Exhaled nitric oxide in premature and mature infants during the first months of life

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Exhaled Nitric Oxide in Premature and Mature Infants During the First Months of Life
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Short title: Exhaled Nitric Oxide in neonates – a review

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Abbreviations:
NO = Nitric Oxide
FeNO = Fractional Exhaled Nitric Oxide
V’NO = FeNO x flow
BPD = Bronchopulmonary dysplasia
BILD Cohort= Bern Infant Lung Development Cohort
SBE = Single Breath Exhalation
CF = Cystic Fibrosis
NOS= Nitric oxide Synthetase
ABSTRACT
Goth F E M, Schmidt B J, Agertoft L, Jørgensen I M. Exhaled Nitric Oxide in premature and mature infants during the first months of life

Aim: In this review, we aim to describe how exhaled Nitric Oxide(NO) changes during the first months of life in premature and mature infants.

Method: Review of the literature up to August 2020, on online, tidal breathing NO measurements in unsedated infants. The association between Fractional exhaled NO(FeNO) values, postnatal age, and prematurity was analysed using linear mixed modeling and Spearman’s rank correlation.

Results: Median FeNO during the first months of life was 5.9 and 8.5 ppb in premature and mature infants, respectively. The linear mixed model analysis showed a significant effect of postnatal age on FeNO (p = 0.007).

Conclusion: Our study suggests that FeNO is higher in mature infants than premature infants, and FeNO increased with postnatal age at approximately the same pace in both groups.

Keywords: Exhaled Nitric Oxide, Neonatal, Lung development, Premature

Key Notes:
- Exhaled Nitric Oxide increases with age during the first months of life.
- Premature infants have lower values of exhaled Nitric Oxide than mature infants.
- Exhaled NO might be a biomarker for the developing respiratory system in infants.
- Additional clinical, longitudinal follow-up studies are needed to evaluate the importance and clinical relevance of the difference in exhaled Nitric Oxide between premature and mature infants.
Title: Exhaled Nitric Oxide in premature and mature infants during the first months of life

1.0 INTRODUCTION

Exhaled Nitric Oxide (NO) is considered a predictor of airway eosinophilia and type 2 allergic response in children and adults. The method of measuring exhaled NO is non-invasive and well-tolerated, and therefore the method has gained ground as an inflammatory biomarker in airway diseases in pediatric pulmonology [1]. High Fractional exhaled NO (FeNO) is seen in asthma and other inflammatory disorders, and low FeNO is seen in cystic fibrosis and survivors of Bronchopulmonary dysplasia (BPD) [2-5]. It has been shown that exhaled NO increases with age in childhood from 4 to 17 years of age in a healthy (non-asthmatic) population [6].

The issue concerning poorly developed lungs and lack of surfactant is well known when born prematurely [7,8]. The prenatal and neonatal period is crucial to the development of the lung [9,10]. NO has multiple physiological functions, besides being a predictor of eosinophil airway inflammation, including angiogenesis and pre-and postnatal lung development [11]. Fractional exhaled NO (FeNO) is one of the possible biomarkers for early detection of respiratory morbidity investigated in the literature [9,12,13]. To which extent FeNO is useful as a neonatal marker or predictor for respiratory morbidity or lung development is still unknown.

Exhaled Nitric Oxide in neonates and infants can be measured in multiple ways, and the following technical aspects and methods for measurement should be taken into consideration when planning a study or interpreting results from FeNO measurements:

Nasal FeNO or mixed facemask FeNO: The mucosae in the nose and sinuses produce large amounts of NO contaminating the exhaled air from the lower airways [14]. Nasal contamination can be reduced by exhalation against a resistance [15]. Neonates have less developed sinuses, why the contamination is smaller in this group of patients. When using a mixed facemask, air from both mouth and nose is collected. This method is well tolerated by infants and newborns, who are generally nose-breathers [16].

