Tidal exhaled nitric oxide in healthy, unsedated newborn infants with prenatal tobacco exposure

GRAHAM L. HALL,[1,2] BENJAMIN REINMANN,[1]
JOHANNES H. WILDHABER,[2] AND URS FREY[1]
[1]*Swiss Paediatric Respiratory Physiology Research Group, University Children's Hospital Bern, CH-3010 Bern; and* [2]*Swiss Paediatric Respiratory Physiology Research Group, University Children's Hospital, 8032 Zürich, Switzerland*

Hall, Graham L., Benjamin Reinmann, Johannes H. Wildhaber, and Urs Frey. Tidal exhaled nitric oxide in healthy, unsedated newborn infants with prenatal tobacco exposure. *J Appl Physiol* 92: 59–66, 2002.—Tidal fractional exhaled nitric oxide ($F_{E_{NO}}$) changes were investigated in healthy, unsedated infants with or without prenatal tobacco exposure. Tidal flow ($\dot{V}$), $F_{E_{NO}}$, and CO_2 were measured in 20 healthy, unsedated infants [age: 25–58 days, length: 56.5 ± 2.5 (SE) cm]. NO output ($\dot{V}_{NO}$) was calculated ($\dot{V}_{NO} = F_{E_{NO}} \times \dot{V}$). Two approaches were used to investigate within-breath changes of $F_{E_{NO}}$ and $\dot{V}_{NO}$. First, we identified phases II and III from the expiratory capnogram. Second, we divided expiration into time-based quartiles. Tidal $F_{E_{NO}}$ (range: 14.5 ± 1.6 to 17.6 ± 2.1 parts/billion: *quartile 4* and phase II, respectively) was not different between portions and exhibited significant negative $\dot{V}$ dependence. $\dot{V}_{NO}$ was significantly dependent on the expiratory portion, with *quartile 4* being significantly lower than the remaining expiratory portions. Infants exposed to prenatal cigarette smoke ($n = 7$) exhibited significantly lower $F_{E_{NO}}$ and $\dot{V}_{NO}$ compared with nonexposed ($n = 13$) infants. We conclude that tidal $F_{E_{NO}}$ is $\dot{V}$ dependent and that $\dot{V}_{NO}$ may be a more suitable outcome parameter in variable $\dot{V}$ conditions. Prenatal tobacco exposure resulted in a decreased $F_{E_{NO}}$ and $\dot{V}_{NO}$ in infants.

tidal breathing; lung function

THERE IS EXTENSIVE INTEREST in the measurement of exhaled nitric oxide (NO; $F_{E_{NO}}$) as a noninvasive marker of airway inflammation. In adults and older children, measurement involves exhaling at a constant flow ($\dot{V}$) against a resistance and obtaining a plateau in the $F_{E_{NO}}$ signal (1, 14). Whereas this test is noninvasive, in uncooperative patients, such as young children and infants, it is unsuitable. In young children, tidal $F_{E_{NO}}$ has been used to discriminate between healthy and asthmatic patients (4). This technique involves the patient breathing through a mouthpiece and the exhaled breath being sampled on-line to produce tidal $F_{E_{NO}}$. An expiratory resistance is added to close the soft palate, hence removing contamination by nasal NO. Guidelines state that techniques for sampling pulmonary $F_{E_{NO}}$ should exclude contamination of the sample with nasal NO (1). Infants are preferential nasal breathers, and lung function measurements in this age necessitate the use of a face mask, increasing the difficulty of nasal NO exclusion. The addition of an expiratory resistance to ensure closure of the soft palate during tidal breathing in quiet sleep may result in failed or poor quality measurements in this age group. Wildhaber et al. (24) have reported an adaptation of the single-breath technique that excludes nasal NO and allows $F_{E_{NO}}$ to be obtained at a constant $\dot{V}$ in infants. The technique, however, requires the sedation of the infant, and specialized equipment is needed to raise the infant's lung volume and, therefore, has limited use, particularly in large epidemiological studies. Baraldi et al. (5) have measured offline tidal $F_{E_{NO}}$ in infants and small children using a collection reservoir connected to a face mask, which is placed over the mouth while the infant's nose is closed. The disadvantage of this technique is that closure of the nostrils may disturb the infant, inducing highly variable breathing patterns, and the subsequent expiratory times (T_E), tidal volumes (V_T), and $\dot{V}$ may influence the resulting $F_{E_{NO}}$ values. This appears critical, as the $\dot{V}$ dependency of NO is well established in both adults (20, 22) and children (9). Furthermore, differences in T_E and $\dot{V}$ due to disease may influence the subsequent tidal $F_{E_{NO}}$ concentrations. These studies suggest that $F_{E_{NO}}$ measurements in infants should fulfill the following criteria: *1*) be simple to apply, *2*) be noninvasive, *3*) have negligible impact on the infant's natural breathing patterns, and *4*) account for the $\dot{V}$ dependence of NO. It appears that, whereas the techniques described above have their advantages and disadvantages, they may not fulfill all of these criteria.

The aim of this study was to initially develop a method for the on-line collection of tidal $F_{E_{NO}}$ in infants that would allow breath-to-breath monitoring of tidal $F_{E_{NO}}$ and $\dot{V}$ in natural sleep without disturbing the infant's normal breathing pattern (*criteria 1–3*) and to determine whether tidal $F_{E_{NO}}$ is $\dot{V}$ dependent

(*criterion 4*). The washout characteristics of CO_2 were then used to identify separate regions of the NO profile; these regions were subsequently utilized to test the ability of the developed analysis methods to detect differences in F_{ENO} and NO output ($\dot{V}_{NO}$) in a group of healthy infants with or without prenatal tobacco exposure (PTE), as chronic cigarette exposure is known to reduce NO in adults (7, 15, 19).

METHODS

Patients. Twenty healthy infants, aged between 25 and 58 days, without a positive maternal history of asthma or atopy, were studied unsedated, during quiet sleep, in a supine position, with the head in the midline position. Seven of the infants had PTE (PTE group) and received variable exposure to passive, postnatal tobacco smoke, whereas the remaining 13 infants had no tobacco exposure (control group). The two groups of infants were matched for postnatal age, weight, and length. Heart rate and arterial O_2 saturation (Biox 3700; Datex-Ohmeda, Helsinki, Finland) were monitored throughout the study. Tidal measurements could be obtained in non-rapid eye movement sleep in all infants. The ethics committee of the University Hospital of Berne approved the study, and the parents were generally present during testing. Anthropometric data are shown in Table 1.

