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Exhaled nitric oxide is not reduced in infants with cystic fibrosis

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ABSTRACT: Fractional exhaled nitric oxide (F_{ENO}) has been reported to be reduced in cystic fibrosis (CF) patients. However, data from young children are conflicting and it is not clear whether this is a primary feature of the disease or a secondary response. The present study compared F_{ENO} between CF and healthy infants using a validated single-breath technique.

A total of 23 healthy infants (11 females; mean age 40.1 weeks) and 18 infants with CF (nine females; 64.9 weeks) underwent tests of lung function and F_{ENO}. Bronchoalveolar lavage (BAL) was collected from all CF infants 2–5 days after lung function testing.

There was no significant difference in F_{ENO} between the CF and healthy infants (geometric mean: 23.1 parts per billion (ppb) and 17.0 ppb, respectively). There was an inverse relationship between age and F_{ENO} in the CF patients, but not in the healthy group. Within the CF group, there was no association between F_{ENO} and any marker of airway inflammation measured in the BAL.

Exhaled nitric oxide is not reduced in cystic fibrosis infants, but does decrease with age. The current data indicate that F_{ENO} is not a good marker of airway inflammation in cystic fibrosis.

KEYWORDS: Airway inflammation, cystic fibrosis, exhaled nitric oxide, infants

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Exhaled nitric oxide (fractional exhaled nitric oxide (F_{ENO})) has been proposed as a marker of airway inflammation [1]. However, in cystic fibrosis (CF), an inflammatory airway disease, F_{ENO} is either reduced or not significantly different from healthy controls [2–7]. As nitric oxide (NO) plays an important role in host defence through both its anti-microbial activity and regulation of ciliary motility [8], reduced F_{ENO}, and by implication NO production, may predispose CF airways to subsequent bacterial colonisation [4, 7, 9].

One reason for reduced F_{ENO} in CF may be decreased expression and/or activity of the inducible NO synthase (NOS) II gene [4, 7]. In healthy and asthmatic children, NOSII is the predominant contributor to F_{ENO} levels [10]. The present authors have shown that NOSII expression is downregulated in CF compared with healthy infants [11]. Therefore, it would be expected that F_{ENO} would also be reduced in young CF children. However, the data are conflicting. ELPHICK *et al.* [12] found that F_{ENO} was reduced in CF infants, while WOOLDRIDGE *et al.* [13] found no difference in F_{ENO} between young CF children and a disease control group. In these studies, two different methods were used to measure F_{ENO} (tidal breathing and direct lower airway sampling). The aim of the current study was to test the hypothesis that F_{ENO}, measured using a validated single-breath method, is reduced in CF infants.

METHODS

Subjects and protocol

A total of 18 infants with CF (nine females; age range 5.5–114.5 weeks) were compared with 23 healthy infants (11 females; age range 15–66 weeks) whose F_{ENO} results have been published previously [14]. All children underwent pulmonary function testing that included measures of forced expiratory volume in 0.5 seconds ($FEV_{0.5}$) and F_{ENO}. All children were well at the time of testing. Details of all infants are presented in table 1.

Infants were studied in the supine position, asleep following an oral dose of chloral hydrate (60–100 $mg \cdot kg^{-1}$). F_{ENO} was always measured prior to lung function. The study was approved by the Medical Ethics Committee of the Princess Margaret Hospital for Children (Perth, Australia) and written informed consent was obtained from the parents.

Lung function

Lung function ($FEV_{0.5}$) was measured using the raised volume rapid thoracoabdominal compression technique as previously described [15]. Results are reported as z-scores (FEV_Z) using normative data calculated from healthy children tested at Princess Margaret Hospital, Perth, Australia [16].

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TABLE 1 Anthropometric, lung function and fractional exhaled nitric oxide (FeNO) data for cystic fibrosis (CF) and healthy infants

	CF	Healthy
Subjects n	18	23
Sex M/F	9/9	12/11
Age weeks	64.9±32.0*	40.1±21.2
Height cm	73.5±10.5	71.1±5.7
Weight kg	9.6±2.9	8.9±1.6
FEVz	0.20±1.45	0.51±0.77
FeNO ppb	17.0 (12.8–22.5)	23.1 (18.2–29.3)

Data are presented as arithmetic mean±SD or geometric mean (95% confidence interval), unless otherwise stated. M: male; F: female; FEVz: forced expiratory volume in 0.5 second, z-score; ppb: parts per billion. *: $p<0.05$.