Sedated, intubated, or awake with spontaneous breathing: Infants and newborns, especially premature infants, have an irregular breathing pattern easily affected by external impacts. Therefore, many lung function and FeNO studies have been performed on sedated and intubated infants [17]. However, intubation seems to affect the levels of FeNO, and the clinical relevance of testing lung function in a sedated state is doubtful [17].

Single Breath Exhalation (SBE) or Tidal Breathing: In adults and older children able to cooperate, SBE is the standard method to measure FeNO. However, this technique is not possible in infants and newborns who cannot cooperate to the procedure. Schmidt et al. validated a method measuring FeNO by tidal breathing in infants and newborns, which proved well tolerated, with a low subject-specific-prediction-variance and high success rates [16].

Offline or Online FeNO: Offline means collecting FeNO into a collapsible bag allowing for later analysis. This method is portable and convenient in a clinical setting. With the online method, FeNO is analysed instantly by direct exhalation into a chemiluminescence analyser. A Japanese study showed that the offline method is comparable to the online
method when correcting for flow-rate and resistance [18]. MacBean et al. confirmed this hypothesis when the subject could fill the whole bag in one breath (SBE). The results differed when using multiple breaths to fill the bag, and it is therefore not recommended when measuring FeNO in neonates and infants who cannot fill the entire bag in one breath [19].

Flow: For reproducibility, the exhalation flow can be adjusted by continuously adjusting the exhalation resistance. The expired flow aim is $50 \text{ mL} \cdot \text{s}^{-1}$ (range 40-60) [1].

In this non-systematic review, we aim to review the literature on exhaled NO in neonates and infants to provide a basis for future research on the subject. We wish to enlighten possible differences between premature and mature neonate’s lung development by describing characteristics of FeNO distribution among premature and mature infants within the first months of life. Chosen measurement methods and technical aspect were selected with regards to potential future clinical application. We consider this review important since a difference in FeNO could indicate a potential clinical use in the future and focus research on the field of early neonatal biomarkers for long-term respiratory health.

2.0 METHOD

2.1

Literature searching strategy: We searched PubMed using the MESH terms (exhaled nitric oxide) AND ((infant) OR (neonatal)) for papers published in English. The last search was carried out on August 27th, 2020. The reference list of all selected papers was screened to ensure the inclusion of all relevant studies.

Inclusion criteria: Age 0 to 3 months old at the time of FeNO measurement, mixed facemask, multiple breaths (tidal) exhaled NO, online method, mean or median FeNO values presented in the paper, papers published in English.

Exclusion criteria: Sedated, intubated, nasal NO, offline method, Single Breath exhaled NO, known disease-specific cohort (CF, BPD), and age range at FeNO measurement > one month. Papers were not included if mean or median FeNO values were not presented in the paper.

Outcome measure: We selected mean, or median FeNO values for non-BPD or control group infants from all included studies, premature and mature, respectively. Premature infants were defined as born before 36 weeks and six days of gestation. Mature infants were defined as born at 37 weeks gestational age or later.

We did not include $V'NO$ (FeNO x flow) values as they were not reported in most papers.

2.2

Statistics: Differences between groups were calculated using the t-test or Wilcoxon rank test. FeNO mean and SD for each group as well as median and IQR are presented. Associations between FeNO, postnatal age, and prematurity were analysed using Spearman’s rank correlation. To investigate changes in FeNO over time (postnatal age), we applied a linear mixed model, including age and prematurity (categorical) as fixed effects as well as their interaction.
To account for the correlation in the repeated measurements and possible dependence within cohorts, we included the latter as a random intercept. All statistical analysis was performed using the Statistical software R version 3.6.1.

3.0 RESULTS

Initially, 217 papers were found in the search, and all abstracts were read, after which we included 74 papers reviewed in full text. Seven papers fulfilled all inclusion criteria and none of the exclusion criteria. By cross-reference, another five papers meeting all inclusion criteria were found. In total, twelve papers were included in this review (Figure 1).