Study design. Tidal F_{ENO} concentration may depend on which part of expiration is examined. To determine which part of the expiratory NO signal shows concentrations most similar to the NO concentrations collected under constant $\dot{V}$ conditions in infants (24), we analyzed the on-line tidal F_{ENO} profiles using two approaches to identify discrete portions of expiration (as described in detail below) and compared the values with published data.

It is well known that infant tidal breathing patterns are influenced by a complex neurorespiratory control system, as well as rapidly changing lung mechanics (18). Therefore, tidal F_{ENO} washout characteristics may vary from breath to breath. We, therefore, determined the breath-to-breath vari-

ability of tidal F_{ENO}. To determine which part of the tidal signal exhibited the least variability and thus could potentially be the most discriminatory, we examined the variability of discrete tidal F_{ENO} portions as described in detail below.

The $\dot{V}$ dependence of tidal F_{ENO} can easily be determined by measuring $\dot{V}$ synchronously, allowing the breath-to-breath $\dot{V}$ dependence to be assessed. The parallel assessment of F_{ENO} and $\dot{V}$ also allows the determination of $\dot{V}_{NO}$ calculated as the tidal F_{ENO} by the tidal $\dot{V}$ ($\dot{V}_{NO} = F_{ENO} \times \dot{V}$). We assessed the breath-by-breath $\dot{V}$ dependence of F_{ENO} in each expiratory portion.

The analysis of the tidal F_{ENO} signal was performed by using two approaches. The first was based on the known washout characteristics of CO_2 from the lung, which includes three distinct phases. Phase I equates to the gas expired from the convective airways, phase II (PII) is due to progressive washout of the airways with alveolar gas, whereas phase III (PIII) represents emptying of CO_2 from alveolar compartments. We identified PII and PIII from the expiratory capnograph and used these to examine discrete portions of the tidal F_{ENO} signal. This method of identification will take into account differences in expiratory washout characteristics (for example, those caused by changes in tidal $\dot{V}$, volume, and T_E) between individuals and disease groups. The second approach used a time-based, rather than volume-based, analysis by dividing each expiration into four equal portions. The latter approach would allow examination of the on-line tidal F_{ENO} signal without the need for additional capnography and thus possibly allow a more simple clinical application of the method.

The detection of alterations in tidal F_{ENO} due to changes in respiratory physiology may differ, depending on the type of disease. In disease, the alterations in tidal breathing patterns, including T_E, $\dot{V}$ profiles, and V_T, may cause changes in the tidal F_{ENO} concentration unrelated to NO production. To ascertain if these factors played a significant concomitant role between the control and PTE groups, we tested for differences in T_E, $\dot{V}$, V_T, and peak expiratory flow (PEF). The ability of a method to distinguish between disease states can

Table 1. *Individual and group anthropometric and tidal breathing data*

Patient No.	Age, days	Weight, kg	Length, cm	V_T, ml	PEF, ml/s	RR, breaths/min	T_E, s	$EE_{T_{CO_2}}$, %
1	25	4.29	53	25.6 ± 1.7	63.6 ± 8.6	50.9 ± 7.8	0.87 ± 0.09	4.25 ± 0.17
2	33	5.34	58	28.7 ± 6.4	52.3 ± 9.5	44.8 ± 8.2	0.64 ± 0.10	4.15 ± 0.64
3	**37**	**5.1**	**56.8**	**22.1 ± 5.2**	**44.7 ± 5.5**	**39.0 ± 6.4**	**0.48 ± 0.06**	**4.97 ± 0.11**
4	38	4.6	56.4	31.4 ± 2.3	44.2 ± 6.3	30.9 ± 4.6	1.24 ± 0.10	5.28 ± 0.50
5	43	5.34	58	34.0 ± 2.5	49.4 ± 6.7	46.3 ± 6.9	0.87 ± 0.07	5.15 ± 0.40
6	37	6.3	61.5	27.5 ± 2.1	62.7 ± 8.4	36.6 ± 5.9	0.79 ± 0.05	4.65 ± 0.42
7	**34**	**4.45**	**54**	**37.2 ± 5.8**	**87.4 ± 13.7**	**53.0 ± 8.7**	**0.68 ± 0.08**	**4.86 ± 0.46**
8	40	3.76	52.4	35.9 ± 3.9	43.5 ± 5.1	38.7 ± 6.1	0.99 ± 0.11	4.90 ± 0.47
9	37	4.76	56.4	26.0 ± 4.0	54.2 ± 11.9	39.2 ± 6.4	0.98 ± 0.11	4.80 ± 0.11
10	**31**	**4.52**	**57**	**28.5 ± 2.6**	**90.4 ± 10.9**	**66.2 ± 9.8**	**0.45 ± 0.05**	**4.18 ± 0.34**
11	28	4.43	57.8	31.5 ± 1.8	42.7 ± 5.0	36.5 ± 6.3	1.08 ± 0.06	5.27 ± 0.11
12	**47**	**4.86**	**56**	**28.7 ± 3.7**	**41.0 ± 10.4**	**35.2 ± 6.7**	**1.04 ± 0.15**	**4.84 ± 0.90**
13	37	4.78	57.3	36.0 ± 2.9	42.5 ± 6.6	25.7 ± 5.3	0.86 ± 0.08	5.73 ± 0.51
14	58	4.13	59	23.9 ± 1.9	50.2 ± 13.3	48.1 ± 13.5	0.69 ± 0.06	4.70 ± 0.72
15	41	3.92	53	21.8 ± 2.7	40.0 ± 2.6	37.3 ± 4.6	0.65 ± 0.16	4.22 ± 0.09
16	**37**	**5.4**	**57**	**40.7 ± 7.4**	**81.9 ± 22.4**	**40.3 ± 9.3**	**0.71 ± 0.17**	**4.49 ± 0.59**
17	37	5.72	59.3	23.7 ± 3.7	52.0 ± 6.6	35.6 ± 5.2	0.73 ± 0.09	5.46 ± 0.09
18	**45**	**4.95**	**56**	**33.0 ± 2.8**	**74.0 ± 11.4**	**45.7 ± 6.8**	**0.68 ± 0.08**	**5.64 ± 0.54**
19	47	5.07	58	33.1 ± 5.0	63.9 ± 6.1	38.6 ± 6.0	1.27 ± 0.19	4.31 ± 0.10
20	**36**	**3.87**	**52.3**	**22.0 ± 2.2**	**39.8 ± 4.5**	**44.2 ± 5.9**	**0.65 ± 0.07**	**4.58 ± 0.44**
Mean ± SD	38.4 ± 7.3	4.78 ± 0.7	56.5 ± 2.5	29.6 ± 5.6	56.0 ± 16.2	41.6 ± 8.8	0.81 ± 0.23	4.82 ± 0.49

