

# Airway nitric oxide in infants with acute wheezy bronchitis

Ratjen F, Kavuk I, Gärtig S, Wiesemann H-G, Grasemann H. Airway nitric oxide in infants with acute wheezy bronchitis. *Pediatr Allergy Immunol* 2000; 11: 230–235. © Munksgaard, 2000

Concentrations of nitric oxide (NO) in exhaled air are increased in children and adults with asthma, and NO measurements are used as a non-invasive marker to monitor airway inflammation in these patients. To define the role of NO in infants with acute wheezy bronchitis, we measured nasal and end-tidal NO concentrations in 17 infants with acute virus-associated wheezy bronchitis, in 22 term infants without respiratory disease, and in nine premature infants. Nasal NO measurements were performed with an olive placed in the infant's nose; end-tidal NO concentrations were assessed during tidal breathing through a snugly fitting face mask. Both end-tidal NO concentrations and nasal NO concentrations were reduced in infants with acute wheezy bronchitis. There were no differences in NO concentrations between term infants and premature infants. Measurements by both techniques were highly reproducible, as assessed by repeated measurements three times daily on three consecutive days in eight premature infants. Reduced airway NO concentrations in infants with virus-associated acute wheezy bronchitis are in contrast to findings in adults where both upper and lower airway NO levels are increased in patients with asthma. Whether this reflects a different inflammatory reaction to upper airway infections in acutely wheezy infants or pathophysiologic differences in airway response remains to be determined.

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Key words: nitric oxide; infants; asthma;  
wheezing

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Accepted 3 May 2000

Nitric oxide (NO), which is formed by enzymes in a variety of tissues, is involved in many physiologic processes within the respiratory tract, including regulation of bronchomotor tone, inflammation, and host defence (1). Airway NO concentrations are increased in diseases associated with airway inflammation, such as asthma and upper respiratory tract infections (2,3). Recent studies suggest that the elevated levels of NO in exhaled air may reflect the degree of airway inflammation in both paediatric and adult patients with asthma and, in some centres, NO is currently used to monitor response to treatment (4–7).

Wheezing associated with viral upper airway infection is very common in infants. Epidemiological studies suggest that  $\approx 30\%$  of all children under 3 years of age will have at least one episode of wheezing (8). Only a fraction of these children will have continuous symptoms compatible with the diagnosis of

childhood asthma. Currently there is no simple and non-invasive technique available to monitor the evolution of airway inflammation in wheezy infants. Techniques to measure airway NO in infants differ from those used in older children and adults as infants breathe mainly through the nose. Airway NO is therefore invariably contaminated by upper airway NO, the concentration of which is known to be much higher than that of the lower airway (9,10). In the present study we used two different techniques to measure airway NO in infants, and report our findings on airway NO concentrations in normal infants and in infants with acute virus-associated wheezy bronchitis.

## Materials and methods

The study population consisted of three groups of infants:

**1** Term infants (n=22) aged 1–10 months (mean age $\pm$ SD: 3.6 $\pm$ 2.6 months) without respiratory diseases, hospitalized for non-pulmonary causes without signs of acute respiratory or systemic infections (Table 1).

**2** Infants (n=17) aged 1–10 months (mean age: 5.4 $\pm$ 2.6 months) with acute wheezy bronchitis associated with an acute virus airway infection and no reported previous episodes of wheezing.

**3** Premature infants (n=9) aged 1–3 months (mean age 1.4 $\pm$ 0.7 months) without respiratory disease. The gestational age of these infants ranged from 27 to 35 weeks (mean 31 $\pm$ 2.7 weeks).

To test the variability of NO measurements over time, eight premature infants (mean age: 1.5 $\pm$ 0.4 months) were studied three times daily (morning, noon, and afternoon) on three consecutive days.

All infants were in-patients at the Children's Hospital, University of Essen. None of the infants received inhaled or systemic steroids either during the study or in the 4 weeks prior to entry to the study. Infants with acute virus-associated wheezy bronchitis were studied when in a stable clinical condition; all had received only  $\beta_2$ -agonist as a therapeutic agent on the days prior to the study. The study was approved by the ethical committee of our institution; informed parental consent was obtained in all cases.

