

Letter to the Editor

Age-stratified comparison of large and peripheral airway/alveolar nitric oxide levels in children and young adults

To the Editor:

Measurement of exhaled nitric oxide (NO) is a relatively simple, reproducible, and noninvasive clinical test for monitoring endogenous inflammatory airway signals in asthmatic patients and the response to inhaled and oral corticosteroids.¹ The currently accepted method of measuring the fraction of exhaled nitric oxide (FENO [in parts per billion]) at a single constant expiratory flow rate, usually 50 mL/s, is a surrogate of predominantly large airway NO flux. However, it is incapable of separating whether the source of normal or increased NO production is originating from the large/central airway versus the peripheral airway/alveolar sites, or both.¹ Therefore we developed newer techniques to better discriminate the source of NO gas exchange and to correct for axial back-diffusion of NO from the large to peripheral airway/alveolar sites.² This attempts to avoid overestimating peripheral NO concentrations and underestimating large-airway NO flux.²

The current prospective outpatient study evaluates baseline exhaled NO gas exchange in healthy children and adolescents age 10 to 19 years. We insisted on normal physical examination results, absence of all medication use, and no history of allergies, eczema, rhinitis, lung disease (including asthma), cigarette smoking (cotinine levels were not measured), or respiratory tract infection within the 6 weeks before testing. On the basis of our previous experience, children age less than 10 years have less than optimal cooperation for NO testing. We wanted to obtain age-stratified data for comparison with results previously obtained in our laboratory in healthy young and older adults age 20 years or greater by using the same methods to measure exhaled NO levels.³ These baseline data have not been previously published and are needed for both research and clinical studies.

As previously described in detail,¹ NO production in large and peripheral airway/alveolar sites was measured with a Sievers NOA 280 chemiluminescent analyzer (GE Analytical Instruments, Boulder, Colo). All volunteer healthy subjects provided informed consent, and if they were less than 18 years of age, approval of both parents was also required. Data were assessed for normality of distribution and correlation with age by using Spearman analysis. Data for FENO values at 50 mL/s, large-airway NO flux (J'aw_{NO} [in nanoliters per second]), and peripheral airway/alveolar NO (C_{ANO}) for different age groups were analyzed by using 1-way ANOVA on ranks with repeated measurements. The unpaired *t* test (Mann-Whitney) was also used to compare age groups. This study was approved by both Lakewood Regional Medical Center and Western IRB (Olympia, Wash) and registered as NCT#00576069 and NCT#00568347.

Results are summarized in Table I,³ and comparison with previously published data⁴⁻¹⁰ in children and adolescents appears in Table II. Forty-seven (33 female) healthy, asymptomatic, children who never smoked and were age 15 ± 3 years (mean $\pm$ SD; median, 15 years [1-3 interquartile] 12-17 years]) were studied. Their height was 64 inches (60-67 inches), weight was 119 lbs (100-143 lbs), and body mass index was 20 kg/m² (19-24 kg/m²). Ethnicity was 26% Hispanic, 37% non-Hispanic white,

22% African American, and 15% Asian. FEV₁ was 2.8 L (2.4-3.5 L) and 87% (80% to 99%) of predicted value, forced vital capacity was 3.3 L (2.9-3.9 L) and 91% (83% to 100%) of predicted value, and FEV₁/forced vital capacity ratio was 84% (81% to 89%) based on normal predictions from Hsu et al.¹¹