Values are means ± SD. V_T, tidal volume; PEF, peak tidal expiratory flow; RR, respiratory rate; T_E, expiratory time; $EE_{T_{CO_2}}$, end-expiratory tidal CO_2. Bold rows, infants exposed to prenatal tobacco smoke.
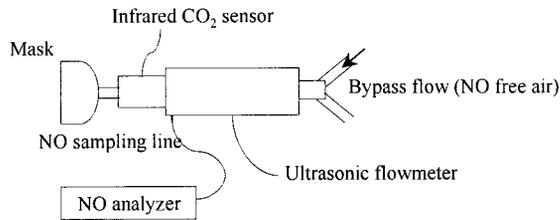
Fig. 1. Schematic diagram of the measurement setup used to record tidal flows, exhaled nitric oxide (NO; F$_{ENO}$), and CO$_2$.

be quantified by defining the absolute change between the groups, normalized for the variability of the control group [i.e., the sensitivity index (SI)]. Furthermore, to ensure that the additional $\dot{V}$ dependence of F$_{ENO}$ was accounted for (as described above), we included $\dot{V}_{NO}$ in the SI analysis.

Equipment. Tidal $\dot{V}$, volume, F$_{ENO}$, and CO$_2$ were measured using commercially available infant lung function equipment (Fig. 1) (Exhalyser; EcoMedics, Duernten, Switzerland). $\dot{V}$ was determined using an ultrasonic flowmeter (Spiroson model M30.8001; EcoMedics), NO was measured in exhaled air with a rapid response chemiluminescence analyzer (CLD 77 AM; EcoMedics) in the range of 0–100 parts/billion (ppb) with a sensitivity of 0.05 ppb. The response and delay times of the analyzer were 100 ms (10 Hz) and 830 ms, respectively. CO$_2$ was monitored with the use of an infrared analyzer with a resolution of 0.05% and a response time of 60 ms (16 Hz) (Pryon). Volume was calculated from the $\dot{V}$ signal. The dead space of the $\dot{V}$, NO, and CO$_2$ measurement equipment was 3 ml, whereas the face mask had a volume of 15 ml. Current recommendations for infant lung function testing advise that the effective dead space of a face mask equate to 50% of the water displacement volume of the mask (10); hence the effective dead space of the measurement head was 10.5 ml (50% contribution of face mask, i.e., 7.5 ml). The resistance of the measurement equipment (Req) was 0.36 kPa·l^{-1}·s at an $\dot{V}$ of 100 ml/s and was within current recommendations of Req < 0.7 kPa·l^{-1}·s at 100 ml/s in term neonates (10). It is important to note that this resistance will not cause the closure of the vellum and thus exclude nasal NO. A bias $\dot{V}$ of NO-free air was used to ensure that levels of F$_{ENO}$ were not contaminated by ambient NO. As the NO was determined from a sidestream portal and CO$_2$ and $\dot{V}$ were determined in-line, the delay times were determined and adjusted so as to allow for real-time, breath-by-breath inspection of the data. Data were sampled at a rate of 200 Hz with an accuracy of 12 bits.

Measurement and analysis. Infants were studied during quiet sleep, using a compliant silicon mask (size 0 infant mask; Homedica) placed over the nose and mouth. $\dot{V}$-volume loops were inspected for leak before measurement was commenced. Tidal breathing was recorded for a period of 3–4 min using Spiroware software (EcoMedics) and stored for later analysis. Tidal $\dot{V}$, F$_{ENO}$, CO$_2$, and volume recordings were exported, and further analysis was carried out using a custom-designed analysis software (MATLAB; Mathworks). Individual breaths and, hence, expirations were identified, and all further analysis was carried out on the individual expiratory portions of the tidal breathing. To ensure consistency of analysis among infants, only the first 100 breaths in the tidal breathing data were analyzed. To investigate possible information contained within a single expiration, we analyzed the data using two approaches, as follows.

The first approach (*analysis A*) was based on the expiratory capnograph [exhaled CO$_2$ vs. expired volume (VE)] and was used to identify the PII and PIII as described previously