Measurements of airway NO were performed in two ways. Nasal NO concentrations were monitored with a nasal olive (external diameter 12 mm) placed into one nostril of the infant. This technique of sampling nasal NO is similar to that reported previously by Schedin et al. (11). The olive was connected via plastic tubing to a chemiluminescence NO analyzer (280 A; Sievers Instruments, Boulder, Colorado, USA). The sampling flow of the NO analyzer was 200 ml/min. After adjustment of the olive, NO concentrations increased and plateaued within seconds. This plateau was used to judge nasal NO con-

centrations in a given infant. Measurements were performed during regular tidal breathing with the child lying quietly in the supine position and the results were considered acceptable only if a stable plateau of at least 10 s was achieved. NO measurements in each nostril had to be within 5% of each other to be accepted for analysis. Measurements were performed at least three times in both the left and the right nostril. The mean of three measurements in each nostril was used to represent NO concentrations. A snugly fitting face mask was then placed on the infant's face covering both the nose and the mouth. Plastic tubing with an internal diameter of 2 mm was connected via a small hole in the mask to the NO analyzer. A Fleisch 00 pneumotachograph was attached to the face mask to measure respiratory rate and expiratory flow. The dead space of the system was 13 ml. To avoid CO<sub>2</sub> retention in the mask, each measurement was limited to 60 s. The mask was removed and reapplied after each measurement. NO concentrations were registered during phases of quiet tidal breathing over a period of 1 min and displayed on a four-channel chart recorder (Hugo Sachs, March, Germany). The frequency response of the system was flat up to 5 Hz; the response time for sudden increments in NO concentrations was 1.04 s. The chart recorder was calibrated before each measurement with a NO-free gas mixture as well as with a calibration gas containing 200 parts per billion (p.p.b.) of NO. A line was drawn by hand through the peaks of end-tidal NO concentrations displayed on a strip chart recording. The mean of three measurements was used to reflect tidal expiratory NO concentrations in a given infant. Similarly to the criteria used for nasal NO, measurements had to be within 5% to be accepted for analysis.

#### Statistical analysis

All data were tested for normal distribution using the Kolmogorov-Smirnov test. Because of the parametric distribution of all parameters, data were expressed as mean $\pm$ standard deviation (SD). Differences between the two populations of patients were assessed using the *t*-test. Linear regression analysis was used to analyze correlation between room air NO concentrations and those in end-tidal air and in the nose, respectively. The agreement between measurements obtained from the right and left nostril was assessed using the mean difference technique proposed by Bland & Altman (12). Intra-individual variations in NO concentrations were assessed by coefficient of variation analysis. A *p*-value of less than 0.05 was considered significant.

Table 1. Underlying diagnosis of children without respiratory disease

| Diagnosis             | n |
|-----------------------|---|
| Thermal instability   | 3 |
| Seizures              | 3 |
| Commotio cerebri      | 4 |
| Opiate withdrawal     | 3 |
| Jitteriness           | 1 |
| Vesicoureteral reflux | 2 |
| Hypospadias           | 1 |
| Cholesteatoma         | 1 |
| Diarrhoea             | 4 |

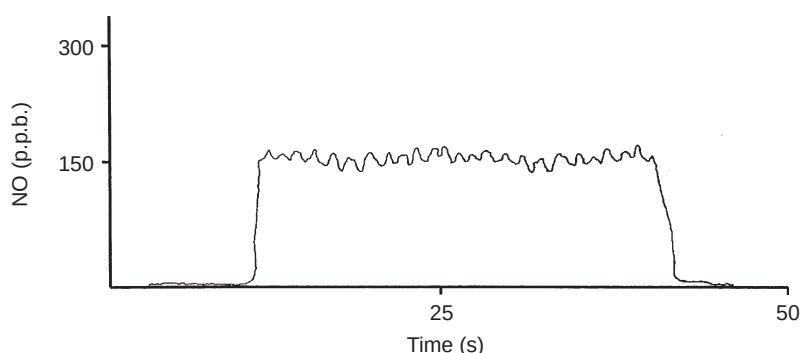


Fig. 1. Representative tracing of nasal nitric oxide (NO) concentrations, in parts per billion (p.p.b.), in an infant without lung disease. The NO concentration rises from when the olive is placed in the infant's nose until a plateau is reached, which is maintained until the olive is removed. The oscillations in the plateau represent changes in nasal NO during tidal breathing.

