

Minimal exhaled nitric oxide production in the lower respiratory tract of healthy children aged 2 to 7 years

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BACKGROUND: Exhaled nitric oxide (eNO) is elevated in inflammatory airway conditions, e.g. asthma. We measured eNO levels in normal preschool children for whom there is little data available and in whom the prevalence of asthma is high.

SUBJECTS AND METHODS: Fifty children, 2-7 years old, undergoing elective surgery, excluding airway procedures, were recruited. Children with known respiratory disease or acute viral infections were excluded. Gas for eNO measurement was collected in a non-diffusion bag via 1) the mask after inhalation induction of anesthesia, 2) endotracheal tube (ETT) or laryngeal mask airway (LMA), and 3) during emergence. Measurement was off-line by chemiluminescent analyzer.

RESULTS: Mean eNO level by mask was 10.23 ppb (mean value±SD of 8.8-11.1 ppb) after induction and 8.35 ppb (mean value±SD of 5.9-10.8 ppb) on emergence. Mean eNO for the intubated group (n=25) was 0.75 ppb (mean value(SD of 0.4-1 ppb) ($P<0.0001$ vs mask); mean eNO for the LMA group (n=25) was 2.6 ppb (mean value±SD of 2-3.2 ppb), which differed from the mask ($P<0.0001$), and from ETT values ($P<0.0001$).

CONCLUSIONS: Most eNO is produced by the upper airway in healthy pre-school children. The lower airway constitutive eNO production is very low. The LMA does not completely isolate the upper airway and current mask collection techniques allow significant contamination of samples by sino-nasal eNO production in young children.

Nitric oxide (NO), produced by endothelial cells, is a potent smooth muscle relaxant in the pulmonary vasculature. Recent work has demonstrated the presence of NO in gas exhaled from the respiratory system, and investigators have postulated that NO is a marker of the inflammatory response in the mucosa of the airway.¹⁻³ Indeed, several authors have documented changes in production of NO with the degree of inflammatory activity in adult asthmatics, although the techniques and measured amounts vary widely between laboratories.⁴ Recently, the European Respiratory Society has produced standards of measurement for exhaled nitric oxide (eNO).⁴

A number of authors⁶⁻⁸ have demonstrated that NO is produced in the airway of children with asthma, and in other inflammatory airway conditions. Moreover, exhaled NO concentrations have been determined in both normal and symptomatic children but the results are variable.⁹⁻¹⁴ To complicate these measurements contamination from the upper airway, already known to be a source of NO production, may occur because of difficulties in performing manoeuvres to isolate the nasal air passage for gas collection.¹⁴

Acute infective processes affecting the lower airway are very common in young children before school age. In our hospital, 50% of patients attending the emergency room for "asthma" are younger than the age of 6 years. In this age group, where objective measurements of severity of inflammatory activity are difficult to obtain, eNO appeared to offer a relatively simple marker of epithelial inflammatory activity. We therefore undertook these measurements of NO levels from the airway as the preliminary data of normal preschool children for a larger study of inflammatory mediators in this age group.

Subjects and Methods

The subjects of the study were 50 pre-school children, aged 2 to 7 years, undergoing intubation as part of elective surgery. The children were free of all respiratory symptoms at the time of the study, and had not had an acute respiratory infection for at least 2 months. Excluded from the group were children with an acute viral infection within 6 weeks of the study, children known to have asthma or other atopic conditions, and children with other chronic respiratory illness (i.e. cystic fibrosis, or bronchopulmonary dysplasia). This study was approved by the Institutional Review Board of the Montreal Children's Hospital, and informed written consent for each patient was obtained from parents/guardians.