by Strömberg and Gustafsson (21). The expiratory capnograph can be divided into three phases. Phase I consists of expired gas from the convective airways and contains minimal CO$_2$, PII is a transitional phase, due to progressive washout of the airways with alveolar gas, whereas PIII represents emptying of CO$_2$ from alveolar compartments. We determined PII and PIII of the expired CO$_2$ vs. VE graph and used these to determine the mean F$_{ENO}$, $\dot{V}$, and $\dot{V}_{NO}$ within the respective phases. Briefly, PII was defined as the interval bounded by an expired CO$_2$ concentration of 0.5% and end-tidal CO$_2$ of 60%, whereas the PIII interval was delimited by the point at which 200% of the airway dead space was expired and 90% of the VE. The mean airway dead space for the entire tidal breathing trace was used and determined with the use of the Bohr equation. A representative trace illustrating the phases is shown in Fig. 2.
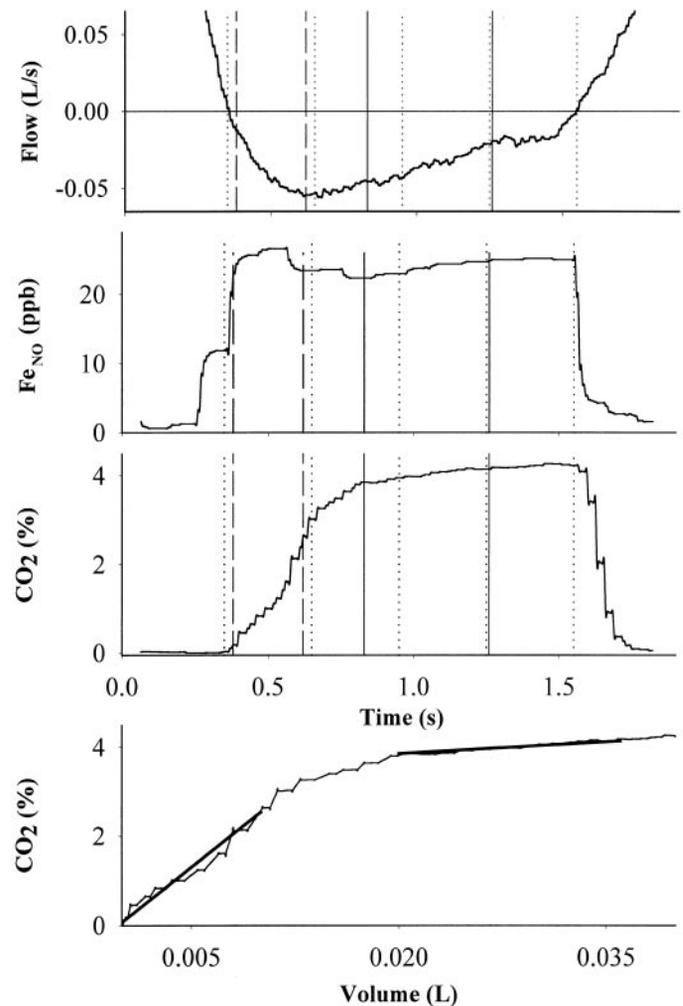

Fig. 2. Representative tidal flow, F$_{ENO}$, and CO$_2$ for an individual infant. The *top* 3 panels show the tidal data vs. time, whereas the *bottom* panel shows the same CO$_2$ expiration plotted as a function of expiratory volume. The 4 quartiles (Q1–Q4), each representing 25% of the expiratory time, are shown as dotted vertical lines. Phases II and III were calculated from the expiratory capnograph and are shown in the CO$_2$ vs. expiratory volume as solid lines. The translation of the phase II (dashed vertical lines) and III (solid vertical lines) portions to their equivalent time-based components is also shown, illustrating that phase II is approximately equivalent to the Q1, whereas phase III overlapped Q3 and a portion of Q2. ppb, Parts/billion.

The second approach (*analysis B*) was time rather than volume based. Each successive expiration was divided into quartiles (Q1, Q2, Q3, and Q4, with each quartile equating to 25% of expiration) as illustrated in Fig. 2. For each individual quartile, we determined the mean F$_{\text{E}_{\text{NO}}}$ concentration (e.g., Q1-F$_{\text{E}_{\text{NO}}}$), $\dot{V}$, and $\dot{V}_{\text{NO}}$.

Statistics. Group data are presented as means ± SE if normally distributed or as median and 25–75th percentiles if not normally distributed. The breath-by-breath $\dot{V}$ dependency of the tidal F$_{\text{E}_{\text{NO}}}$ data was tested by fitting a linear regression equations to the individual breath F$_{\text{E}_{\text{NO}}}$ and $\dot{V}$ for the two groups (PTE and control) for each portion of expiration. A two-way ANOVA was used to determine differences in the group F$_{\text{E}_{\text{NO}}}$ and $\dot{V}_{\text{NO}}$ data between quartiles and phases and to test for differences between infants exposed to prenatal cigarette smoke and controls. The intra- and intersubject coefficients of variation (CV) of the group data were calculated (CV = SD/mean). Differences in intrasubject variability were tested by using one-way ANOVA. We determined the SI of each portion of the PTE group, using SD units, as the absolute change between the two groups in multiples of SD of the control group. To ascertain if the PTE group differed significantly from our control group, a *t*-test was used to test whether the SD units were significantly shifted from zero. A one-way ANOVA of the SD units was performed to determine whether any individual portion was more sensitive to differences between the two groups. Significance was accepted at the $P < 0.05$ level.

RESULTS

The group mean T$_{\text{E}}$ in the infants was 0.82 ± 0.05 (SE) s (range: 0.45–1.27 s), whereas the duration of PII and PIII of the expiratory capnograph was 0.17 ± 0.09 s (0.09–0.25 s) and 0.33 ± 0.027 s (0.15–0.58 s), respectively. Infants receiving PTE had significantly reduced T$_{\text{E}}$ (0.67 ± 0.07 s) compared with controls (0.90 ± 0.06 s; $P < 0.03$), which leads to a tendency for an increased respiratory rate (46.2 ± 4.0 and 39.2 ± 1.9 breaths/min, respectively; $P = 0.09$). Mean $\dot{V}$ within each portion tended to be increased in the PTE group; however, this tendency did not reach signifi-

cance. No significant differences in V$_{\text{T}}$, PEF, or end-expiratory CO$_2$ were noted because of PTE.

Tidal F$_{\text{E}_{\text{NO}}}$ concentrations and output. Individual and group tidal F$_{\text{E}_{\text{NO}}}$ (Fig. 3) and $\dot{V}_{\text{NO}}$ (Fig. 4) for PII and PIII and the four expiratory quartiles are shown. Whereas the group mean F$_{\text{E}_{\text{NO}}}$ tended to be highest in PII (PII-F$_{\text{E}_{\text{NO}}}$) and lowest in the final quartile of expiration (Q4-F$_{\text{E}_{\text{NO}}}$), these differences were not significant. The group mean $\dot{V}$ was significantly dependent on the portion of expiration ($P < 0.001$), with Q4 being significantly lower than all other portions of expiration (Q4-$\dot{V}$: 26.2 ± 2.0 ml/s; $P < 0.05$). The remaining portions of expiration ranged between 34.7 ± 2.3 ml/s (Q1) and 50.3 ± 3.7 ml/s (Q2), with Q3, PII, and PIII being 41.4 ± 3.3, 46.0 ± 3.0, and 40.8 ± 2.9 ml/s, respectively. These significant differences in $\dot{V}$ lead to $\dot{V}_{\text{NO}}$ being significantly influenced by the portion of expiration ($P < 0.001$), with the final quartile (Q4-$\dot{V}_{\text{NO}}$) being significantly lower than the remaining portions. Infants with cigarette exposure in pregnancy exhibited significantly reduced F$_{\text{E}_{\text{NO}}}$ and $\dot{V}_{\text{NO}}$ in all expiratory portions (Figs. 3 and 4, respectively; $P < 0.001$). The breath-by-breath tidal F$_{\text{E}_{\text{NO}}}$ exhibited significant negative $\dot{V}$ dependence in all portions in both groups of infants (Table 2).