## Results

Representative tracings of nasal NO concentrations in an infant without respiratory disease, as well as in an infant with acute virus-associated wheezy bronchitis, are shown in Figs 1 and 2. Nasal NO measurements were feasible and well tolerated in all infants. Measurements of NO via the face mask were performed successfully in all premature infants, in 12 out of 22 infants without respiratory disease, and in 14 of 17 infants with wheezy bronchitis. The respiratory rates were not significantly different between control infants and infants with wheezy bronchitis, as shown by the following mean  $\pm$  SD values:  $45 \pm 8$  vs.  $49 \pm 10$ . In addition, no differences were observed in respiratory rate between end-tidal and nasal NO measurements; there was no correlation between respiratory rate and NO concentration in any of the groups.

Room air NO concentrations were not significantly different among the three groups of infants. No correlation was found between room air and nasal NO concentrations for all groups. At ambient NO concentrations above 20 p.p.b., end-tidal NO concentrations showed a positive correlation with room air NO concentrations. The analysis of end-tidal NO was therefore limited to infants with NO concentration measurements below 20 p.p.b. Mean  $\pm$  SEM

ambient NO levels during measurements of end-tidal NO were  $8.3 \pm 2.4$  p.p.b. in preterm infants,  $5.9 \pm 1.7$  p.p.b. in control infants, and  $3.6 \pm 1.3$  p.p.b. in wheezy infants. No correlation was found between age and NO concentrations in any of the groups of infants. Both nasal and end-tidal NO concentrations were comparable in infants born prematurely and in term infants (Figs 3 and 4). There were no major differences in nasal NO concentrations between the left and the right nostril, although variability was higher for lower levels of nasal NO concentrations (Fig. 5). End-tidal and nasal NO concentrations showed no significant differences at different times of the day or between days. The mean coefficient of variation  $\pm$  SEM for all measurements was  $14.8 \pm 3.6\%$  for end-tidal and  $11.5 \pm 2.3\%$  for nasal NO concentrations.

Infants with viral-associated wheezy bronchitis had significantly lower nasal NO concentrations compared to infants without respiratory disease ( $27.7 \pm 8.0$  p.p.b. vs.  $169.4 \pm 56.1$  p.p.b.,  $p < 0.001$ ,  $t$ -test) (Fig. 3). A similar difference was also observed for end-tidal NO concentrations ( $9.6 \pm 4.1$  p.p.b. vs.  $21.2 \pm 8.2$  p.p.b.,  $p = 0.0012$ ) (Fig. 4). There was a significant correlation between nasal and end-tidal NO concentrations for all groups ( $p < 0.0003$ , linear regression analysis).

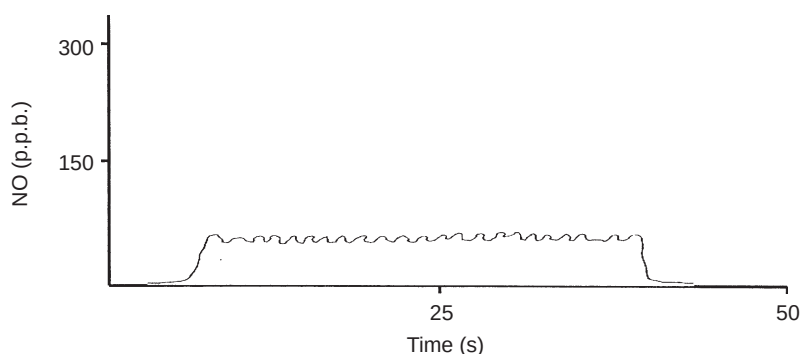


Fig. 2. Representative tracing of nasal nitric oxide (NO) concentrations, in parts per billion (p.p.b.), in an infant with virus-associated wheezing. Nasal NO concentrations were systematically lower in this group of infants. For details see legend of Fig. 1.

