smaller sex difference was found (+4.8 ppb; CI, -2.16 to 11.76; p = 0.17). A regression model, including atopic disease as a linear term (coded as 0 = nonatopic, 1 = atopic, nonasthmatic, 2 = asthmatic), showed a significant interaction between this indicator of atopy and sex (p = 0.021).

ETS. Smoking during pregnancy was associated with significantly decreased eNO levels in infants of mothers without atopic disease (-5.49 ppb; p = 0.023). This was not the case in children of mothers with atopy; if anything, eNO levels were increased if the mother had smoked (+3.83 ppb; p = 0.278). The effect modification by maternal atopy was tested by including interaction terms (prenatal smoking $\times$ maternal atopy) in the model (p = 0.015). In children of mothers with asthma (Table 4), prenatal smoking was associated with a large increase in eNO (+12.01 ppb; p = 0.011). In consequence, the effect modification (maternal asthma $\times$ prenatal smoking) was highly significant (p for interaction = 0.0001).

In infants of mothers with atopic disease other than asthma, smoking during pregnancy tended to be associated with a lower eNO (-7.60 ppb; CI, -18.2 to -3.0 ppb; p = 0.15), similar to offspring of mothers without atopic disease.

Coffee consumption during pregnancy. In infants of mothers

TABLE 4. ASSOCIATION BETWEEN ENVIRONMENTAL EXPOSURES AND EXHALED NITRIC OXIDE IN INFANTS OF MOTHERS WITH AND WITHOUT ATOPIC DISEASE

	Infants of Mothers without Atopic Disease (n = 63)			Infants of Mothers with Atopic Disease (n = 35)				Infants of Mothers with Maternal Asthma (n = 13)			
	Δ eNO (ppb)	95% CI (ppb)	p Value [†]	Δ eNO (ppb)	95% CI (ppb)	p Value [†]	Interaction [‡]	Δ eNO (ppb)	95% CI (ppb)	p Value [†]	Interaction [‡]
Male sex*	-0.23	-3.79 to +3.33	0.90	7.34	+2.44 to +12.23	0.005	0.046	11.91	+3.58 to +20.23	0.011	0.013
Maternal smoking in pregnancy*	-5.49	-10.18 to -0.79	0.023	3.83	-3.25 to +10.91	0.278	0.015	12.01	+3.57 to +20.45	0.011	0.0001
Coffee in pregnancy	1.34	-2.24 to +4.93	0.46	-6.02	-10.35 to -1.70	0.008	0.007	-4.86	-16.09 to +6.36	0.35	0.008

Definition of abbreviation: CI = confidence interval; Δ eNO = mean difference in eNO concentration in exposed compared to unexposed infants in ppb.

All models are stratified by maternal atopic disease, defined as the presence of doctor-diagnosed asthma, hay fever or eczema, and adjusted for $\dot{V}_E$, weight, and sex. Results are presented as parameter estimates (95% CI). Effect modification was tested by including the interaction term in the full model. $\dot{V}_E$ and weight were centered when entered into the model; the estimated constant (not shown), therefore, represents the value of an infant with average $\dot{V}_E$ (1,280 ml/minute) and average weight (4.50 kg). Population mean eNO = 18.1 ppb.

* Adjusted only for $\dot{V}_E$ and weight.

[†] p Values assessing the significance of an effect within subgroups (atopic/nonatopic mothers).

[‡] p Value for interaction between listed exposures and subgroups (atopic/nonatopic) in a model including all children.

with atopic disease, maternal coffee drinking during pregnancy was associated with decreased eNO (-6.02 ppb; $p = 0.008$). No such effect of coffee was seen in infants of mothers without atopic disease ($+1.34$ ppb, $p = 0.46$), with a highly significant interaction between caffeine consumption and atopy ($p = 0.007$). In contrast to male sex and smoking, results from infants of mothers with asthma did not differ from that of those with other atopic disease.

Other exposures and potential risk factors such as spilling, paternal atopic disease, paternal smoking, alcohol or antibiotic intake during pregnancy, respiratory infections before the lung function test, or the presence of pets during pregnancy were examined but showed no independent association with eNO, nor did they act as confounders.

DISCUSSION

We investigated whether eNO in infants measured shortly after birth is influenced by sex, family history of asthma, eczema, hay fever, or prenatal or early postnatal environmental stimuli (e.g., smoking, caffeine intake, exposure to pets in pregnancy, and infections). We have found that male sex and maternal smoking, recommenced postnatally, can increase eNO in the first month in healthy infants before symptoms occur. Smoking during pregnancy was associated with lower levels of eNO in infants of mothers without asthma. Maternal atopic disease *per se* was not associated with eNO in infants, although infants of mothers with asthma tended to have higher eNO levels. This was not statistically significant in this small group ($n = 13$). However, the presence of maternal atopic disease modified the effect of male sex and environmental factors (ETS and caffeine exposure in pregnancy) on eNO in the healthy offspring. Male sex was associated with higher eNO levels only in infants of mothers with atopic disease, although this effect was stronger in infants of mothers with asthma. Smoking during pregnancy by mothers without asthma was associated with lower levels of eNO in their infants, whereas the same exposure in mothers with asthma was associated with higher levels. This difference in effects between groups was statistically highly significant using interaction tests. Similarly, maternal coffee drinking during pregnancy was associated with lower levels of eNO only in the offspring of mothers with atopic disease. Paternal atopic disease and other environmental factors (e.g., exposure to pets, infections, and alcohol during pregnancy) had no influence.