The intrasubject variability was expressed as CV for the F$_{\text{E}_{\text{NO}}}$ and $\dot{V}_{\text{NO}}$ for each portion of expiration in each infant over the 100 analyzed breaths (Fig. 5). There was a significant effect of expiratory portion on the CV of both F$_{\text{E}_{\text{NO}}}$ ($P < 0.002$) and $\dot{V}_{\text{NO}}$ ($P < 0.001$). Q4-F$_{\text{E}_{\text{NO}}}$ was significantly more variable than the remaining phases and quartiles (pairwise comparisons; $P < 0.05$), whereas both Q1- and Q4-$\dot{V}_{\text{NO}}$ demonstrated increased intrasubject variability ($P < 0.05$). The intersubject variability of F$_{\text{E}_{\text{NO}}}$ and $\dot{V}_{\text{NO}}$ was also determined. The intersubject variability in tidal F$_{\text{E}_{\text{NO}}}$ was high, ranging between 34.6 and 48.8%. Similarly, the intersubject variability of $\dot{V}_{\text{NO}}$ ranged between 30.2 and 51.9%. No differences in intersubject variability were noted, ei-
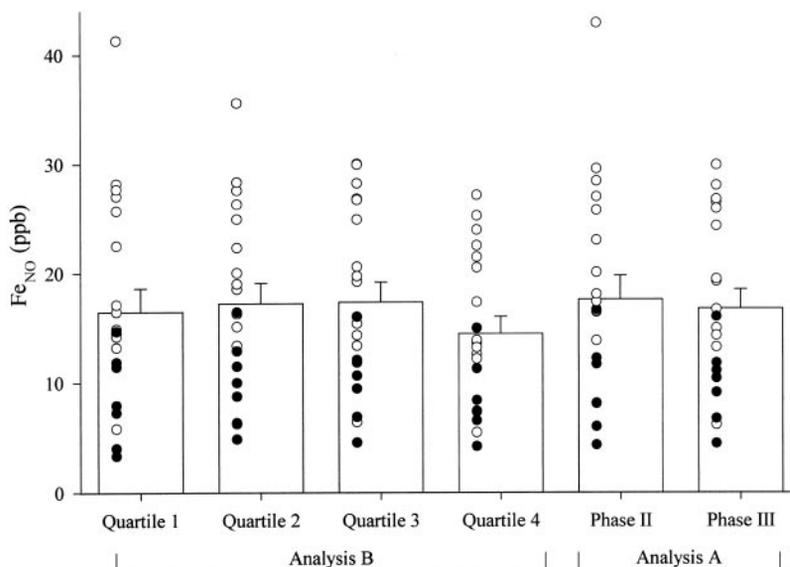

Fig. 3. Relationship between F$_{\text{E}_{\text{NO}}}$ and the portion of expiration. There were no significant differences in F$_{\text{E}_{\text{NO}}}$ between portions. Group mean (± SE) data are shown as columns, whereas individual mean data are shown as either open (controls) or solid (prenatal smoke exposure) circles. Prenatal tobacco exposure significantly reduced F$_{\text{E}_{\text{NO}}}$, irrespective of the portion of expiration tested ($P < 0.001$). See text for explanation of *analyses A* and *B*.
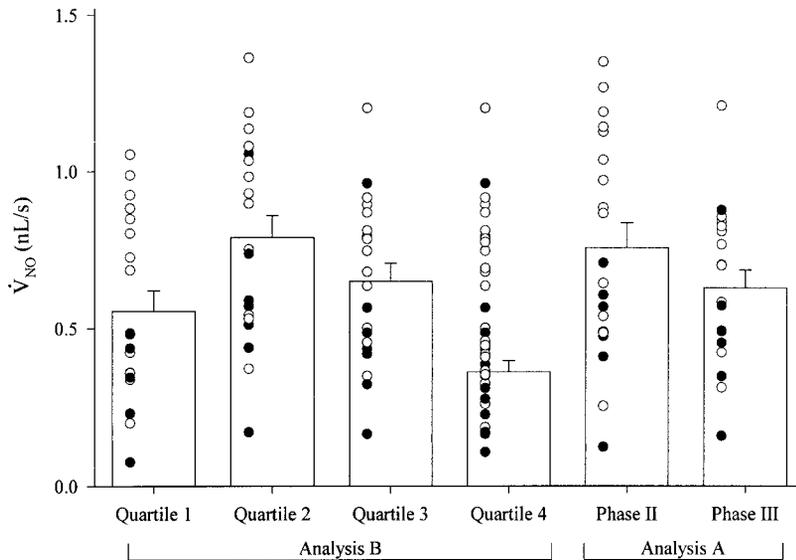
Fig. 4. Relationship between NO output ($\dot{V}_{NO}$) and the portion of expiration. $\dot{V}_{NO}$ was significantly different between portions of expirations ($P < 0.001$), with the Q4 being significantly lower (multiple pairwise comparison: $P < 0.05$) than the remaining portions. Group mean ($\pm$ SE) data are shown as vertical columns, and individual mean data are shown as circles. Infants with prenatal smoke exposure (●) had significantly lower $\dot{V}_{NO}$ ($P < 0.001$) compared with controls (○) in all portions of expiration.

ther in $F_{E_{NO}}$ or $\dot{V}_{NO}$ for the differing portions of expirations. Those infants exposed to prenatal tobacco did not have significantly different CV values in any portion of expiration than those of controls ($P > 0.1$). There were no significant differences in levels of $F_{E_{NO}}$, $\dot{V}_{NO}$, or CV for these parameters in either the volume-based (*analysis A*) or time-based (*analysis B*) analysis methods, due to prenatal smoke exposure.