Exhaled NO

FeNO was measured with a chemiluminescence analyser (NOA 280; Seivers Instruments Inc., Boulder, CO, USA) using the single-breath technique as described previously [14]. Briefly, an inflatable jacket was wrapped around the infant's chest and abdomen. Lung volume was then raised to an inflation pressure of 20 cmH_2O *via* a computer-controlled circuit. Three consecutive inflation cycles were used after which the jacket was inflated manually using a 3-L calibration syringe. Immediately prior to jacket inflation, an *i.v.* cannula (Insyte 16 or 22Ga; Becton Dickinson, Salt Lake City, UT, USA) was inserted into the expiratory limb of the system to increase expiratory resistance. During exhalation, mouth pressure was maintained at 20 cmH_2O to achieve a constant flow of 11 $mL \cdot s^{-1}$. The high positive expiratory pressure minimises nasal NO contamination [17].

Microbiology, inflammation and CF genotype

The pulmonary function tests for the CF infants were performed 2–5 days prior to a routine bronchoalveolar lavage. Therefore, contemporaneous data was available for cytology and bacteriology for these children. For inflammatory markers, data was recorded for total cell count (TCC) and the percentage of neutrophils. Soluble markers in bronchoalveolar lavage (BAL) fluid were analysed using commercially available ELISAs for interleukin (IL)-8 (Becton Dickinson, San Diego, CA, USA) and leukotriene (LT)B_4 (Amersham Biosciences, Piscataway, NJ, USA). Significant microbial colonisation was considered as $>10^4$ colony-forming units$\cdot mL^{-1}$. Using available genotype data, the CF infants were grouped as homozygous for the ΔF508 mutation (in the CF transmembrane conductance regulator) or other.

Statistical analyses

FeNO was log normally distributed. Age, height, weight and FEVz were normally distributed. The percentage of neutrophils was also normally distributed, but TCC, IL-8 and LTB_4 were all log transformed. Normally distributed data are expressed as mean±SD, whereas transformed data are expressed as geometric mean with 95% confidence intervals. Unpaired t-tests were used to compare FeNO, FEVz, age, height and weight between healthy and CF children, as well as between CF children with different genotypes and colonisation status (colonised or uncolonised). The relationship between FeNO and age and FEVz was investigated using Pearson's correlation initially for the whole group and then separately for the CF and healthy groups. As there was a significant age difference between the two groups, the difference in FeNO between the two groups was reassessed using a general linear model (GLM) to control for age. Finally, for the CF group, associations between FeNO and cytology data were determined using Pearson's correlations.

Sample size

Reported differences in FeNO between CF and healthy subjects have varied from more than two-fold [6, 12] to no difference [4]. However, in infants, mean FeNO for CF patients was less than half that of healthy infants [12]. In the present study, using data from healthy controls, the authors calculated a sample size of 18 infants per group for the study to have 80% power, with 95% confidence, to detect a two-fold difference in FeNO between the groups.

RESULTS

Healthy versus *CF infants*

The healthy infants were significantly younger than the CF infants ($p=0.008$); however, there was no difference in height or weight (table 1). There was also no significant difference in FEVz between the two groups (table 1). Geometric mean FeNO was 23.1 parts per billion (ppb) (18.2–29.3 ppb) and 17.0 ppb (12.8–22.5 ppb) for the healthy and CF groups, respectively. This difference was not significant ($p=0.1$). After controlling for age in the GLM, the difference became less pronounced ($\beta=1.06$ ppb; $p=0.8$).

For the combined population, there was a significant inverse relationship between age and FeNO ($r=-0.53$; $p<0.001$). When the healthy and CF groups were analysed separately, the relationship was only evident in the CF group ($r=-0.64$; $p=0.005$; healthy group $r=-0.3$; $p=0.16$; fig. 1).

Within the CF group

There was no significant difference in age, height, weight or FEVz between the ΔF508 homozygotes (n=9) and other genotypes (n=9) (table 2). There was a nonsignificant trend for FeNO to be lower in the homozygotes (13.1 *versus* 22.2 ppb, respectively; $p=0.07$) and this trend remained after controlling for age ($p=0.08$). FeNO was significantly lower in the ΔF508 group compared with healthy controls when the three groups were compared using a one-way ANOVA ($p=0.015$); however, this difference was lost after controlling for age ($p=0.23$).