Influence of Sex

Male sex is a known risk factor for childhood asthma, and sex differences in eNO levels have been reported for adults, with men having higher levels than women (8, 11). An association between variants of NOS1 genes and eNO levels has been described as being influenced by sex, and has only been found in females (8). However, it has never been shown that the sex difference in infants is influenced by maternal atopic disease. Although eNO levels were not increased in male infants of mothers without atopic disease, they were significantly increased in sons of mothers with atopic disease. The latter effect was even present after correction for different flow and expiratory times. The reason for this is not clear but is in accordance with other immunologic evidence (24).

Influence of Smoking

ETS exposure is a known risk factor for childhood asthma (22), and maternal smoking in particular has been associated with asthmatic symptoms in children (22). There is increasing evidence that prenatal ETS exposure is a risk factor for decreased lung function and frequent respiratory symptoms in the first

years of life (25, 26). Although it has been postulated that ETS exposure may affect airway mechanics, little is known about whether prenatal ETS exposure affects NO synthesis. In an earlier methodologic report including 20 infants (14), we found that healthy infants of mothers without atopic disease had lower eNO levels, indicating that prenatal ETS exposure might have an influence on eNO synthesis. In the current larger study, we confirmed those findings in the subgroup of infants of mothers without asthma. This might be analogous to observations reported for adult chronic smokers (13), in which chronic use of tobacco decreased eNO, whereas acute exposure was associated with increased eNO. A new finding of our study was that eNO levels were higher in infants of mothers who recommenced smoking after delivery. We hypothesize that this situation is comparable with that of the acute smoking adult. However, more interesting is the fact that we only found a significant decrease in eNO in infants of mothers without asthma, whereas eNO was significantly increased in infants of mothers with asthma when prenatally exposed to tobacco, with strong statistical evidence for interaction between maternal asthma and prenatal tobacco exposure. In infants of mothers with atopic diseases other than asthma (e.g., eczema and hay fever), prenatal ETS exposure was not significantly associated with eNO levels. These findings suggest a dominant influence of maternal asthma rather than atopy on eNO levels in the offspring in the presence of prenatal ETS exposure. Statistical significance of these findings should be interpreted carefully, as the groups of infants of smoking mothers ($n = 13$) and mothers with asthma ($n = 13$) were relatively small. Nevertheless, because eNO is likely to be a marker of allergic inflammation, these data expand our knowledge of the complex relationship between ETS exposure—an important environmental factor—and the development of allergies (27–30).

Caffeine Consumption

Caffeine consumption during pregnancy has been found to be associated with prevalence of wheeze and asthma in adults (31), and with IgE levels in infant cord blood (30). Although it has been recently shown that low-dose theophylline does not decrease eNO in individuals with mild asthma (32), our data strongly support an interaction between caffeine and eNO in infants of mothers with atopic disease but not in those of mothers with asthma. In mothers with atopy and asthma, caffeine drinking during pregnancy significantly decreased eNO levels in their infants. The effect was not seen in infants of mothers without atopy. These findings are in accordance with findings in adults where caffeine (100 mg, 1 cup) intake was associated with lower levels of eNO (15). As summarized by Bruce and colleagues (15), caffeine can have various effects. In high doses, caffeine inhibits phosphodiesterases, leading to elevation of cAMP and cGMP, which may be the mechanism for its weak bronchodilator effect. Further effects include mobilization of intracellular calcium stores and, at lower concentrations, antagonistic action on adenosine receptors (A_1 , A_{2a}). In addition, an increase in intracellular cGMP upregulates constitutive NOS, whereas an increase in intracellular cAMP downregulates it. Stimulation of adenosine receptors leads to different effects; A_{2a} receptor stimulation increases NO generation, whereas A_1 receptor stimulation leads to reduced NO production by endothelial cells. We found this caffeine effect only in infants of mothers with atopic disease. The reason is unclear, but we hypothesize that these pathways may interact with immunologic phenomena. Theophylline, for example, has antiinflammatory effects and reduces eosinophilic inflammation in individuals with mild asthma (32). However, whether caffeine has an interaction with immunomodulating effects in newborn infants remains unclear. Our data

suggest that this may be possible, and future studies should aim to elucidate these effects.