The SI was used to determine the differences between control and PTE groups. The approaches of *analyses A* and *B* were equally sensitive to changes in $F_{E_{NO}}$ and $\dot{V}_{NO}$ because of PTE (Table 3). $F_{E_{NO}}$ in the PTE group was lower than that in the control group in all portions. Whereas no individual portion was significantly better able to distinguish $F_{E_{NO}}$ in the PTE group from the control group (one-way ANOVA; $P =$

0.98), all portions were significantly shifted from zero ($P < 0.005$). A similar outcome was noted for $\dot{V}_{NO}$, with all portions being significantly able to discriminate between the PTE and control groups ($P < 0.03$) and no portion being able to distinguish differences significantly better than any other ($P = 0.99$).

DISCUSSION

In the present study, we investigated the collection and analysis of tidal $F_{E_{NO}}$ measurements in infants, such that it was feasible to obtain $F_{E_{NO}}$ in unsedated, sleeping infants (*criteria 1* and *2*) without disturbing their breathing patterns (*criterion 3*). Critical to this point is the preferential nasal breathing of infants, requiring the measurement of nasal tidal breathing. We demonstrated that tidal $F_{E_{NO}}$ was $\dot{V}$ dependent (*criterion 4*) and thus should be accounted for when tidal $F_{E_{NO}}$ is monitored. We demonstrated that the presented methods of analysis (volume and time based) could ascertain differences between control infants with no maternal history of smoking and those infants who had been exposed to prenatal cigarette smoke.

Tidal $F_{E_{NO}}$. In unsedated infants, we found $F_{E_{NO}}$ to range between 13.1 ± 1.6 (SE) ppb for Q4-$F_{E_{NO}}$ to 15.8 ± 1.6 ppb for PII-$F_{E_{NO}}$. Sparse data on tidal $F_{E_{NO}}$ values in healthy children are available. Baraldi et al. (5) measured mixed tidal $F_{E_{NO}}$ in infants and young children using a collection reservoir. The investigators reported values of 14.1 ± 1.8 ppb in acutely wheezy subjects and lower values of 5.6 ± 0.5 ppb in healthy controls. In a further study in older children, the same authors reported reference values using an on-line tidal technique in healthy children ranging between 6 and 15 yr and reported a mean $F_{E_{NO}}$ level of 8.7 ppb (3). The tidal values in the present study are higher than those previously reported in healthy infants and children; these differences are most likely explained by the significantly lower tidal $\dot{V}$ values found in young infants and highlight the importance of recording and

Table 2. *Linear regression equations and their corresponding R and significance (P) values for the 6 discrete portions of expiration in the control and PTE groups*

Regression Equations	R	P
Control group		
Q1-$F_{E_{NO}} = 29.27 - (213.96*$Q1-$\dot{V})$	0.24	<0.001
Q2-$F_{E_{NO}} = 43.75 - (472.53*$Q2-$\dot{V})$	0.61	<0.001
Q3-$F_{E_{NO}} = 40.37 - (507.12*$Q3-$\dot{V})$	0.66	<0.001
Q4-$F_{E_{NO}} = 25.71 - (332.93*$Q4-$\dot{V})$	0.40	<0.001
PII-$F_{E_{NO}} = 37.01 - (319.45*$PII-$\dot{V})$	0.34	<0.001
PIII-$F_{E_{NO}} = 36.49 - (411.38*$PII-$\dot{V})$	0.51	<0.001
PTE group		
Q1-$F_{E_{NO}} = 31.45 - (192.84*$Q1-$\dot{V})$	0.82	<0.001
Q2-$F_{E_{NO}} = 30.31 - (156.57*$Q2-$\dot{V})$	0.33	<0.001
Q3-$F_{E_{NO}} = 31.43 - (202.84*$Q3-$\dot{V})$	0.62	<0.001
Q4-$F_{E_{NO}} = 24.70 - (271.19*$Q4-$\dot{V})$	0.30	<0.001
PII-$F_{E_{NO}} = 36.63 - (244.53*$PII-$\dot{V})$	0.87	<0.001
PIII-$F_{E_{NO}} = 27.18 - (83.31*$PII-$\dot{V})$	0.27	<0.001

Q1, Q2, Q3, Q4: quartiles; PII and PIII, phases II and III, respectively; $F_{E_{NO}}$, fractional exhaled nitric oxide; $\dot{V}$, flow; PTE, prenatal tobacco exposure. Linear regressions were fitted to the breath-by-breath individual $F_{E_{NO}}$ (parts/billion) and $\dot{V}$ (l/s) data for each portion.

Fig. 5. Coefficient of variation data for the portions of expiration for FE$_{NO}$ (open bars) and V̇$_{NO}$ (hatched bars). The box plots are bounded by the 25–75th percentiles, with the group median values shown as a solid horizontal line; the error bars equate to the 10–90th percentiles. Significant differences were seen in the coefficient of variation data for FE$_{NO}$ ($P < 0.002$) and V̇$_{NO}$ ($P < 0.001$), depending on the portion of expiration. The variability of Q4-FE$_{NO}$ and Q4-V̇$_{NO}$ was significantly higher than the remaining portions of expiration (multiple pairwise comparison: $P < 0.05$).
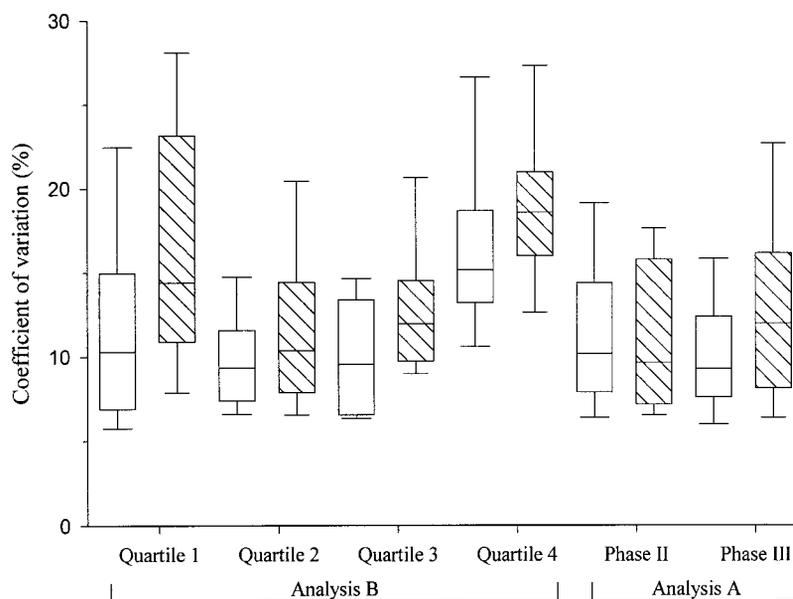
correcting for V̇ in tidal FE$_{NO}$ measurements (details below). The intrasubject variability differed between expiratory phases and ranged between 9.3 and 15.1%. Similar to previous studies in infants (24), the intersubject variability of FE$_{NO}$ was high, ranging from 34.6 to 44.6% in the Q3 and Q1 of expiration, respectively.

