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ORIGINAL ARTICLE

Endogenous Nitric Oxide in the Nasal Airways of Healthy Term Newborn Infants in Taiwan

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Background: Nitric oxide (NO) in the respiratory tract is an important regulator of pulmonary homeostasis during the perinatal transition. In humans, much of the nitric oxide is derived from the upper airways, and autoinhalation of nasal NO has been suggested to influence pulmonary function. No standard methods for measuring nasal NO in neonates currently exist, and previous studies have reported varying levels of nasal nitric oxide in infants, due to the different measuring methods used. The use of nasal olives with a central lumen placed securely in the nares was recommended as a standardized procedure for the measurement of nasal NO in adults and children in 1999. We attempted to establish a safe, convenient and practical method for measuring nasal NO in healthy newborn infants, and investigated possible correlations between nasal NO and gender, postnatal age, gestational age, birth weight, and the differences between the right and left nostrils.
Methods: Nasal NO was studied in healthy newborn infants within the first 3 postnatal days. Gas was sampled from the nostril, and NO concentrations were determined using a fast response chemiluminescence analyzer. Each newborn infant underwent NO measurements on the first, second and the third postnatal days. Ninety-one newborn infants completed the study.
Results: Peak nasal NO in 91 newborn infants was 42.52±16.82 (mean±SD) parts per billion (ppb) (right nostril) and 40.86±16.08 ppb (left nostril) on the first postnatal day, 48.75±17.64 ppb (right nostril) and 49.47±17.26 ppb (left nostril) on the second postnatal day, and 59.65±19.72 ppb (right nostril) and 59.29±20.09 ppb (left nostril) on the third postnatal day. Nasal NO increased significantly with postnatal age ($p<0.001$). There were no significant differences in nasal nitric oxide between sexes, or in relation to gestational age or birth weight, or between left or right nostrils.
Conclusion: We conclude that nasal NO increased significantly in the first 3 days of life.

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1. Introduction

Nitric oxide (NO) is produced endogenously within the respiratory tract and was first detected in exhaled air in humans and mammals in 1991.[1] Measurement of exhaled NO has been proposed as a new test for pulmonary function that could be used to monitor airway inflammation in asthma and other inflammatory respiratory disorders.[2] Most of the NO in adults and children originates from the upper respiratory tract, especially the paranasal sinuses.[3,4] The physiologic role of nasal NO is still unclear, but it has been suggested that it acts as a regulator of pulmonary homeostasis in humans and plays an important role during perinatal adaptation in neonates.[5,6] No standard methods for measuring nasal NO in neonates are currently available, and procedures using velum closure to either prevent the loss of nasal NO via the posterior velopharyngeal aperture, or entry of lower respiratory air into the nasal cavity, are difficult to perform in neonates.[7] The most accurate method of measuring NO concentrations in the upper and lower respiratory tracts in infants is by sampling in intubated infants, though this is often inconvenient or impractical.[8,9] Nasal olives, with a central lumen placed securely in the nares, were recommended as the standardized procedure for the measurement of nasal NO in adults and children in 1999.[10]

Measurements of nasal NO concentrations are helpful for screening children with clinical symptoms suggestive of primary ciliary dyskinesia (PCD), and to aid decisions on the need for further, more invasive testing.[11] The early measurement of nasal NO concentrations may be helpful for screening neonates with PCD.

In this study, we attempted to establish a safe, convenient and practical method of measuring nasal NO in healthy newborn infants. We also investigated possible correlations between nasal NO and gender, postnatal age, gestational age, birth weight, and differences between the right and left nostrils.

2. Materials and Methods

This prospective study was conducted in infants cared for in our hospital nursery between July 1, 2006 and May 31, 2007. One hundred and nine healthy term infants with normal birth weights were enrolled into the study after informed written parental consent was obtained. The study was approved by the Tri-Service General Hospital Institutional Review Board. The mothers were all healthy, with histories of normal pregnancies. To detect any changes in measurable levels of endogenously produced NO in the upper airways during the first, second and third postnatal days, measurements were performed 24 hours, 48 hours and 72 hours after birth, in the nursery.