FENO at 50 mL/s was 16 ppb (11-21 ppb), with a Spearman rho versus age of 0.3. The FENO value at 50 mL/s was significantly decreased ($P = .03$) compared with that seen in 32 healthy young adults (15 female) with an age range of 20 to 39 years (median, 25 years [21-28 years]) and a FENO value of 22 ppb (14-38 ppb), as previously reported.³ In the present study Spearman correlation with height (in inches) and FENO value at 50 mL/s resulted in an *r* value of 0.36 ($P = .02$), and for male versus female sex, the *P* value was .11. After correction for NO axial back-diffusion,² the J'aw_{NO} value was 1.0 nL/s (0.5-1.6 nL/s), and the Spearman rho value versus age was 0.3. The J'aw_{NO} value was significantly decreased ($P = .007$) compared with that seen in 32 healthy young adults with J'aw_{NO} values of 1.5 nL/s (1.0-2.9 nL/s), as previously reported.³ Spearman correlation with height (in inches) and J'aw_{NO} resulted in an *r* value of 0.34 ($P = .02$), and for male versus female sex, the *P* value was .10. After correction for NO axial back-diffusion,² the C_{ANO} value was 3.4 ppb (1.8-4.9 ppb) and was significantly ($P = .01$) increased compared with the C_{ANO} value of 2.0 ppb (1.1-3.1 ppb) seen in the 32 healthy young adults, as previously reported.³ Spearman correlation with height (in inches) and C_{ANO} value resulted in an *r* value of -0.008 ($P = .96$), and for male versus female sex, the *P* value was .24. The C_{ANO}/FENO ratio was 21% (9% to 37%) and significantly increased ($P = .0004$) compared with 8% (3% to 15%) in the 32 healthy young adults, as previously reported.³ Spearman correlation between the FENO value at 50 mL/s and the J'aw_{NO} value resulted in an *r* value of 0.97 ($P < .000$), and FENO value at 50 mL/s and C_{ANO} resulted in an *r* value of 0.02 ($P = .89$).

We observed a significant increase in C_{ANO} values in healthy children and adolescents age 10 to 19 years compared with published data in healthy subjects aged 20 to 39 years, as previously obtained in our laboratory using similar equipment.³ We did not probe the underlying mechanism of this observation; however, our results are consistent with an increase in peripheral airway/alveolar NO tissue production (increased inducible nitric oxide synthase or possibly constitutive nitric oxide synthase levels), a reduced NO lung diffusing capacity, or both. NO lung diffusing capacity is relatively insensitive to changes in capillary volume because the transfer rate across the alveolar capillary surface area between NO and hemoglobin is much greater compared with that between carbon monoxide and hemoglobin and therefore reflects primarily membrane conductance.³ However, there is some recent evidence that suggests alveolarization continues through adolescence, and thus NO membrane conductance might be reduced in children and adolescents because of a smaller alveolar surface area.¹²

J'aw_{NO} is the product of the NO concentration in bronchial wall mucosa and bronchial NO diffusing capacity, which reflects transfer of NO from the airway wall to the lumen of expired air and is proportional to the lumen surface area and the transfer coefficient. Although we did not measure NO concentrations in bronchial wall mucosa and bronchial NO diffusing capacity, J'aw_{NO} in healthy subjects aged 10 to 19 years is significantly decreased

TABLE I. Exhaled NO gas exchange in healthy volunteers of varying age

Parameter	Age (y)	No.	Mean	SD	Minimum	Median	Maximum	Q1	Q3	P value
J'aw _{NO} (nL/s)	10-19	47	1.3	1.2	0	1	6.4	0.5	1.6	
	20-39	32	2.3	2.4	0.51	1.53	10.88	0.5	2.9	
	40-59	26	1.52	0.76	0.44	1.36	3.06	1	2	
	10-19 vs 20-59									.003
	10-19 vs 20-39									.007
	20-39 vs 40-59									.6
FENO at 50 mL/s (ppb)	10-19	47	19.4	14.4	3.5	16.1	80.9	11	21	
	20-39	32	31.2	30.5	9	21.5	137	14	38	
	40-59	26	22.52	7.55	11	22	36	18	30	
	10-19 vs 20-59									.003
	10-19 vs 20-39									.03
	20-39 vs 40-59									.9
C _{ANO} (ppb)	10-19	47	3.49	2.14	0	3.4	8.2	1.8	4.9	
	20-39	32	2.35	2.04	0	1.95	9.5	1.1	3.1	
	40-59	26	3.1	2.64	0	2.8	9.9	0.8	5.1	
	10-19 vs 20-59									.03
	10-19 vs 20-39									.01
	20-39 vs 40-59									.01
C _{ANO} /FENO ratio	10-19	47	0.26	0.20	0	0.21	1	0.1	0.4	
	20-39	34	0.13	0.19	0	0.08	1	0	0.1	
	40-59	26	0.18	0.17	0	0.14	0.55	0	0.3	
	10-19 vs 20-59									.0009
	10-19 vs 20-39									.0004
	20-39 vs 40-59									.3

All C_{ANO} and J'aw_{NO} values were corrected for axial NO back-diffusion. Data for healthy subjects aged greater than 19 years has been published previously.³

TABLE II. Results of current and previous studies of large versus peripheral/alveoli nitric oxide corrected for NO axial back-diffusion² in children