V̇ dependence of tidal FE$_{NO}$ and V̇$_{NO}$. The present study demonstrated that breath-to-breath tidal FE$_{NO}$ exhibits significant negative V̇ dependence in all portions of expiration. The negative V̇ dependence of FE$_{NO}$ is well recognized and has been demonstrated in numerous studies (9, 16, 20). In the present study, we used linear regression equations to test for V̇ dependency, whereas studies in adults have shown that FE$_{NO}$ is exponentially related to expiratory V̇ (20, 22). The use of exponential regression analysis in this population did not improve the description of alterations in FE$_{NO}$ with V̇. This most likely relates to the very small V̇ range found in infant tidal breathing (24.2 ± 0.3 to 59.5 ± 0.5 ml/s for Q4 and Q2, respectively) compared with extended V̇ ranges used in adult studies (5–1,500 ml/s). The V̇ dependence of tidal FE$_{NO}$ suggests that the measurement of V̇ and the calculation of V̇$_{NO}$ are essential in methods not controlling the expiratory V̇,

such as the collection of FE$_{NO}$ using reservoir bags (5) or the on-line tidal breathing method (3, 13).

V̇$_{NO}$ was found to be significantly dependent on the portion of expiration and ranged between 0.36 ± 0.04 and 0.79 ± 0.07 nl/s for Q4 and Q2, respectively. Wildhaber and coworkers (24) measured forced FE$_{NO}$ in healthy infants [18.8 ± 12.4 (SD) ppb] at a V̇ of 50 ml/s, equating to a V̇$_{NO}$ of 0.94 nl/s. These authors demonstrated a significant effect of parental atopy on FE$_{NO}$, irrespective of respiratory history. The population studied by these authors included three infants with no respiratory history and no parental atopy and had a mean (range) FE$_{NO}$ of 12.9 ppb (2.6–26.3 ppb), equating to a mean V̇$_{NO}$ of 0.65 nl/s (0.13–1.32 nl/s). Previous studies measuring tidal FE$_{NO}$ in infants have not reported the corresponding V̇ values, and, hence, comparisons cannot be made. Franklin et al. (9) reported FE$_{NO}$ values in 116 healthy, nonatopic children (aged 7–13 yr) of 7.2 ppb (6.4–8 ppb) at 75 ml/s, representing a V̇$_{NO}$ range of 0.48–0.6 nl/s. Artlich and coworkers (2) reported similar values of V̇$_{NO}$ (mean: 0.31 nl/s) in 11 healthy children. V̇$_{NO}$ levels ranging between 0.2 and 0.65 nl/s have been reported in studies of healthy nonatopic adults (7, 8, 17). The current data are in good agreement with studies that use standardized measurement techniques and would suggest that the presented analysis methods of tidal FE$_{NO}$, when corrected for tidal V̇ values, provide similar information as the forced techniques. The variability of V̇$_{NO}$ was dependent on the phase of expiration. We found that Q1 and Q4 were significantly more variable than the remaining phases. This may be due to the more stable V̇ conditions occurring during midexpiration.

PTE. Our study population included seven infants who had been exposed to tobacco smoke during pregnancy, and these infants exhibited significantly decreased FE$_{NO}$ and V̇$_{NO}$ compared with nonexposed controls. Studies conducted in adults have demonstrated that chronic cigarette exposure decreases FE$_{NO}$, irre-

Table 3. *Sensitivity indexes for FE$_{NO}$ and V̇$_{NO}$ for discrete portions of expiration*

Expiratory Portion	FE$_{NO}$	V̇$_{NO}$
Q1	−1.30 ± 0.17	−1.16 ± 0.20
Q2	−1.43 ± 0.20	−1.09 ± 0.36
Q3	−1.50 ± 0.19	−1.18 ± 0.43
Q4	−1.41 ± 0.21	−1.08 ± 0.22
PII	−1.35 ± 0.17	−1.26 ± 0.21
PIII	−1.47 ± 0.20	−1.11 ± 0.37

Values are means ± SD. V̇$_{NO}$, nitric oxide output. No individual portion was more sensitive to changes in FE$_{NO}$ or V̇$_{NO}$ caused by PTE (1-way ANOVA). Conversely, all portions of expiration were significantly shifted from zero (*t*-test), indicating that the PTE group was significantly lower, in all portions, than the control group.

spective of the subject's health status (6, 15, 19). The role of passive smoke exposure is less clear. Franklin et al. (9) found no effect of passive cigarette smoke exposure in a community study of 7- to 13-yr-old children with no history of respiratory disease, possibly indicating that the documented effects of cigarette smoke may only apply to direct inhalation. We are not aware of any studies investigating the role of PTE on neonatal $F_{E_{NO}}$. Hasan et al. (12) demonstrated that prenatal cigarette smoke reduced neuronal NO synthase (nNOS), but not endothelial NO synthase expression, in the caudal brain stem of neonatal rats. The same group demonstrated that a downregulation of nNOS resulted in a diminished ventilatory response to hypoxia in developing rats (11). These results may provide indirect evidence of the underlying mechanisms resulting in decreased respiratory drive and hypoxic ventilatory response in infants of smoking mothers (23). Similarly, prenatal exposure to tobacco products may downregulate nNOS expression in infants and hence potentially reduce the contribution of nNOS to $F_{E_{NO}}$. Whereas at this time there is no known role of NO in smoke-exposed infants, who are particularly prone to wheezing disorders, we conclude from the present population that future studies of $F_{E_{NO}}$ in infants need to take smoke exposure into account as a confounding variable.