In non-cooperative children, nasal NO can be measured during normal tidal breathing if sedation is not to be used.[12,13] The infants were studied while lying in their cots, with a room air temperature of 24–25°C. During the study period, the NO concentrations in the ambient air were always below 5 parts per billion (ppb). A bead was inserted approximately 5 mm inside one nostril (right first), ensuring a tight seal, while the contralateral nostril was left open. The bead was made of a smooth, non-traumatizing material, and was of a sufficient diameter and appropriate size to occlude the nares (Figure 1).

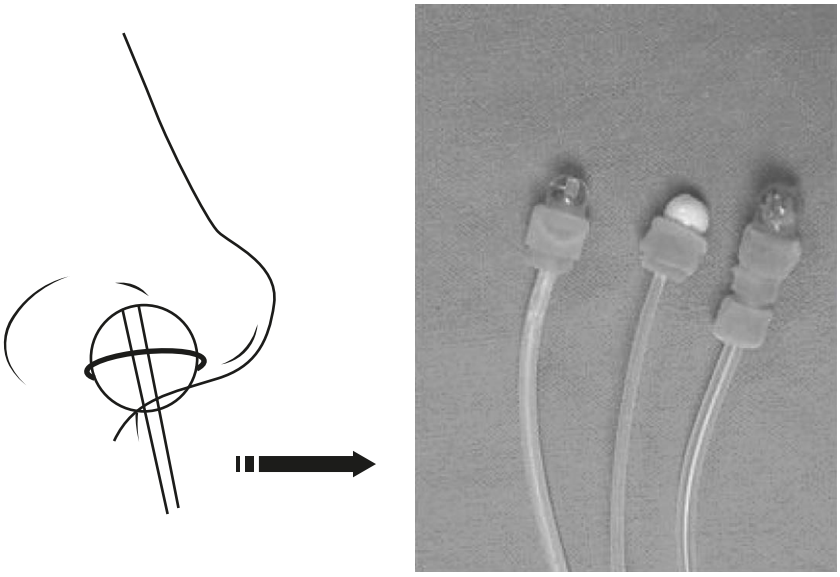

Figure 1 Appropriate sizes of beads introduced to occlude nares for nasal aspiration.

Measurements were performed during regular tidal breathing, with the neonates lying quietly in the supine position with mouths closed. The results were considered acceptable only if a stable plateau of at least 10 seconds was achieved. Measurements were performed at least three times in both the right and left nostrils. This technique for sampling nasal NO is similar to that reported previously by Schedin et al.[14] The bead was connected by a Teflon tube to a suction pump with a side port connecting to the NO analyzer. The levels of nasal NO were measured using a fast response (0.02 seconds) chemiluminescence analyzer (NOA 280; Sievers Instruments, Inc., Boulder, CO, USA). The sampling flow rate was 200 mL/minute. NO levels were simultaneously displayed on the front panel of the analyzer and on a computer attached to the RS-232 output. Data were stored and analyzed using computer software (NO Analysis 3.21 BREATH; Sievers Instruments, Inc.). A 2-point calibration was performed daily, first to zero with air passing through an NO scrubber tube containing $KMnO_4$ (potassium permanganate) and activated charcoal, and then with certified standardized NO gas (9.45 ppm) for the span (Linde Gas LLC; Maumee Specialty Gas Plant, Maumee, OH, USA). The amount of NO in the ambient air (baseline) was recorded before each measurement. We present the data for peak nasal NO concentrations, i.e., for each peak level detected in the plateau periods (Figure 2). The peak nasal NO level was the maximal value for the exhalation, as calculated by the software. Within-subject variation in NO levels was quantified in 31 subjects. A series of nasal NO examinations was performed in the same nostril, which revealed no significant differences (Table 1). Our methods were therefore reproducible.

Data were analyzed using Sigma Stat (Version 3.1, SPSS, Chicago, IL, USA). Statistical evaluation was performed using the paired *t* test and linear

Table 1 Within-group variation based on three measurements in each nostril in 31 subjects

Nostril	Nasal NO	*p*
Day 1 R	46.55±18.72	0.158
	46.65±18.64	
	46.68±18.66	
Day 1 L	45.17±18.55	0.804
	45.23±18.46	
	45.21±18.49	
Day 2 R	61.30±26.26	0.179
	61.23±26.30	
	61.42±26.19	
Day 2 L	60.86±27.77	0.369
	60.72±27.63	
	60.84±27.57	
Day 3 R	68.68±24.62	0.507
	68.81±24.73	
	68.81±24.51	
Day 3 L	66.62±27.64	0.384
	66.69±27.71	
	66.76±27.67	

*Mean±SD.