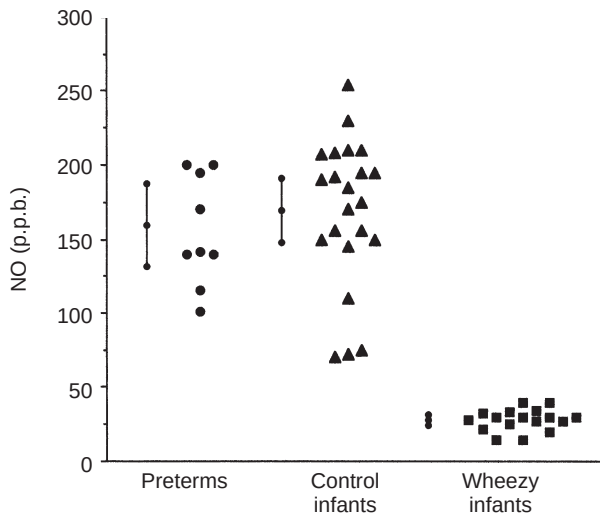


Fig. 3. Nasal nitric oxide (NO) concentrations, in parts per billion (p.p.b.), in preterm infants (circles), infants without lung disease (triangles), and infants with wheezy bronchitis (squares). Each symbol represents one individual. The mean and 95% confidence interval for each group is also shown. Nasal NO concentrations were reduced in infants with wheezy bronchitis ( $p < 0.001$ ,  $t$ -test).

### Discussion

Our results demonstrate that infants with acute virus-associated wheezy bronchitis have lower NO concentrations both in the nose and in end-tidal air compared to infants without respiratory disease. This is an unexpected finding as both upper respiratory tract infections and asthma are associated with an increase in airway NO concentrations in adults (12–19).

Concentrations of airway NO vary according to the methodology used. Recommendations for standard techniques have been developed for adults but these are not suitable for infants (20). In our study, both nasal and tidal expiratory NO measurements were well tolerated and highly reproducible within infants, and showed little diurnal variation. Levels of nasal NO in our population of infants without lung disease were

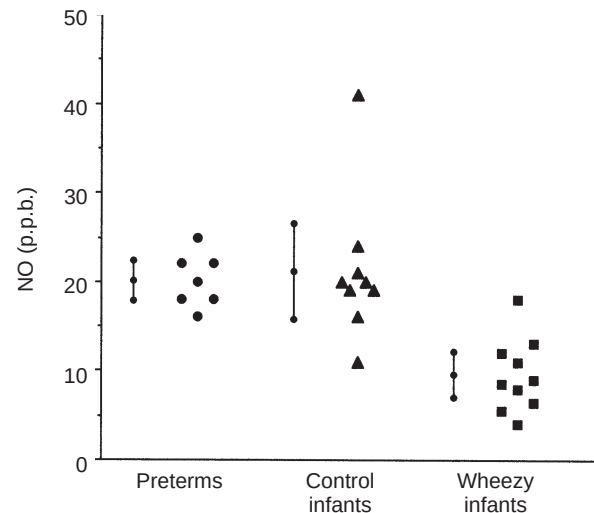
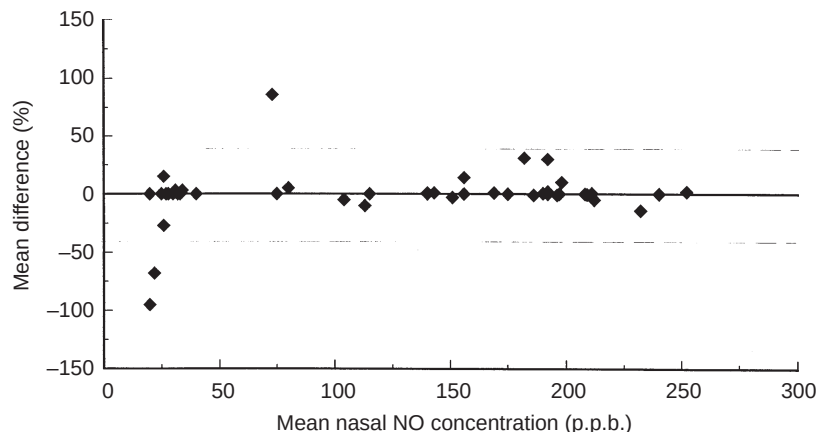