Prior to induction of anaesthesia, parents/guardians were interviewed and requested to complete the ISAAC questionnaire¹⁵ for familial and patient history of atopy. Each child was weighed and measured prior to an inhalational induction of anaesthesia with sevoflurane. The sample of 50 asymptomatic children was divided into two equal groups of 25 based upon the means of airway control, either endotracheal tube (ETT) or laryngeal mask (LMA) during anaesthesia. The collection of exhaled gas for measurement of eNO was performed in the operating room after induction of anaesthesia. Aliquots were obtained on three occasions in each child: 1) at induction using a mask and a 10-cm H₂O/L per second expiratory resistor according to standard methods;^{5,16} 2) when the airway was controlled either by endotracheal intubation (ETT) or with a laryngeal mask (LMA), and 3) using the mask system at the end of the procedure, during emergence from anaesthesia. The mixed expired gas was collected for 30 seconds into a non-diffusing collecting bag (Model series 6000, Hans Rudolf Inc., Kansas City MO, USA,) with mean exhaled concentration determined by a chemiluminescent analyser (Sievers 280NOA; Sievers Instruments Co., Boulder CO, USA) within 30 minutes of collection of the sample. The analyser was calibrated each day by the two-point method using gases with known concentrations of nitric oxide. The values obtained were reported as mean concentrations of eNO (in parts per billion, ppb).

The demographic questionnaire data was evaluated to ensure that a sample representative for age, gender, and size was evaluated. Correlations were determined between the concentration of eNO, the technique of collection, and the demographic data. In addition, the repeated measures of eNO for each subject were

analysed by analysis of covariance. Statistical significance was assumed at a $P < 0.05$ level.

Results

There were significant differences in age distribution between the two groups (mean ETT group 3.2 ± 2.1 years, versus mean LMA group 5.1 ± 2.6 years) (Table 1). Following induction of anaesthesia, the eNO concentration, measured by the mask system, for the 50 subjects was a mean of 10.23 ppb with no significant difference between the two groups (unpaired t-test, $P > 0.1$) (Figure 1). After airway instrumentation the mean eNO in the intubated subjects (ETT group) was 0.75 ppb with 19 of 25 subjects having a mean eNO < 1.0 ppb. In contrast, the mean eNO for patients with a LMA was 2.6 ppb with 4 of 25 subjects with eNO levels < 1.0 ppb (Figure 1). There was a significant difference in eNO

Table 1. Demographic data for the 50 healthy children.

	ETT group*		LMA group†	
	Mean	SD	Mean	SD
Age (yr)	3.2	2.31-4.01	5.1	4.07-6.19
Weight (kg)	15.8	13.1-18.6	20.4	16.8-24.0
Time of surgery (min)	66.1	52.4-74.8	41.2	36.8-45.5
Total	13	12	18	7

ETT, endotracheal intubation; LMA, laryngeal mask
*13 males, 12 females, †18 males, 7 females

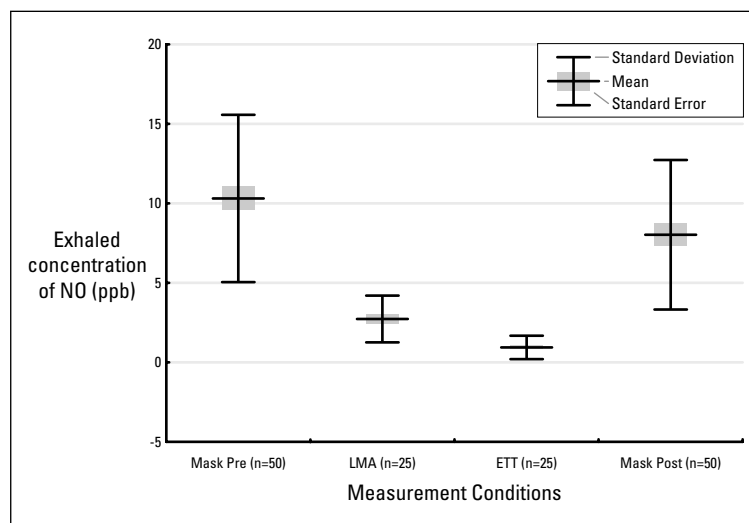


Figure 1. Mean exhaled nitric oxide concentrations (eNO, ppb) with different techniques of collection (LMA, laryngeal mask; ETT, endotracheal intubation), before (pre) and after (post) surgical procedure.

levels between ETT and LMA groups ($P < 0.0001$). On emergence from anesthesia the eNO concentration in the two groups was 8.35 ppb (mean with SD of 5.9 ± 10.8 ppb), again measured by mask and resistor, versus 7.32 ppb (mean with SD of 6.2-8.5 ppb) for the ETT group versus LMA group respectively. These results pre- and post-anesthesia were not significantly different ($P > 0.4$ by paired t-test).