Only four of the infants had evidence of significant colonisation from the subsequent BAL (two with candida, one with pseudomonas and one with aspergillus); however, their FeNO was not different from the noncolonised infants (geometric mean 16.1 *versus* 17.1 ppb). Finally, there was no association between FeNO and any of the cellular markers of inflammation (fig. 2a–c).

DISCUSSION

In this study, FeNO, obtained using a constant flow, single-breath method, was not reduced in CF compared with healthy

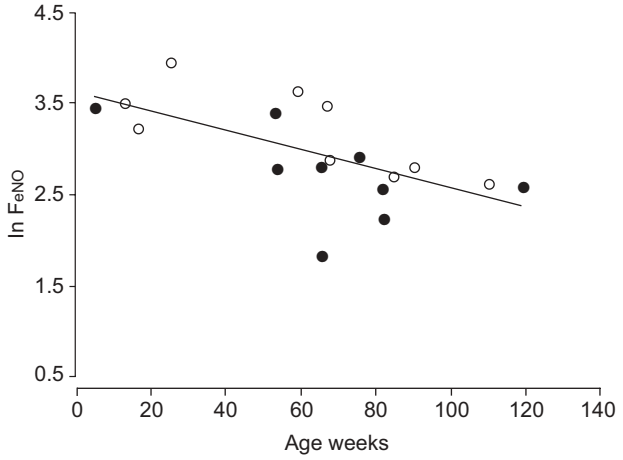

FIGURE 1. Relationship between age and fractional exhaled nitric oxide (FeNO) in cystic fibrosis (CF) infants (r=0.64). ●: ΔF508 homozygotes; ○: CF infants with other genotypes. The relationship was similar in both groups.

infants. This contrasts with the findings of ELPHICK *et al.* [12] but agrees with those of WOOLDRIDGE *et al.* [13], although they did not make any comparisons with a healthy control group. Interestingly, FeNO decreased with age in the CF group but not the healthy group and this may account for reports of reduced FeNO in older CF patients [3, 6, 7].

Only two other studies have investigated CF in very young children [12, 13]. In the study by ELPHICK *et al*. [12], FeNO was measured during tidal breathing. The current authors have previously reported a number of problems interpreting FeNO levels collected during tidal breathing in infants [18]. WOOLDRIDGE *et al*. [13] measured FeNO directly from the lower airways *via* a bronchoscope; however, they made comparisons with a control group of children with other airway disease. In the present study, the control infants had no history of respiratory disease and the authors measured FeNO using a single-breath technique that has been demonstrated to be more sensitive, for comparisons between groups, than tidal breathing measurements [14].

TABLE 2 Anthropometric, lung function and fractional exhaled nitric oxide (FeNO) data for cystic fibrosis infants with different genotypes

	Homozygote F508	Other
Subjects n	9	9
Sex M/F	4/5	5/4
Age weeks	70.2±30.4	59.6±34.3
Height cm	73.9±12.1	73.0±9.4
Weight kg	9.9±3.3	9.3±2.6
FEVz	0.30±0.82	0.11±1.95
FeNO ppb	13.1 (8.9–19.2)	20.1 (15.6–31.6)

Data are presented as arithmetic mean±SD or geometric mean (95% confidence interval), unless otherwise stated. M: male; F: female; FEVz: forced expiratory volume in 0.5 second, z-score; ppb: parts per billion.