Sensitivity of the presented methods. We demonstrated that, whereas the differences in $F_{E_{NO}}$ and $\dot{V}_{NO}$ between the control and PTE groups were significant in all portions, no particular portion was able to distinguish between the differences of the two groups better. This result is not unexpected, if PTE does indeed downregulate nNOS. The subsequent reduction in NO production would be spread throughout the entire respiratory system and not be restricted to a particular location. Whereas $F_{E_{NO}}$ appeared to be more discriminatory than $\dot{V}_{NO}$, these differences were not significant. This apparent increased sensitivity in $F_{E_{NO}}$ probably relates to the increased $\dot{V}$ values found in the PTE group, causing reductions in $F_{E_{NO}}$ unrelated to actual NO production. We would stress the importance of measuring $\dot{V}_{NO}$ in conditions associated with variable tidal $\dot{V}$ values (such as on-line tidal $F_{E_{NO}}$ measurements) or alterations in tidal breathing patterns caused by respiratory disease.

Limits of the method. A number of technical aspects may influence our results. The delay times of the NO and CO_2 sensors will influence the accuracy of the analysis. We determined the delay times before measurement and corrected these signals, with respect to $\dot{V}$, before carrying out any further analysis. The Req used (0.36 $kPa \cdot l^{-1} \cdot s$ at 100 ml/s) was insufficient to close the vellum, and thus we were able to measure $F_{E_{NO}}$ via the nose of the unsedated infants, allowing tidal breathing to be monitored without disturbing the infant's natural breathing patterns. However, should the expiratory $\dot{V}$ be sufficiently high, it is conceivable that the subsequently increased resistance may close the vellum and hence alter the physiological system being measured, possibly explaining the differing $F_{E_{NO}}$

levels between the groups studied. An airway pressure >4–5 cmH_2O will cause the vellum to close. The present equipment configuration would increase airway pressure >4–5 cmH_2O at $\dot{V}$ values >120–140 ml/s. Whereas the PTE group had a tendency for increased PEF, the highest PEF was 90.4 ml/s, and thus vellum closure will not have occurred in any of the studied infants.

We demonstrated that infants exposed to tobacco had significantly decreased $F_{E_{NO}}$ and $\dot{V}_{NO}$. Alterations in control of breathing or tidal breathing patterns in smoke-exposed infants may have potentially contributed to the noted differences in $F_{E_{NO}}$ parameters. There was a tendency for mean $\dot{V}$ within each portion to be higher in infants exposed to prenatal tobacco; these differences were, however, not significant. It is conceivable that this nonsignificant increase in $\dot{V}$ may have contributed to the noted reduction in $F_{E_{NO}}$ levels but cannot explain the significant differences in $\dot{V}_{NO}$ found between the control and PTE groups. Indeed, the use of $\dot{V}_{NO}$, rather than $F_{E_{NO}}$, as the parameter of choice would enable any differences in $\dot{V}$ between groups to be accounted for and may enhance the ability of the presented methods to detect changes in NO production beyond simple alterations in tidal breathing patterns.

Conclusions. This study has demonstrated that on-line tidal $F_{E_{NO}}$ and $\dot{V}_{NO}$ can be measured, via a face mask, in unsedated newborn infants. This represents the first on-line tidal $F_{E_{NO}}$ data in healthy infants in this age group and in infants before an episode of respiratory disease. The on-line measurement of tidal $F_{E_{NO}}$ and $\dot{V}_{NO}$ proved to be a simple (*criterion 1*) and noninvasive (*criterion 2*) technique that was able to be applied to the population studied and allowed undisturbed $F_{E_{NO}}$, $\dot{V}$, and CO_2 breathing patterns (*criterion 3*) to be collected. The basis of the technique, therefore, satisfied the requirements of the application of any infant lung function methodology.

The use of CO_2 washout characteristics allowed the identification of airway (PII) and alveolar (PIII) emptying and hence the different regions of the $F_{E_{NO}}$ profile. These phases could be related to specific quartiles, with PII corresponding most closely to the Q1, whereas PIII overlapped the Q2 and Q3. Therefore, in the absence of a CO_2 washout signal, a time-based analysis may be used as an approximation of the PII and PIII. Tidal $F_{E_{NO}}$ was found to be significantly $\dot{V}$ dependent throughout expiration, indicating the importance of measuring $\dot{V}$ (*criterion 4*) in tidal $F_{E_{NO}}$ conditions. Furthermore, this significant $\dot{V}$ dependence of $F_{E_{NO}}$ highlights the importance that the technique of measuring the on-line tidal $F_{E_{NO}}$ does not alter the breathing pattern of the subjects.

The simultaneous collection of $F_{E_{NO}}$ and $\dot{V}$ allowed tidal $\dot{V}_{NO}$ to be characterized in this group of infants. $\dot{V}_{NO}$ values proved to be comparable to previously reported values derived from standardized techniques. In respiratory disease, differences in tidal breathing may alter the pattern of $F_{E_{NO}}$ clearance from the air spaces and, hence, cause changes in $F_{E_{NO}}$ and $\dot{V}_{NO}$

levels and variability. No individual expiratory portion discriminated the PTE group from controls better than any other portion. This result was not unexpected and may relate to the effect of PTE on nNOS throughout the airway tree. However, this may not be the case in future studies of lower airway disease. Finally, we conclude that both $F_{E_{NO}}$ and $\dot{V}_{NO}$ were reduced in those infants exposed to maternal smoking, compared with healthy, nonatopic controls, suggesting that the presented analysis methods are able to detect differences between normal and abnormal physiological systems.

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