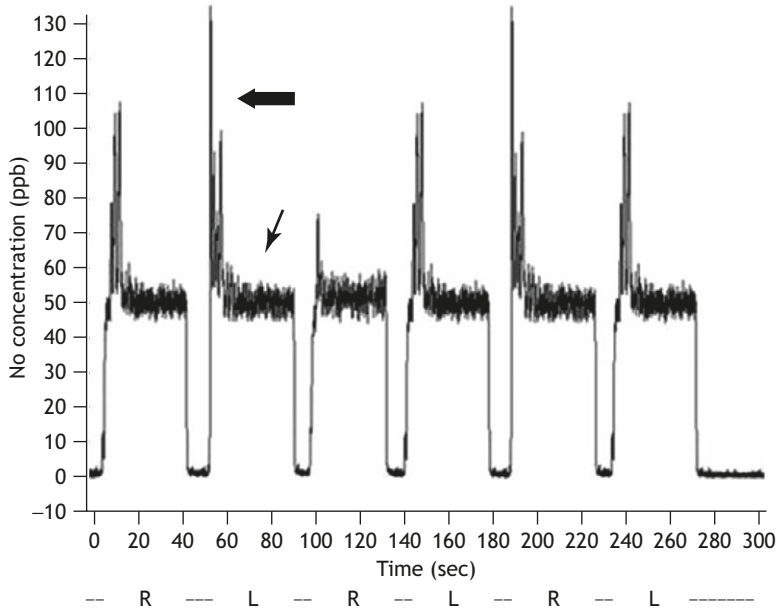

Figure 2 A typical recording of nasal NO derived from an infant. Nasal sampling periods from right and left nostril (right first) and interspersed non-aspirated periods (–) are indicated by horizontal bars. Ambient air was aspirated during non-aspirated periods. A postocclusive nasal NO maximal level (large arrow) followed by a plateau period (small arrow) was shown.

regression analysis. Data are reported as mean±SD. Statistical significance was defined as $p<0.05$.

3. Results

A total of 91 infants (46 male), with a median gestational age of 39.4 weeks (39.4±1.2) and a median birth body weight of 3140.5 g (3140.5±376), were entered into the study (Table 2). NO concentrations in the upper airways of the healthy newborn infants increased with postnatal age during the first 3 days of life. The ambient air NO concentrations were 2.27±0.3 ppb during the experiments. Peak nasal NO in the 91 newborn infants was 42.52±16.82 (mean± SD) ppb (right nostril) and 40.86±16.08 ppb (left nostril) on the first postnatal day, 48.75±17.64 ppb (right nostril) and 49.47±17.26 ppb (left nostril) on the second postnatal day, and 59.65±19.72 ppb (right nostril) and 59.29±20.09 ppb (left nostril) on the third postnatal day (Figure 3). Peak nasal NO increased significantly with postnatal age during the first 3 days, with the lowest levels on the first postnatal day and the highest levels on the third postnatal day ($p<0.01$). There was no significant difference in nasal NO between sexes, or in relation to gestational age or birth weight, or between and left and right nostrils.

Table 2 Characteristics of healthy subjects ($n=91$)

Male/female	46/45
Gestational age (wk)	39.4±1.2*
Birth body weight (g)	3140.5±376

*Mean±SD.