Fig. 4. End-tidal nitric oxide (NO) concentrations, in parts per billion (p.p.b.), in preterm infants (circles), infants without lung disease (triangles), and infants with wheezy bronchitis (squares). Each symbol represents one individual. The mean and 95% confidence interval for each group is also shown. End-tidal NO concentrations were reduced in infants with wheezy bronchitis ( $p < 0.0012$ ,  $t$ -test).

very similar to those previously reported by Schedin et al. (11,21). Contrary to the approach used in their study, we did not include measurements of peak nasal NO concentrations during airway occlusions as, during airway occlusion, NO concentration increases in a time-dependent manner and does not reach an equilibrium. Nasal NO concentrations during tidal breathing are systematically lower than those reported for peak nasal NO levels, both in infants and in adults (20–22), owing to continuous dilution of upper airway NO by both lower airway and ambient air as the infant breathes through the contralateral nostril (23).

In contrast to nasal NO levels, end-tidal NO concentrations are influenced by the NO concentration in ambient air. If sampling is carried out by using a face mask, inhalation of NO free

Fig. 5. Comparison of nasal nitric oxide (NO) concentrations determined in the right and the left nostril. The average NO concentrations are displayed on the abscissa; the differences between the two nostrils (expressed as percentage of the mean concentration) are shown on the ordinate. Each triangle represents one individual. The dotted lines represent 2 standard deviations of the mean. There were no systematic differences in NO concentrations between the two nostrils.



air would probably reduce contamination of airway gas by ambient NO. However, this technique will not allow sampling of isolated lower airway NO, as infants usually exhale through the nose. The upper airways contain much higher NO concentrations than the lower respiratory tract, and airway NO measured in a face mask will therefore invariably be contaminated with upper airway NO. To avoid this contamination, Wildhaber et al. used a modification of the rapid thoracoabdominal compression technique to measure lower airway NO (24). This technique facilitates sampling of lower airway NO, although infants still exhale through the nose during forced expiratory manoeuvres. Contamination with upper airway NO may explain the higher concentrations of NO in normal infants in this study compared to reference data in older children or adults (20,25). Recently, Baraldi et al. reported a technique for measuring exhaled NO in infants using a combination of NO-free air, positive expiratory pressure with an external resistor, and a mask that covers only the mouth of the infant (26). This approach may limit contamination of lower airway NO with upper airway NO. Both studies have found increased levels of NO in infants with recurrent wheezing, which is in contrast to our results. Comparison with these two studies is, however, limited as both assessed infants with a history of wheezing rather than infants in the acute phase of the disease, which is different from the population of infants with a first episode of virus-associated wheezing included in our study.

Several factors may have influenced NO concentrations in our study. First, anti-asthmatic therapy may have affected NO levels in the group of wheezy infants. Previous studies in older children and adults have shown that corticosteroids down-regulate NO production in asthma and this was also observed in infants with recurrent wheezing (4–7,26). We therefore did not include infants who had received this form of therapy. As albuterol (salbutamol) was the only form of treatment administered to these infants, and previous studies have shown increased levels of nitric oxide in exhaled air of asthmatics despite treatment with  $\beta_2$ -agonist, it appears unlikely that the low NO concentrations in this population were caused by this therapeutic intervention.

Although both upper and lower airway concentrations of NO have been found to be increased in adults with asthma, nasal NO was not elevated in a study of children (16–19). As it is not possible to sample only lower airway NO concentration in infants non-invasively, a lack of

increase in nasal and end-tidal NO concentrations may merely reflect a lack of NO-mediated inflammation in the upper airways. However, this does not explain why NO concentrations were significantly reduced in wheezy infants with acute upper airway infection. Therefore, airway NO concentrations may also reflect pathophysiologic differences between this group of virus-associated wheezy infants and older children or adults with asthma.