Mask values during induction and emergence from anesthesia of eNO were significantly different from eNO levels with the airway controlled (i.e. intubated or LMA) ($P < 0.0001$), with the post-hoc test (Tukey HSD). There was no significant difference between pre-anesthesia and post-anesthesia eNO concentrations measured by mask. There was no correlation of eNO levels with age, weight, technique of anesthesia, nor length of anesthesia when measured by mask or controlled airway conditions.

Discussion

These data show that in healthy children between the age of 2 years and 7 years there is effectively no eNO produced in the lower airway below the vocal cords (i.e., the constitutive production of eNO was barely detectable using this analytical technique). In addition, the mixed eNO level collected by mask does not vary with age, weight, or gender. The importance of these results is that the constitutive production of eNO produced in the lung is barely detectable in healthy infants and young children, a finding that has been reported previously for a very small number of infants. Thus, eNO measures by mask seem to be inadequate to isolate the sino-nasal contamination of lower airway gas.

eNO is a ubiquitous, endogenously produced substance used as a neurotransmitter and local paracrine modulator. The concentration of eNO, which is produced in the lower airways, has been found to be elevated in asthmatic patients,⁸ in untreated bronchiectasis, in seasonal and allergic rhinitis¹⁷ and with viral respiratory illnesses.¹⁰ Furthermore, the nasal airway produces large quantities of eNO, requiring careful attention to detail to exclude sino-nasal gas.^{5,8,18} Thus the current concept of eNO is that it is locally produced in the epithelium of the conducting airways, not the alveoli, by macrophages, neutrophils, fibroblasts and epithelial cells, altering vascular permeability and enhancing extravasation of cells.^{8,18} In contrast to a number of recent publications, we chose to use an "off-line" gas collection technique because of the difficulty in reliably obtaining uncontaminated

lower respiratory tract exhalations in an infant or young child unwilling to accept a mouthpiece.

The two groups that we evaluated were different in age and mass. In part this is a result of anesthesiologist's choice of technique in that the LMA is more difficult to place accurately in small children due to anatomical size constraints in the larynx. In other respects the groups were similar in that children with a history of atopic abnormalities, or those with a family history of atopy, were actively excluded. Furthermore, because these studies were performed on children anesthetized for elective surgical procedures, other acute or intercurrent illnesses were eliminated from the sample.

The sample size (50 patients) in our study was small, but more than adequate for the results obtained. To obtain a significant difference of 50% between the smallest measurements (i.e. between ETT and LMA) a sample size of 5 would be necessary. Thus our data avoids a type 1 error even though the sample is small. Furthermore, by excluding all patients who had a family history of atopy, the evaluation of the "normal" status of the subjects was sustained.

Throughout the study, one investigator used the same technique and all samples were analyzed using the same analyzer. The technique of collection for the mask was standard.^{5,16} The measurements were not significantly different pre-surgery to post-surgery ($P > 0.1$) even when analyzed as a paired t-test. Indeed these results by mask collection were similar to those reported as mean or "off-line" eNO levels which averaged around 7 to 10 ppb.^{8,9}

The lack of relationship of eNO with age, gender, or weight has been observed previously⁹ and the eNO level may be explained by the different techniques used to measure eNO levels. These results are expressed as a concentration of eNO per unit exhaled ventilation (VE). Since VE changes with body mass, then the absolute mass of eNO will increase, but the VNO appears to increase in concert with the VE such that the concentration of eNO does not change. This assumption can only be evaluated by separately measuring VE and VNO in a separate cohort of children.

In summary, this data derived when the source of exhaled gas is limited to the lower airway portion, would suggest that the constitutive eNO production by the airways is very small (< 1.0 ppb) in normal children. Given these results, then the higher eNO levels detected in the gas specimens collected by the facemask technique (~ 10 ppb) must represent

eNO produced elsewhere—presumably from the upper airway. These observations are consistent with published data that demonstrate that the nasopharyngeal and nasal cavities produce large quantities of eNO. However, these data also would suggest that the current techniques for isolating eNO produced in the nasal/paranasal cavities appear to be inadequate, at least in young children.

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