Eur Respir J 2008; 32: 252–254
DOI: 10.1183/09031936.00073608

EDITORIAL

Environmental exposure in relation to exhaled nitric oxide in newborns: is it all about timing?

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Exhaled nitric oxide (eNO) is a marker of eosinophilic airway inflammation. It is increasingly used for the diagnosis and monitoring of asthmatic lung disease in adults as well as children [1, 2] and is believed to be helpful in titrating steroids or in predicting relapse in established asthma [3, 4]. Although it is known that eNO is increased in healthy atopics [5], its role during the development of asthmatic airway disease is not understood. Only a few studies have examined whether eNO is already elevated before the occurrence of respiratory symptoms or in children that are prone to developing asthma [6, 7]. Data published so far showed an interaction with smoke exposure, suggesting that, at least in high-risk children, eNO may play a role in the very initial phases of recurrent respiratory symptoms [6]. It is tempting to speculate that eNO is a pre-existing marker before allergic inflammation of the airways occurs and that children can be ''eNO phenotyped'' early in life [8], enabling the identification of those with a high risk for later asthma development. However, before this can be done it is necessary to understand the natural course of eNO during the time period of fastest lung development, and the influence of external environmental stimuli on this course with possible long-term consequences [9].

In this issue of the *European Respiratory Journal*, GABRIELE *et al.* [10] help to understand the issues of smoke exposure, airway symptoms and eNO in healthy infants [10]. GABRIELE *et al.* [10] examined the effect of pre- and post-natal smoke exposure and respiratory tract infections on eNO measured at age 4–17 weeks in an unselected birth cohort. They found that compared with unexposed infants, post-natal smoke exposure alone led to an increase in eNO, whereas exposure to smoke both pre- and post-natally led to a sharp decrease in eNO. Interestingly, eNO values in children only exposed to smoke pre-natally were not altered compared with values in nonexposed children. On the basis of their data, GABRIELE *et al.* [10] concluded that the influence of smoking on eNO depends on the timing of the exposure.

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STATEMENT OF INTEREST: A statement of interest for P. Latzin can be found at www.erj.ersjournals.com/misc/statements.shtml

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A clear strength of the study by GABRIELE *et al.* [10] lies in the detailed information on smoke exposure. Data on smoking habits was gathered prospectively from the point of pregnancy onwards. In contrast to previous studies, this enabled GABRIELE *et al.* [10] to nicely disentangle the exact effects of pre- and/or post-natal smoke exposure on eNO levels. Although they did not validate the smoking history by cotinine levels, GABRIELE *et al.* [10] correctly stated that any misclassification would veer towards smoking parents being categorised as nonsmokers and, thus, would have caused the effects of smoking to be underestimated rather than overestimated in their study.

In addition, GABRIELE *et al.* [10] found that eNO levels were decreased in infants with upper respiratory tract symptoms requiring a visit to a doctor or with lower respiratory tract symptoms. This is in line with studies from other cohorts [11], but has to be interpreted cautiously as symptoms were assessed retrospectively and the effect of age and timing on eNO cannot be determined with this data. One clear weakness of the study by GABRIELE *et al.* [10] is the fact that infants inspired two breaths of nitric oxide (NO)-free air only on days with an ambient NO of >10 ppb. Using a filter at an arbitrary cut-off close to the group mean eNO may have introduced a nonsystematic error. However, findings in the multivariable analysis adjusted for ambient eNO provide some support that the observed associations are real and not due to methodological issues. Another weakness of the study by GABRIELE *et al.* [10] is that the sampling procedure does not enable eNO to be adjusted for expiratory flow. Thus, possible changes in expiratory flow as a reason for the differences between groups remain unknown, which is unfortunate, especially given the very small changes in eNO values.