At present, the role of NO during an asthmatic response is unclear. NO may play a role in perpetuating the inflammatory response through inhibition of T helper 1 (Th1) cells (27). It has recently been shown that NO modulates antigen-induced allergic responses by increasing eosinophilic inflammation, a process that can be augmented by administration of L-arginine, the substrate of NO synthases (28–30). It remains to be determined whether down-regulation of NO synthesis reflects a different type of inflammatory reaction to viral antigens or an age-related difference in the airway response. Interestingly, Azevedo et al. found no elevation of the bronchoalveolar (BAL) eosinophil count in wheezy infants (31). Likewise, Stevenson et al. demonstrated an elevation in BAL eosinophils and mast cells only in children with atopic asthma and not in those with virus-associated wheezing (32), which would also support the concept that these two clinical entities have different underlying pathophysiologic mechanisms.

In summary, we detected reduced levels of airway NO in infants with virus-associated wheezy bronchitis. As NO is predominantly produced in the upper airways, this may reflect down-regulation of NO production in the nasopharynx during acute viral infection. Measurement of nasal and end-tidal NO concentrations therefore appear to be unsuitable for monitoring activity of airway inflammation in these patients, but may provide new insights into the pathophysiology of airway inflammation in infants with virus-associated wheezing.

## References

1. GASTON B, DRAZEN JM, LOSCALZO J, STAMLER JS. The biology of nitric oxide in the airways. *Am J Respir Crit Care Med* 1994; 149: 538–51.
2. GUSTAFSSON LE, LEONE AM, PERSSON MG, WIKLUND MP, MONCADA S. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. *Biochem Biophys Res Commun* 1991; 181: 852–7.
3. BARNES P, KHARITONOV SA. Exhaled nitric oxide: a new lung function test. *Thorax* 1996; 51: 233–7.
4. KHARITONOV SA, YATES DH, BARNES P. Inhaled glucocorticoids decrease nitric oxide in exhaled air of