In one study [16], mean FeNO values were only published as the estimated standard deviations of log FeNO. However, we obtained geometric mean FeNO values from the originally published PhD thesis [20]. Six papers presented results from the same cohort, the Bern Infant Lung Development (BILD) cohort in Bern, Switzerland. The authors were contacted, and they recommended one of the papers [21] as reference-data for the full cohort [21-26].

Only two papers presented a non-European cohort –The South African and Chinese studies [27,28]. Nine studies had a cross-sectional design, and three studies were longitudinal.

The included papers are presented in Table 1. Exhaled NO measurements of total 189 premature and 772 mature infants were included in this review. The gestational age of the infants ranged from 27 weeks to term. We included papers with similar methods of measuring FeNO (see selected inclusion criteria section 2.1), however, they did differ in how they validated the method by breaths or over time. In the included papers, ten to 100 breaths or one-minute tidal breathing was used to measure FeNO.
The mean postnatal age at FeNO sampling was 16.9 days in the mature group and 10.0 days in the premature group. Altogether, ten mean or median values of FeNO were reported in the mature group and 18 values in the premature group (Table 2). Mean FeNO was significantly lower in premature infants (6.3 ppb) compared to mature infants (8.4 ppb) (p=0.01).
Table 2. Characteristics and numbers of mean and median values of FeNO from included papers for both premature and mature infants

<table>
<thead>
<tr>
<th></th>
<th>Premature</th>
<th>Mature</th>
<th>All infants</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age in days at the</td>
<td>Mean (SD)</td>
<td>10.0 (9.8)</td>
<td>16.9 (16.9)</td>
<td>12.5 (12.9)</td>
</tr>
<tr>
<td>measurement</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FeNO (ppb)</td>
<td>Median [IQR]</td>
<td>5.9 [5.1, 7.0]</td>
<td>8.5 [7.3, 9.2]</td>
<td>6.6 [5.4, 8.6]</td>
</tr>
<tr>
<td>FeNO (ppb)</td>
<td>Mean (SD)</td>
<td>6.3 (1.7)</td>
<td>8.4 (2.7)</td>
<td>7.1 (2.3)</td>
</tr>
<tr>
<td>Total number of mean/median values in all included papers</td>
<td>18</td>
<td>10</td>
<td>28</td>
<td></td>
</tr>
</tbody>
</table>

| Number of mean/median FeNO values per included paper | Figueras-Aloy (2003)[29] | 1 | 1 | 2 |
|                                                     | Fuchs (2011)[21]         | 0 | 1 | 1 |
|                                                     | Gray (2015)[28]          | 0 | 1 | 1 |
|                                                     | Leipaelae (2004)[30]     | 1 | 1 | 2 |
|                                                     | Liu (2011)[27]           | 4 | 0 | 4 |
|                                                     | Schmidt (2018)[16]       | 6 | 6 | 12 |
|                                                     | Williams (2007)[31]      | 6 | 0 | 6 |

Linear mixed model analyses showed a significant effect of postnatal age on FeNO (estimate = 0.07, SE = 0.02, p = 0.007). There was no significant effect on prematurity on FeNO (estimate = 0.07, SE = 0.02, p = 0.06) Spearman’s rank test showed a significant correlation between prematurity and FeNO and postnatal age and FeNO (p= 0.03 and p=0.02, respectively).

The mean or median FeNO values on different days of life reported in the different studies are presented in Figure 2 for premature and mature infants, respectively.

*Infants born prematurely:* A total of five studies included premature infants. Among these studies, there were three longitudinal studies. FeNO values ranged from 4.2 to 10.18 in premature infants during the first months of life. Williams et al.[31] found a stable FeNO throughout the first month of life in premature infants. Schmidt et al.[20] found an increase in FeNO during the first month, from 5.2 ppb to 10.2 ppb. Figueras-Aloy et al.[29] found higher FeNO values on day three than the other studies. Liu et al.[27] found higher FeNO values during the first week compared to Williams et al. and Schmidt et al.[20,31]. Schmidt et al. found higher FeNO values from day 14-30 than Williams et al. and Leipala et al.[16,27,31].