So, why is this study important? It is not the first study showing an influence of smoke exposure or respiratory tract infection on eNO measurements in infants [11], it is not the first study showing different influences of pre- and post-natal smoke exposure on eNO in newborn infants [12], and it is clinically not relevant to show that smoking behaviour or respiratory infections may change eNO values within the magnitude of a few ppb. Furthermore, due to low numbers, GABRIELE *et al.* [10] could not examine an interaction between pre-natal smoke exposure and maternal asthma on eNO levels, as has been reported previously [12]. However, what makes the study by GABRIELE *et al.* [10] interesting is that it confirms the increasingly apparent role of timing in addition to intensity of environmental exposure in determining eNO levels [12, 13]. In addition, with the inclusion of this study, these results have

not only been obtained in three different cohorts, but also by using three different methods of measuring eNO [10, 12, 13]. Even though the raw levels of eNO cannot be compared among the different methods (mean eNO levels of 11 ppb in the study by GABRIELE *et al.* [10], 17 ppb in the study by FREY *et al.* [12] and 33 and 42 ppb, respectively, in the study by FRANKLIN *et al.* [13]), the effects of smoke exposure relative to the mean were surprisingly comparable among study groups. The use of different eNO measurement methods is significant for this age group because of the lack of standards available and the disparity from the single-breath method used later in life [14].

Finally, these studies further suggest that eNO is a useful marker to determine effects of isolated post-natal passive smoke exposure, which are usually small and not easily detectable by other lung function tests in this age group. Perhaps passive smoke exposure has a weaker effect on lung mechanics, or perhaps eNO is simply a more sensitive test. As the mechanisms for change in eNO following smoke exposure or respiratory tract infection are not entirely understood, this remains speculative. In contrast to post-natal smoke exposure, isolated maternal smoking during pregnancy has no effects on eNO, even though influences on lung mechanics are well known. Although once again the mechanisms involved are unclear, one could imagine the systemic regulation of growth hormones to be different than that of mediators inducing NO-synthase. Furthermore, this finding may hint at the possibility that inflammatory processes in the airways are induced more efficiently by direct exposure (after birth) than indirectly *via* the placenta. It is unknown whether the opposing effects related to pre- or post-natal exposure are indeed due to influences mediated *via* the placenta. However, a very tempting hypothesis for this would be a change in susceptibility of mediators that induce NO-synthase with birth or in general depending on (the correct) timing. This may be based on gene-expression/environment interaction (switching on and off certain genes during critical times of exposure) or on different activities of the three forms of NO synthase during different time phases of pregnancy. Some evidence from animal studies exist for the latter possibility, but studies in humans are limited [15, 16].

In any case, the results of the study by GABRIELE *et al.* [10] lend weight to eNO as a sensitive and relevant measure of the small effects caused by exposures at low levels, as also occurs with other environmental exposures, *e.g.* air pollution, and confirming data from older children [17]. It is important to have such a measure already available at an early age as lung growth and development are most rapid during this time.

Furthermore, the importance of timing may also hold true for the influence of other environmental exposures on lung development, as is known for the development of allergies. Here it has been shown that, for example, the protective effects of a farm environment are dependent when they occur during pregnancy or the first years of life [18].

Whether it is smoke exposure, air pollution, infections, medication or nutrition, it may not only be important whether an exposure occurs but also when it occurs during lung development. It seems plausible that the same environmental stimulus may have opposite effects depending on when it occurs. This has implications both for understanding lung growth and development as well as clinical management, *e.g.* when during the course of a disease a certain drug is most effective (or has the least side-effects). These complex environment-timing interactions can only be assessed with larger longitudinal cohort studies, regular measurements of eNO (or of other lung function parameters) and carefully assessed exposures. The effects of various environmental insults and their timing, and probably also the potential interaction with genetic influences, can then be disentangled using appropriate statistical approaches.

Further research is needed before treatment and prevention studies can be planned to determine the value of exhaled nitric oxide-phenotyping, and surely before exhaled nitric oxide can be used in individuals for prediction purposes [8].

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