The Role of Maternal Atopic Disease

Maternal atopic disease *per se* was not associated with increased eNO in healthy infants despite the fact that maternal atopic disease is a strong risk factor for later asthma and other atopic disease in children. Thus, simply speaking, infants with such an increased risk of developing atopic disease are not *per se* born with increased levels of eNO in exhaled air. Nevertheless, the presence of maternal atopic disease can modify the effects of male sex and environmental factors (e.g., tobacco smoke and caffeine) on eNO in the healthy infants. These findings are interesting in light of the concept that childhood asthma might be determined by the interaction of maternal and environmental factors (18–21). A new and interesting aspect is that these interactions occur so early in life. It has been hypothesized that altered or disrupted immune maturation in this early phase of life may play a role in the pathogenesis of atopic disease. Many studies suggest that initial priming of the T cell system to environmental allergens may occur before birth (18, 19). Transplacental immune compounds, such as cytokines, or intrauterine allergen exposure have a modulating effect on the immune system. Many cytokines also have the potential to induce NOS (17). We hypothesize that the in utero environment or other maternal factors play a modulating role in the eNO metabolism of infants. Interestingly, paternal atopic diseases had no influence at all, despite the fact that surely the father has a genetic influence on the child. This is in accordance with studies showing that maternal influence on childhood asthma is dominant (33). We have not investigated the influence of genes associated with NOS or asthma on eNO in infants.

Technical Aspects of the Study, Concomitant Factors, and Limits of the Method

In unsedated infants, we found eNO to be 16.6 ppb (SD = 7.17) and a calculated NO output (NO output = $\dot{V} \times \text{eNO}$) of 0.643 nl/second (0.248) at 50–75% of expiration. Mean flow during this phase was 38.7 ml/second. Only sparse data on tidal eNO values in healthy infants and toddlers are available (4, 5, 14, 34). Wildhaber and coworkers (5) measured forced eNO in healthy infants (18.8 ± 12.4 ppb [mean $\pm$ SD]) at a flow of 50 ml/second through a face mask, equating to a NO output of 0.94 nl/second, and demonstrated a significant effect of parental atopy on eNO irrespective of respiratory history. In healthy 2–5-year-old children measured at a flow rate of 40–60 ml/second using a resistor, tidal eNO (50–90% of expiration) was found to be 3 ppb, equating to an NO output of 0.12–0.18 nl/second in the mouthpiece (4). The results from these older children are difficult to compare due to methodologic differences. Nevertheless, it became obvious in many studies that eNO is flow dependent. The advantage of our method, which assesses eNO in the third quartile of a tidal breath, is the relative steadiness of the flow and the minimal intraindividual breath-by-breath variation (14). Furthermore, in contrast to the CO₂ washout signal (14), airway dead space is mainly washed out in this phase. In the current study, tidal breathing parameters (breathing frequency, expiratory time, $\dot{V}_E$, V_T , PEF) were significantly associated with eNO. This has been statistically accounted for in all models but is particularly important where groups with differences in expiratory flow or breathing pattern are compared (e.g., ETS-exposed or male infants) (25, 26, 35). The calculation of NO output (14) only partly accounted for this problem and, thus, it was more useful to account for $\dot{V}_E$ in a multivariate regression of eNO. With NO output as outcome, the findings were similar. A limitation of the study

was that maternal atopy was not verified with skin-prick testing. Most mothers had positive prick tests performed by their family doctors at the time of diagnosis. However, history was taken before the lung function test and a potential error could therefore only be a nondifferential misclassification, leading to a weakening of the effect found in the study rather than the induction of a false positive result. Interviews at the time of recruitment verified the general practitioners' diagnosis of maternal atopic disease by our team.

As always, interpretation of findings of effect modifications must be done with due caution because of the large number of potential statistical comparisons. We have targeted our analyses to interactions between parental atopic disease and a limited number of risk factors known to influence eNO in adults. Nevertheless, our findings certainly need to be replicated in a different population before they can be generalized.

Conclusions

In this prospective study (36), we showed that male sex and environmental factors, such as maternal smoking and coffee drinking during pregnancy, influenced eNO in healthy offspring before recurrent infections or the onset of manifest allergic disease in a very early phase of immune development. The presence of maternal atopic disease—a strong, known risk factor for atopic childhood asthma—was not associated with increased eNO in the infants *per se*, however, it did modify the effects of male sex and environmental factors (e.g., tobacco smoke and caffeine) on eNO in the healthy infants. It appears that mainly environmental factors, such as ETS exposure, caffeine, and maternal intrauterine environment, are crucial for eNO in infants. Since eNO is a noninvasive marker attributed to airway inflammation at least in adults and children, it may be a sensitive tool with which to study the effects of timing and dosage of exposure to various environmental factors dependent on maternal predisposition and intrauterine environment. This marker may help to find answers in studies of gene–environment interactions in the pathogenesis of wheezing disorders and allergic disease in infants, and help to test preventative measures (37) and therapeutic strategies.

Conflict of Interest Statement: U.F. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; C.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; H.R. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; M.C. received, from June 30, 2000 to June 30, 2001, a research grant from University of Ljubljana that was supported by GlaxoSmithKline, Slovenia in the amount of \$10,000; B.R. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; J.H.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; G.L.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this.

Acknowledgment: The authors thank the staff of the Pediatric Respiratory Department, University of Berne for their help in obtaining the data presented in this study. In particular, H. Straub, C. Becher, M. Graf, G. Wirz, H. Gehr, M. Stampfli, and Dr. D. Baldwin.

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