*Infants born at term:* FeNO values included in this review ranged from 4.0 to 14.3 ppb in mature infants. During the first week of life, FeNO was between 4.0 ppb to 9.9 ppb. There was only one longitudinal study of mature infants[20]. Fuchs et al. reported higher values than the other studies [21].
4.0 DISCUSSION

This non-systematic review indicates that mature infants have a higher FeNO value during the first months of life than premature infants. FeNO for both groups of infants increased with postnatal age during the first months of life.

The lower measured FeNO values in premature infants can indicate lower NO production in the immature lungs and airways than in the mature lungs. NO's role in the airway physiology of the human body is still not completely understood. Nitric oxide Synthetase (NOS) that produces NO in endothelial, epithelial, and inflammatory cells is present in constitutive (cNOS), constitutive endothelial (eNOS), and inducible (iNOS) forms. cNOS and eNOS are Ca²⁺ and calmodulin-dependent enzymes and, when stimulated, releases small NO concentrations [11]. On the contrary, iNOS releases large NO concentrations over a more extended period (hours to days) after stimulation of proinflammatory cytokines [11]. Therefore, the concentration of exhaled NO from the airways is generally determined by the expression of iNOS in the airway endothelial and epithelial cells and macrophages. eNOS is abundant in endothelial cells of pulmonary blood vessels [32]. The expression of eNOS increases during fetal lung development, and it has been suggested that this increase in eNOS may enhance the angiogenesis and airway function in the postnatal period [33]. NO works as the main vasodilator in the transition from fetal to neonatal breathing and decreases vascular resistance [34].

A Canadian study, including 73 infants, published data indicating that younger postnatal age increases treatment effectiveness with inhaled NO unaffected by gestational age and reduces the need for respiratory support [35]. This result could be interpreted as the effect of inhaled NO increases when the endogen production of NO in the airways is lower. However, then we would expect that a lower gestational age would have an impact as well. Moreover, there is not enough evidence for the use of inhaled NO as treatment or prophylaxis for respiratory disease in premature infants, and guidelines advise against it [36].

A recently published meta-analysis showed no difference in FeNO in children from age two and adults born respectively premature and mature, suggesting that the difference in exhaled NO disappears over time [37]. However, this Meta-analysis cannot enlighten this review's subject and did include studies using different methods of measuring FeNO. The included studies had a substantial heterogeneity both in the range of age and cohort-definitions. To avoid this, we choose only to include studies with similar methods of measuring FeNO.

4.1 Differences between studies included in this review:

4.1.1 Age: Generally, FeNO levels were higher in the BILD cohort [21] than in the Danish cohort [20]. The infants in the BILD cohort were older and had a wider age span of 25-58 postnatal days than Schmidt et al., where all subjects were tested longitudinally during the first month of life and finally at postnatal age 28 days [20,21]. Schmidt et al. had a longitudinal design and reported an increase in FeNO with age. This could explain the higher FeNO values found in the
BILD cohort as the infants were generally older. Both studies reported geometric mean values. Fuchs et al. included participants over a more extended calendar period (1999-2009), making this cohort potentially more heterogenic compared to Schmidt et al. that included all participants over one year (2013-2014) [16,21].

4.1.2

**Cohort characteristics:** In this review, we choose to include mean or median FeNO values from control groups, if possible. However, not all papers had a control group. The BILD cohort included unselected healthy infants, and Schmidt et al. included unselected infants admitted to the Neonatal Unit at the hospital for different causes (uncomplicated prematurity, mild respiratory symptoms, 2 cases of mild BPD, and other non-respiratory diseases) which could affect the FeNO values [27,38]. Figueras-Aloy et al. included subjects with a high risk of infection, which could explain the higher FeNO levels in their cohort [29]. Viral infections in the upper airways have been shown to increase FeNO by inducing NO Synthase (NOS) in airway epithelial cells [11,39]. Whether other kinds of infections also increase FeNO is still unknown. However, NOS is induced by cytokines and, therefore, likely to increase the production of NO in all infections, to some extent [11,40]. Liu et al. found slightly higher FeNO values than the European studies, which could be explained by genetic differences and environmental exposure [41].