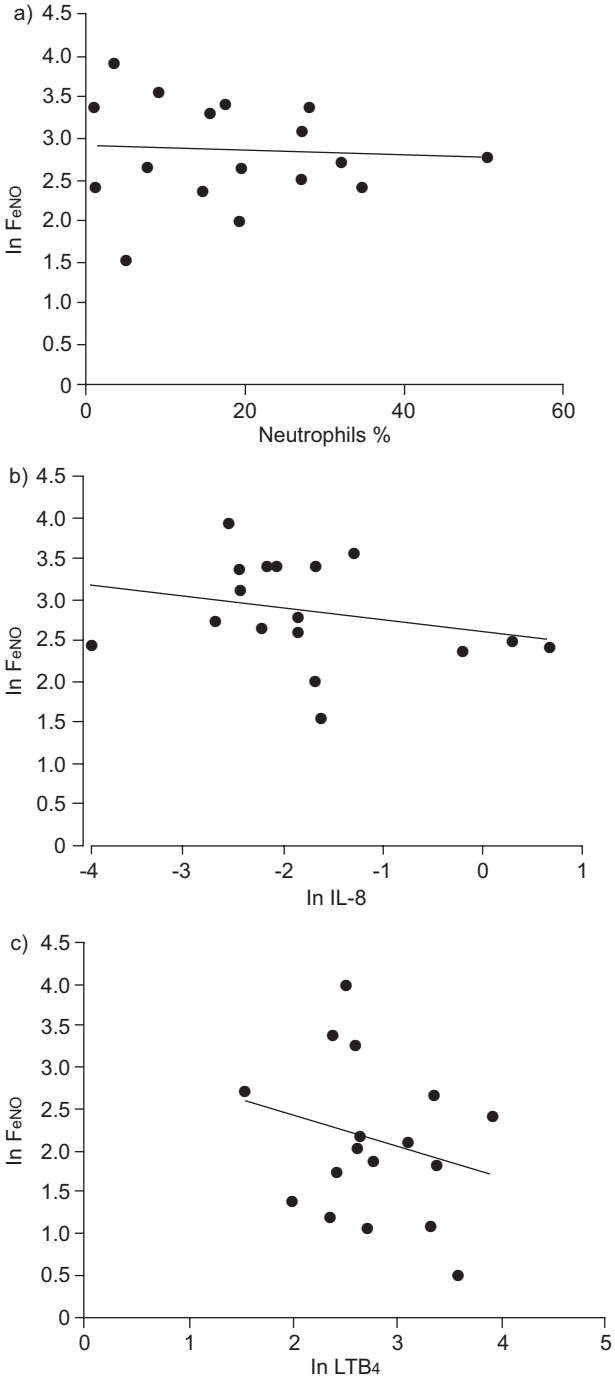

FIGURE 2. Association between the natural log (ln) of fractional exhaled nitric oxide (FeNO) and a) percentage of neutrophils (r^2=0.006), b) interleukin (IL)-8 (r^2=0.07) and c) leukotriene (LT)B_4 (r^2=0.06) in cystic fibrosis infants.

In non-CF children, NOSII is the main contributor of FeNO [10]. There is evidence for decreased NOSII activity in CF airways [19, 20], including infants [11], and, therefore, low NOSII expression may be an innate defect in CF. However, in CF, NOSII may not be the major determinant of FeNO. Indeed, polymorphisms in another of the NOS isoforms, NOSI, can significantly influence levels of FeNO in CF adults [21, 22]. Furthermore, WOOLDRIDGE *et al.* [13] found no association with

lower airway FeNO and NOSII in CF children. In this situation, given that a number of factors contribute to the final concentration of NO in exhaled breath, the single-breath technique in infants might not be sensitive enough to detect small differences in basal production of NO that might be expected from differences in NOSII expression and activity.

An interesting finding was an inverse relationship between age and FeNO in the CF children but not in healthy children. The reduction in FeNO with age observed in this group of young children may explain why FeNO is often reported reduced in older CF subjects [3, 6, 7]. The reasons for this reduction are unclear; however, it may relate to increased mucous plugging or bacterial infection. In this study, there was no difference in FeNO in CF children with and without significant colonisation.

Within the CF group, no significant difference in FeNO between children who were homozygous for ΔF508 and those who were not was found, although homozygotes had a trend for lower FeNO. This is consistent with the results of THOMAS *et al.* [7] who studied a larger group of CF adults. The implications of these differences are unknown but, in this study, it did not seem to be a function of the severity of disease. Although only four of the CF children had evidence of bacterial colonisation, FeNO in these children was not different from the rest of the group. Again, this is in agreement with THOMAS *et al.* [7]. Furthermore, there was no association between FeNO and any markers of inflammation in the BAL. WOOLDRIDGE *et al.* [13] also found that FeNO was not associated with any of these markers in CF children. This suggests that FeNO is not a good marker of airway inflammation in CF.

In the present study, fractional exhaled nitric oxide was not reduced in cystic fibrosis compared with healthy infants. Exhaled nitric oxide in this group of cystic fibrosis infants decreased with age, suggesting that changes in cystic fibrosis airways with age influence nitric oxide dynamics in the airways. Finally, this study confirmed that fractional exhaled nitric oxide does not reflect airway inflammation in cystic fibrosis infants.

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