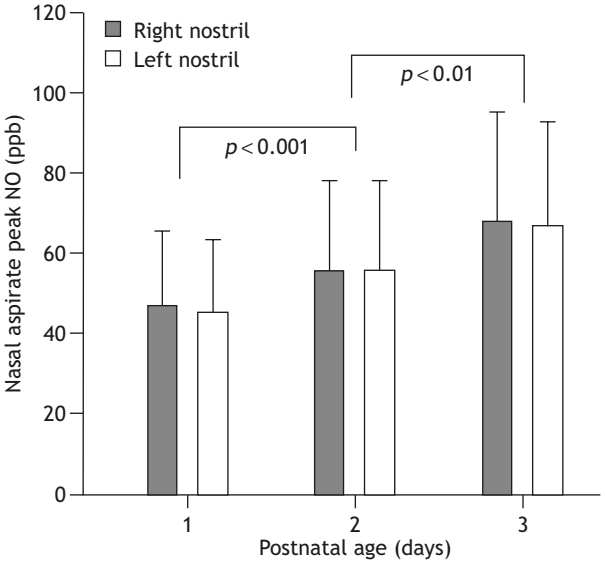

Figure 3 Average peak nasal NO concentrations (mean± SD) in ppb. The peak nasal NO concentrations increased with postnatal ages ($p<0.01$). There was no significant difference in NO concentrations between nostrils.

4. Discussion

In this study, we developed a modified method of measuring NO concentrations in the upper airways of neonates, using a bead instead of an olive.[12–14] The vestibula of newborn infants are shorter and narrower than in adults, and a bead fitted the vestibulum of neonates better than an olive. Newborn infants, like adults, excrete considerable amounts of NO through their nasal airways. NO concentrations in the ppb range can be measured as early as the first postnatal day.

We measured the NO concentrations from nasal airways without the velum closure procedures recommended by the American Thoracic Society.[10] There is currently no validated non-invasive technique for measuring nasal NO concentrations in infants or young children who are unable to perform velum closure maneuvers. Our non-invasive method provides a safe, convenient and practical means of measuring NO concentrations in the nasal airway of newborns. The nasal-aspirated NO measured without velum closure procedures, included NO from both the upper and lower airways. However, most of the NO in exhaled air originates from the nasal airways, with only a minor contribution from the lower airways (<5 ppb) and the oral cavity.[8] The measured nasal NO concentrations were distinctly higher than those in the expired air from mechanically ventilated infants.[8] High concentrations of NO are found in the noses of neonates shortly after birth, even before the sinuses have developed.[15] We thus considered that the NO measured reflected nasal concentrations, and that these were already present in the ppb range on the first postnatal day, and possibly even earlier. We therefore introduced a non-invasive method for the detection of nasal NO concentrations. This has been considered to play several hypothetical roles, including enhancing non-specific host defense mechanisms, reducing pulmonary vascular resistance,[16,17] modulating the pulmonary vascular tone as well as improving ventilation-perfusion matching, regulating ciliary function,[18] and being a surrogate marker of nasal inflammations such as allergic rhinitis.[19]

The plateau nasal NO levels have been thought to reflect a balance between NO production, absorption and removal of the sampling pump, and were not affected by breathing conditions or occlusion time.[20] Colnaghi et al measured the nasal NO plateau level using a higher sampling flow rate (1 L/min). Their results showed lower nasal NO levels than those found

in our study (10.91 ± 0.65 ppb *vs.* 53.02 ± 21.98 ppb). The authors also found that the nasal NO levels were significantly higher in term than in preterm infants.[5] Schedin et al used a lower sampling flow rate (20 mL/minute) to evaluate the nasal NO concentration in healthy term neonates, and they analyzed the changes of postocclusive nasal NO peak levels. The authors concluded that postocclusive peak nasal NO levels increased with postnatal age and occlusion time.[14] In our study, we found that NO concentrations in the nasal aspirates increased with postnatal age. This provides additional information about the normal levels of NO present in the nasal airways of healthy newborn infants in Taiwan.

Screening may be performed for PCD. Measurement of nasal NO concentrations in patients with PCD are extremely low compared with those in healthy children or children with other respiratory disorders,[21,22] though the reason for this low nasal NO concentrations has not yet been clarified. Several observations suggest that NO plays an important role in signal transduction associated with ciliary motility.[23] Measurements of nasal NO concentrations are helpful for screening infants with clinical symptoms suggestive of PCD, and can aid decisions on the need for further, more invasive testing.

In conclusion, we found that NO concentrations in the nasal airways of healthy newborn infants increased with postnatal age during the first 3 days of life. There were no significant differences in nasal NO between sexes, or in relation to gestational age or birth weight, or between left and right nostrils.

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