4.1.3

**Method validation:** When measuring FeNO, the BILD cohort, Leipala et al., and Williams et al. counted breaths and excluded irregular breathing, sighs, and short apneas [21,30,31]. Schmidt et al. and Liu et al. measured FeNO continuously for 60 seconds a minimum of 3 times, including sighs and apneas, to make the inclusion of premature infants with irregular respiratory pattern possible [20,27]. Liu et al. used ambient NO and not NO free air, which might explain their slightly higher FeNO values compared to the other cohorts.

4.1.4

**Gestational age in the premature cohorts:** Williams et al. found stable FeNO levels from day 7 to 28 in premature infants, whereas Schmidt et al. found an increase in FeNO over the same period of time. The lower GA in William's cohort (mean GA 27 weeks, and all infants below 32 weeks compared to GA range from 28-36 in Schmidt's cohort) could explain this difference since FeNO increases with postnatal age [16]. The mean gestational age in the Chinese cohort was higher than in William's and Schmidt's cohorts, explaining the slightly higher reported FeNO values [20,27,31].

4.1.5

**Reported FeNO values:** Some of the papers included reported FeNO as median [29-31] and some as geometric mean [16,21,27,28]. FeNO is not considered normally distributed but rather right skewed, and when data is distributed this way, a geometric mean tends to overestimate the true mean compared to the median.

4.2
Strengths and limitations

Overall, the studies' direct comparison is compromised by the many different factors discussed above. The use of geometric means and medians for comparison is not optimal. However, since the full data set from the papers were rarely presented, we had to accept this limitation. We have tried to avoid bias by choosing only to include studies using similar methods of measuring FeNO and only including cohorts with a small range in age. However, there is still some heterogeneity in methods, as well as the periods of included samples differ.

When measuring FeNO with a facemask, it is impossible to eliminate the nasal and upper airways' contribution, where the NO concentration is considerably higher than in the lower airways [42]. Although the neonatal sinuses are less developed, neonates are nose-breathers, and we cannot rule out that there is a contribution from the upper airways. However, all the included studies used multiple breath and facemask, thus, measured mixed FeNO. A comparison between the studies in this context is, therefore, less problematic. Furthermore, the upper airway contribution to mixed FeNO may be of less interest in angiogenesis and lung development.

Multiple breath FeNO is flow-dependent, and it is suggested that V'NO (FeNO x flow) is a better measurement than FeNO when using the multiple breath technique [43]. V'NO is not included in this review, as only a few of the studies included reported V'NO values.

The data in this review show FeNO values' trends over time for groups of infants and cannot be interpreted as individual tracking of FeNO values.

5.0 CONCLUSION

This review suggests that premature infants have lower FeNO values than mature infants at birth and during the first months of life. FeNO increased with postnatal age at approximately the same pace in both groups resulting in a lower FeNO in premature infants the first months of life compared to mature infants. Many factors influence the level of FeNO during the first few months of life, and the role of NO is still not fully understood. This should be taken into consideration when evaluating FeNO levels in infants. The potential relevance of these findings on the long-term respiratory outcome is still not unveiled.

We consider this review important since a difference in FeNO could indicate a potential clinical use in the future. To fully understand and explain FeNO changes during the first months of life, there is a need for standardised testing methods, further additional longitudinal measurements, and follow-up studies of larger cohorts. Including studies with premature and mature infants examined at defined postnatal ages and the correlation to subsequent respiratory morbidity.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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**References**


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Figure captions:

**Figure 1.** Literature search. Inclusion and exclusion of papers, resulting in the inclusion of 12 papers in this review.

**Figure 2.** Mean and/or median FeNO values from included papers for premature and mature cohorts separately.