- asthmatic patients. *Am J Respir Crit Care Med* 1996; 153: 454-7.
5. MASSARO AF, MEHTA S, LILLY CM, KOBZIK L, REILLY JJ, DRAZEN JM. Elevated nitric oxide concentrations in isolated lower airway gas of asthmatic subjects. *Am J Respir Crit Care Med* 1996; 153: 1510-4.
6. GASTON B. Expired nitric oxide in pediatric asthma: emissions testing for children? *J Pediatr* 1997; 131: 343-4.
7. BARALDI E, AZZOLIN NM, ZANCONATO S, DARIO C, ZACCHELLO F. Corticosteroids decrease exhaled nitric oxide in children with acute asthma. *J Pediatr* 1997; 131: 381-5.
8. MARTINEZ FD, WRIGHT AL, TAUSSIG LM, et al. Asthma and wheezing in the first 6 years of life. *N Engl J Med* 1995; 332: 133-8.
9. GERLACH H, ROSSAINT R, PAPPERT D, KNORR M, FALKE KJ. Autoinhalation of nitric oxide after endogenous synthesis in the nasopharynx. *Lancet* 1994; 343: 518-9.
10. LUNDBERG JON, WEITZBERG E, NORDVALL SL, KUYLENSTERN R, LUNDBERG JM, ALVING K. Primarily nasal origin of exhaled nitric oxide and absence in Kartegener's syndrome. *Eur Respir J* 1994; 7: 1501-4.
11. SCHEDIN U, NORMAN M, GUSTAFSSON LE, HERIN P, FROSTELL C. Endogenous nitric oxide in the upper airways of healthy newborn infants. *Pediatr Res* 1996; 40: 148-51.
12. BLAND JM, ALTMAN DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; i: 307-9.
13. KHARITONOV SA, YATES DH, BARNES P. Increased nitric oxide in exhaled air of normal subjects with upper respiratory tract infections. *Eur Respir J* 1995; 8: 295-7.
14. KHARITONOV SA, WELLS AU, O'CONNOR BJ, HANSELL DM, COLE PJ, BARNES P. Elevated levels of exhaled nitric oxide in bronchiectasis. *Am J Respir Crit Care Med* 1995; 151: 1889-93.
15. ALVING K, WEITZBERG E, LUNDBERG JM. Increased amount of nitric oxide in exhaled air of asthmatics. *Eur Respir J* 1993; 6: 1268-70.
16. KHARITONOV SA, YATES DH, ROBBINS RA, LOGAN-SINCLAIR R, SHINEBOURNE EA, BARNES P. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 1994; 343: 133-5.
17. LUNDBERG JON, NORDVALL SL, WEITZBERG E, KOLLBERG H, ALVING K. Exhaled nitric oxide in paediatric asthma and cystic fibrosis. *Arch Dis Child* 1996; 75: 323-6.
18. ARTLICH A, HAGENAH JU, JONAS S, AHRENS P, GORTNER L. Exhaled nitric oxide in childhood asthma. *Eur J Pediatr* 1996; 155: 698-701.
19. KHARITONOV SA, CHUNG KF, EVANS D, O'CONNOR BJ, BARNES P. Increased exhaled nitric oxide in asthma is mainly derived from the lower respiratory tract. *Am J Respir Crit Care Med* 1997; 153: 1773-80.
20. KHARITONOV S, ALVING K, BARNES PJ. Exhaled and nasal nitric oxide measurements. The European Respiratory Society Task Force. *Eur Respir J* 1997; 10: 1683-93.
21. SCHEDIN U, NORMAN M, GUSTAFSSON JONSSON B, FROSTELL C. Endogenous nitric oxide in the upper airways of premature and term infants. *Acta Paediatr* 1997; 86: 1229-35.
22. GRASEMANN H, GÄRTIG SS, WIESEMANN HG, TESCHLER H, KONIETZKO N, RATJEN F. Effect of L-arginine infusion on airway NO in cystic fibrosis and primary ciliary dyskinesia syndrome. *Eur Respir J* 1999; 13: 114-8.
23. DUBOIS AB, DOUGLAS JS, STITT JT, MEHSEIN V. Production and absorption of nitric oxide gas in the nose. *J Appl Physiol* 1998; 84: 1217-34.
24. WILDHABER JH, HALL GL, SICK SM. Measurement of exhaled nitric oxide with the single-breath technique and positive expiratory pressure in infants. *Am J Respir Crit Care Med* 1998; 159: 74-8.
25. BARNES P, LIEW FY. Nitric oxide and asthmatic inflammation. *Immunol Today* 1995; 16: 128-30.
26. BARALDI E, DARIO R, SCOLLO M, et al. Exhaled nitric oxide concentrations during treatment of wheezing exacerbation in infants and children. *Am J Respir Crit Care Med* 1999; 159: 1284-8.
27. FEDER LS, STELTZ D, CHAPMAN RW, et al. Role of nitric oxide on eosinophilic lung inflammation in allergic mice. *Am J Respir Cell Mol Biol* 1997; 17: 436-42.
28. IJIMA H, UCHIDA Y, ENDO T, et al. Role of endogenous nitric oxide in allergen induced airway responses in guinea-pigs. *Br J Pharmacol* 1998; 124: 1019-28.
29. FERREIRA HH, BEVILACQUA E, DE GAGIOTI SM, et al. Nitric oxide modulates eosinophilic infiltration in antigen-induced airway inflammation in rats. *Eur J Pharmacol* 1998; 358: 253-9.
30. TAKANO H, LIM HB, MIYABARA Y, ICHINOSE T, YOSHIKAWA T, SAGAI M. Oral administration of L-arginine potentiates allergen-induced airway inflammation and expression of interleukin-5 in mice. *J Pharmacol Exp Ther* 1998; 286: 767-71.
31. AZEVEDO I, de BLIC J, SCHEINMANN P, VARGRAFTIG BB, BACHELET M. Enhanced arachidonic acid metabolism in alveolar macrophages from wheezy infants. *Am J Respir Crit Care Med* 1995; 152: 1208-14.
32. STEVENSON EC, TURNER G, HEANEY LG, et al. Bronchoalveolar lavage findings suggest 2 different forms of childhood asthma. *Clin Exp Allergy* 1997; 27: 1027-35.