Age (no. studied)	J'aw _{NO} (nL/s), large-airway NO	C _{ANO} (ppb), peripheral/alveolar NO	FENO (ppb) at 50 mL/s	C _{ANO} (ppb)/FENO (ppb) at 50 mL/s	Reference
10 (6-17) y, median (minimum-maximum), n = 21 (9 female)	0.7 (0.1-1.4), median (minimum-maximum)	1.5 (0.1-2.2), median (minimum-maximum)	8.5 (2.2-15.3), median (minimum-maximum)	18% (5% to 14%), median (minimum-maximum)	Puckett et al ⁴
7-13 y, range, n = 66 (34 female)	0.7 (median) 0.85 ± 0.5 (mean ± SD)	1.2 (median) 1.1 ± 0.2 (mean ± SD)	10.3 (median) 11.7 ± 5.4 (mean ± SD)	17% (median)	Sepponen et al ⁵
11 (8.8-13.5) y, median (1-3 interquartile); range, 4-18 y, n = 107	0.56 (0.35-0.78), median (1-3 interquartile)	3.5 (2.7-4.1), median (1-3 interquartile)	11.9 (8.2-16.8), median (1-3 interquartile), n = 63 subjects at 45 mL/s	31% (25% to 33%), median (1-3 interquartile)	Latzin et al ⁶
13 y (7.5-16), median (range), n = 25 (16 female)	0.82 (0.32-3.2), median (range)	0.7 (0.08-0.0), median (range)	10.4 (3.3-29), median (range)	7% (0% to 24%), median (range)	Paraskakis et al ⁷
12 (6-18) y, median (1-3 interquartile), n = 22 (9 female)	0.83 ± 0.34, mean ± SD	1.0 ± 0.10, mean ± SD	11 ± 4, mean ± SD	9% ± 0.03%, mean ± SD	Radhakrishnan et al ⁸
14 (3.2) y, mean (SD), n = 74 (36 female)	0.66 (0.13-2.3), median (range)	1.4 (0.1-3.6), median (range)	12.4 (5.2-40.1), median (range)	11% (1.9% to 8.9%), median (range)	Keen et al ⁹
9.7 (7-13) y, mean (range), n = 66 (34 female)	1.7 ± 0.09, mean ± SEM	2.3 ± 0.08, mean ± SEM	11.7 ± 1.4, mean ± SEM	20% ± 6%, mean ± SEM	Linkosalo et al ¹⁰
15 (12-17) y, median (1-3 interquartile), n = 47 (33 female)	1.0 (0.5-1.6), median (1-3 interquartile) 1.3 ± 1.2, mean ± SD	3.4 (1.8-4.9), median (1-3 interquartile) 3.5 ± 2.1, mean ± SD	16 (11-21), median (1-3 interquartile) 19 ± 14, mean ± SD	21% (17% to 23%), median (1-3 interquartile) 18%, mean	Current study

Corrections for NO axial back-diffusion² were made by the original authors only in references 4 and 8 to 10 and in the current study. The corrections for axial back-diffusion² in references 5 to 7 were estimated, as described in the Methods section of Gelb et al.¹

when compared with our healthy subjects aged 20 to 39 years.³ However, the paradoxical increase in peripheral airway/alveolar C_{ANO} contributes to an increased C_{ANO}/FENO ratio compared

with healthy subjects aged 20 to 39 years.³ C_{ANO} adds directly to FENO at 50 mL/s because it is the starting contribution of a bolus of air as it traverses the alveolar region through the airways and is

exhaled. Furthermore, in healthy subjects aged less than 20 years, only 4 studies^{4,8-10} have previously used the 2-compartment model and corrected for NO axial back-diffusion,² and comparison data are shown in Table II. We estimated the correction² in the remaining 3 studies.⁵⁻⁷ The variable results contributed from individual studies is likely due to differences in the age range, laboratory method, and equipment, and precludes combining data.

In summary, our results indicate FENO at 50 mL/s and J'aw_{NO} increase with age 20 to 39 years in healthy control subjects compared with values seen in healthy subjects aged 10 to 19 years using the same laboratory method and equipment to measure exhaled NO levels. In contrast, there is an increase in the peripheral airway/alveolar C_{ANO} value in healthy subjects aged 10 to 19 years compared with healthy subjects aged 20 to 39 years. These age-stratified data have not been available previously but could affect the interpretation of clinical data in similar age groups in patients with prevalent diseases, such as asthma.

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